

ANTI-TSLP ANTIBODY CONSTRUCTS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of, and priority to U.S. Provisional Application No. 63/578,947, filed August 25, 2023, the content of which is incorporated herein by reference in its entirety.

REFERENCE TO AN ELECTRONIC SEQUENCE LISTING

[0002] The content of the electronic sequence listing (253272000240seqlist.xml; Size: 121,903 bytes; and Date of Creation: August 19, 2024) is herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0003] The present invention relates to isolated antibody constructs (*e.g.*, anti-TSLP antibody constructs) that specifically recognize thymic stromal lymphopoietin (TSLP), pharmaceutical compositions comprising the isolated anti-TSLP antibody constructs, methods of treating diseases using the isolated anti-TSLP antibody constructs, and kits comprising the isolated anti-TSLP antibody constructs. Also provided are methods of making thereof.

BACKGROUND OF THE INVENTION

[0004] Many abnormal cells and tissues as well as many diseases display unique antigens that can be leveraged for immune cell-mediated clearance, including inflammatory diseases such as autoimmune disorders or asthma. These inflammatory diseases can each display one or more different target antigens or one or more different epitopes of the same target antigen. For example, some antigens are over-expressed, mutagenized, or selectively mutagenized in inflamed tissues. Therefore, antibodies targeting specific antigens present in diseases with systemic or localized inflammation can be used as therapeutics.

[0005] Thymic stromal lymphopoietin (TSLP) is a pleiotropic cytokine characterized as a lymphocyte growth factor. TSLP binds to a high-affinity heteromeric complex composed of thymic stromal lymphopoietin receptor (TSLPR) chain and interleukin 7 receptor- α (IL-7R α). TSLP is expressed predominantly by gut and lung epithelial cells, skin keratinocytes, and dendritic cells (DCs), although it can be produced also by (*e.g.*) airway smooth muscle cells, mast cells, monocytes, macrophages, granulocytes, synovial fibroblasts, etc. Depending on

the immune cells targeted by TSLP, it is reported not only to promote T-helper 2 cell (Th2) responses but also is associated with autoimmune disorders.

[0006] Tezepelumab (Tezspire™) is a fully human monoclonal antibody that binds to TSLP and prevents its interaction with the TSLPR chain. Subsequently, the IL-7R α chain is not recruited and a functional TSLP receptor complex is not formed. *See*, H.K. Lehman and C.M. Sabella, “Allergic and Immunologic Diseases, A Practical Guide to the Evaluation, Diagnosis and Management of Allergic and Immunologic Diseases,” 2022, Pages 1111-1145, “Chapter 38 - New biologics in allergy.” It has been approved by US-FDA as an add-on maintenance treatment for severe asthma.

BRIEF SUMMARY OF THE INVENTION

[0007] In one aspect of the present invention, there is provided an isolated antibody construct (“anti-TSLP antibody construct”) comprising an antibody moiety that specifically recognizes thymic stromal lymphopoietin (TSLP; “anti-TSLP antibody moiety”), wherein the anti-TSLP antibody moiety comprises a heavy chain variable region (VH) and a light chain variable region (VL), and wherein: (a) the VH comprises (i) a CDR-H1 comprising an amino acid sequence of SEQ ID NO:2, or a variant thereof comprising up to 3 amino acid variations; (ii) a CDR-H2 comprising an amino acid sequence of SEQ ID NO:3, or a variant thereof comprising up to 3 amino acid variations; and (iii) a CDR-H3 comprising an amino acid sequence of SEQ ID NO:4, or a variant thereof comprising up to 3 amino acid variations; and the VL comprises (i) a CDR-L1 comprising an amino acid sequence of SEQ ID NO:6, or a variant thereof comprising up to 3 amino acid variations; (ii) a CDR-L2 comprising an amino acid sequence of SEQ ID NO:7, or a variant thereof comprising up to 3 amino acid variations; and (iii) a CDR-L3 comprising an amino acid sequence of SEQ ID NO:8, or a variant thereof comprising up to 3 amino acid variations; (b) the VH comprises (i) a CDR-H1 comprising an amino acid sequence of SEQ ID NO:12, or a variant thereof comprising up to 3 amino acid variations; (ii) a CDR-H2 comprising an amino acid sequence of SEQ ID NO:13, or a variant thereof comprising up to 3 amino acid variations; and (iii) a CDR-H3 comprising an amino acid sequence of SEQ ID NO:4, or a variant thereof comprising up to 3 amino acid variations; and the VL comprises (i) a CDR-L1 comprising an amino acid sequence of SEQ ID NO:6, or a variant thereof comprising up to 3 amino acid variations; (ii) a CDR-L2 comprising an amino acid sequence of SEQ ID NO:7, or a variant thereof comprising up to 3 amino acid variations; and (iii) a CDR-L3 comprising an amino acid sequence of SEQ ID NO:8, or a variant thereof comprising up to 3 amino acid variations; (c)

the VH comprises (i) a CDR-H1 comprising an amino acid sequence of SEQ ID NO:20, or a variant thereof comprising up to 3 amino acid variations; (ii) a CDR-H2 comprising an amino acid sequence of SEQ ID NO:21, or a variant thereof comprising up to 3 amino acid variations; and (iii) a CDR-H3 comprising an amino acid sequence of SEQ ID NO:22, or a variant thereof comprising up to 3 amino acid variations; and the VL comprises (i) a CDR-L1 comprising an amino acid sequence of SEQ ID NO:24, or a variant thereof comprising up to 3 amino acid variations; (ii) a CDR-L2 comprising an amino acid sequence of SEQ ID NO:25, or a variant thereof comprising up to 3 amino acid variations; and (iii) a CDR-L3 comprising an amino acid sequence of SEQ ID NO:26, or a variant thereof comprising up to 3 amino acid variations; (d) the VH comprises (i) a CDR-H1 comprising an amino acid sequence of SEQ ID NO:28, or a variant thereof comprising up to 3 amino acid variations; (ii) a CDR-H2 comprising an amino acid sequence of SEQ ID NO:29, or a variant thereof comprising up to 3 amino acid variations; and (iii) a CDR-H3 comprising an amino acid sequence of SEQ ID NO:30, or a variant thereof comprising up to 3 amino acid variations; and the VL comprises (i) a CDR-L1 comprising an amino acid sequence of SEQ ID NO:32, or a variant thereof comprising up to 3 amino acid variations; (ii) a CDR-L2 comprising an amino acid sequence of SEQ ID NO:33, or a variant thereof comprising up to 3 amino acid variations; and (iii) a CDR-L3 comprising an amino acid sequence of SEQ ID NO:34, or a variant thereof comprising up to 3 amino acid variations; (e) the VH comprises (i) a CDR-H1 comprising an amino acid sequence of SEQ ID NO:36, or a variant thereof comprising up to 3 amino acid variations; (ii) a CDR-H2 comprising an amino acid sequence of SEQ ID NO:37, or a variant thereof comprising up to 3 amino acid variations; and (iii) a CDR-H3 comprising an amino acid sequence of SEQ ID NO:38, or a variant thereof comprising up to 3 amino acid variations; and the VL comprises (i) a CDR-L1 comprising an amino acid sequence of SEQ ID NO:40, or a variant thereof comprising up to 3 amino acid variations; (ii) a CDR-L2 comprising an amino acid sequence of SEQ ID NO:41, or a variant thereof comprising up to 3 amino acid variations; and (iii) a CDR-L3 comprising an amino acid sequence of SEQ ID NO:42, or a variant thereof comprising up to 3 amino acid variations; or (f) the VH comprises (i) a CDR-H1 comprising an amino acid sequence of SEQ ID NO:44, or a variant thereof comprising up to 3 amino acid variations; (ii) a CDR-H2 comprising an amino acid sequence of SEQ ID NO:45, or a variant thereof comprising up to 3 amino acid variations; and (iii) a CDR-H3 comprising an amino acid sequence of SEQ ID NO:46, or a variant thereof comprising up to 3 amino acid variations; and the VL comprises (i) a CDR-L1 comprising an amino acid sequence of SEQ ID NO:48, or a variant thereof comprising up to

3 amino acid variations; (ii) a CDR-L2 comprising an amino acid sequence of SEQ ID NO:49, or a variant thereof comprising up to 3 amino acid variations; and (iii) a CDR-L3 comprising an amino acid sequence of SEQ ID NO:50, or a variant thereof comprising up to 3 amino acid variations.

[0008] In some embodiments according to the isolated anti-TSLP antibody constructs described above, the anti-TSLP antibody moiety comprises: (a) a VH comprising (i) a CDR-H1 comprising an amino acid sequence of SEQ ID NO:2; (ii) a CDR-H2 comprising an amino acid sequence of SEQ ID NO:3; and (iii) a CDR-H3 comprising an amino acid sequence of SEQ ID NO:4; and a VL comprising (i) a CDR-L1 comprising an amino acid sequence of SEQ ID NO:6; (ii) a CDR-L2 comprising an amino acid sequence of SEQ ID NO:7; and (iii) a CDR-L3 comprising an amino acid sequence of SEQ ID NO:8; (b) a VH comprising (i) a CDR-H1 comprising an amino acid sequence of SEQ ID NO:12; (ii) a CDR-H2 comprising an amino acid sequence of SEQ ID NO:13; and (iii) a CDR-H3 comprising an amino acid sequence of SEQ ID NO:4; and a VL comprising (i) a CDR-L1 comprising an amino acid sequence of SEQ ID NO:6; (ii) a CDR-L2 comprising an amino acid sequence of SEQ ID NO:7; and (iii) a CDR-L3 comprising an amino acid sequence of SEQ ID NO:8; (c) a VH comprising (i) a CDR-H1 comprising an amino acid sequence of SEQ ID NO:20; (ii) a CDR-H2 comprising an amino acid sequence of SEQ ID NO:21; and (iii) a CDR-H3 comprising an amino acid sequence of SEQ ID NO:22; and a VL comprising (i) a CDR-L1 comprising an amino acid sequence of SEQ ID NO:24; (ii) a CDR-L2 comprising an amino acid sequence of SEQ ID NO:25; and (iii) a CDR-L3 comprising an amino acid sequence of SEQ ID NO:26; (d) a VH comprising (i) a CDR-H1 comprising an amino acid sequence of SEQ ID NO:28; (ii) a CDR-H2 comprising an amino acid sequence of SEQ ID NO:29; and (iii) a CDR-H3 comprising an amino acid sequence of SEQ ID NO:30; and a VL comprising (i) a CDR-L1 comprising an amino acid sequence of SEQ ID NO:32; (ii) a CDR-L2 comprising an amino acid sequence of SEQ ID NO:33; and (iii) a CDR-L3 comprising an amino acid sequence of SEQ ID NO:34; (e) a VH comprising (i) a CDR-H1 comprising an amino acid sequence of SEQ ID NO:36; (ii) a CDR-H2 comprising an amino acid sequence of SEQ ID NO:37; and (iii) a CDR-H3 comprising an amino acid sequence of SEQ ID NO:38; and a VL comprising (i) a CDR-L1 comprising an amino acid sequence of SEQ ID NO:40; (ii) a CDR-L2 comprising an amino acid sequence of SEQ ID NO:41; and (iii) a CDR-L3 comprising an amino acid sequence of SEQ ID NO:42; or (f) a VH comprising (i) a CDR-H1 comprising an amino acid sequence of SEQ ID NO:44; (ii) a CDR-H2 comprising

an amino acid sequence of SEQ ID NO:45; and (iii) a CDR-H3 comprising an amino acid sequence of SEQ ID NO:46; and a VL comprising (i) a CDR-L1 comprising an amino acid sequence of SEQ ID NO:48; (ii) a CDR-L2 comprising an amino acid sequence of SEQ ID NO:49; and (iii) a CDR-L3 comprising an amino acid sequence of SEQ ID NO:50.

[0009] In some embodiments according to any of the isolated anti-TSLP antibody constructs described above, (a) the VH comprises an amino acid sequence having at least about 80% identity with SEQ ID NO:1, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:5; (b) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:9, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:10; (c) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:11, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:15; (d) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:19, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:23; (e) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:27, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:31; (f) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:35, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:39; (g) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:43, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:47; (h) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:63, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:53; (i) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:63, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:54; (j) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:56, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:52; (k) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:55, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:51; (l) the VH comprises an amino acid

[illegible]

about 80% sequence identity with SEQ ID NO:54; (z) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:62, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:54; (aa) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:64, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:54; (bb) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:63, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:52; (cc) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:188, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:189; or (dd) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:188, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:190.

[0010] In some embodiments according to any of the isolated anti-TSLP antibody constructs described above, (a) the VH comprises an amino acid sequence of SEQ ID NO:1, and the VL comprises an amino acid sequence of SEQ ID NO:5; (b) the VH comprises an amino acid sequence of SEQ ID NO:9, and the VL comprises an amino acid sequence of SEQ ID NO:10; (c) the VH comprises an amino acid sequence of SEQ ID NO:11, and the VL comprises an amino acid sequence of SEQ ID NO:15; (d) the VH comprises an amino acid sequence of SEQ ID NO:19, and the VL comprises an amino acid sequence of SEQ ID NO:23; (e) the VH comprises an amino acid sequence of SEQ ID NO:27, and the VL comprises an amino acid sequence of SEQ ID NO:31; (f) the VH comprises an amino acid sequence of SEQ ID NO:35, and the VL comprises an amino acid sequence of SEQ ID NO:39; (g) the VH comprises an amino acid sequence of SEQ ID NO:43, and the VL comprises an amino acid sequence of SEQ ID NO:47; (h) the VH comprises an amino acid sequence of SEQ ID NO:63, and the VL comprises an amino acid sequence of SEQ ID NO:53; (i) the VH comprises an amino acid sequence of SEQ ID NO:63, and the VL comprises an amino acid sequence of SEQ ID NO:54; (j) the VH comprises an amino acid sequence of SEQ ID NO:56, and the VL comprises an amino acid sequence of SEQ ID NO:52; (k) the VH comprises an amino acid sequence of SEQ ID NO:55, and the VL comprises an amino acid sequence of SEQ ID NO:51; (l) the VH comprises an amino acid sequence of SEQ ID NO:56, and the VL comprises an amino acid sequence of SEQ ID NO:51; (m) the VH

comprises an amino acid sequence of SEQ ID NO:63, and the VL comprises an amino acid sequence of SEQ ID NO:51; (n) the VH comprises an amino acid sequence of SEQ ID NO:64, and the VL comprises an amino acid sequence of SEQ ID NO:51; (o) the VH comprises an amino acid sequence of SEQ ID NO:55, and the VL comprises an amino acid sequence of SEQ ID NO:52; (p) the VH comprises an amino acid sequence of SEQ ID NO:58, and the VL comprises an amino acid sequence of SEQ ID NO:52; (q) the VH comprises an amino acid sequence of SEQ ID NO:60, and the VL comprises an amino acid sequence of SEQ ID NO:52; (r) the VH comprises an amino acid sequence of SEQ ID NO:64, and the VL comprises an amino acid sequence of SEQ ID NO:52; (s) the VH comprises an amino acid sequence of SEQ ID NO:55, and the VL comprises an amino acid sequence of SEQ ID NO:53; (t) the VH comprises an amino acid sequence of SEQ ID NO:57, and the VL comprises an amino acid sequence of SEQ ID NO:53; (u) the VH comprises an amino acid sequence of SEQ ID NO:58, and the VL comprises an amino acid sequence of SEQ ID NO:53; (v) the VH comprises an amino acid sequence of SEQ ID NO:62, and the VL comprises an amino acid sequence of SEQ ID NO:53; (w) the VH comprises an amino acid sequence of SEQ ID NO:64, and the VL comprises an amino acid sequence of SEQ ID NO:53; (x) the VH comprises an amino acid sequence of SEQ ID NO:55, and the VL comprises an amino acid sequence of SEQ ID NO:54; (y) the VH comprises an amino acid sequence of SEQ ID NO:61, and the VL comprises an amino acid sequence of SEQ ID NO:54; (z) the VH comprises an amino acid sequence of SEQ ID NO:62, and the VL comprises an amino acid sequence of SEQ ID NO:54; (aa) the VH comprises an amino acid sequence of SEQ ID NO:64, and the VL comprises an amino acid sequence of SEQ ID NO:54; (bb) the VH comprises an amino acid sequence of SEQ ID NO:63, and the VL comprises an amino acid sequence of SEQ ID NO:52; (cc) the VH comprises an amino acid sequence of SEQ ID NO:188, and the VL comprises an amino acid sequence of SEQ ID NO:189; or (dd) the VH comprises an amino acid sequence of SEQ ID NO:188, and the VL comprises an amino acid sequence of SEQ ID NO:190.

[0011] In some embodiments according to any of the isolated anti-TSLP antibody constructs described above, the anti-TSLP antibody moiety is selected from the group consisting of a full-length antibody, a Fab, a Fab', a F(ab')₂, a diabody, and an scFv.

[0012] In some embodiments according to any of the isolated anti-TSLP antibody constructs described above, the anti-TSLP antibody moiety is a full-length antibody ("anti-TSLP full-length antibody"). In some embodiments, the anti-TSLP full-length antibody comprises a CL

comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:73-75. In some embodiments, the anti-TSLP full-length antibody comprises an Fc domain derived from a human IgG (e.g., human IgG1, IgG2, or IgG4). In some embodiments, the Fc domain is derived from human IgG1, wherein: i) a first subunit and a second subunit of the Fc domain each comprises the amino acid sequence of any one of SEQ ID NOs:76-79; ii) a first subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO:80, and a second subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO:81; iii) a first subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO:81, and a second subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO:80; iv) a first subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO:95, and a second subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO:96; or v) a first subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO:96, and a second subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO:95. In some embodiments, the first subunit and the second subunit of the Fc domain each comprises the amino acid sequence of SEQ ID NO:77 or 79. In some embodiments, the anti-TSLP full-length antibody comprises: i) two heavy chains each comprising the amino acid sequence of SEQ ID NO:104, and two light chains each comprising the amino acid sequence of SEQ ID NO:103; ii) two heavy chains each comprising the amino acid sequence of SEQ ID NO:105, and two light chains each comprising the amino acid sequence of SEQ ID NO:103; or iii) a first heavy chain comprising the amino acid sequence of SEQ ID NO:136, a second heavy chain comprising the amino acid sequence of SEQ ID NO:137, and two light chains each comprising the amino acid sequence of SEQ ID NO:103.

[0013] In some embodiments according to any of the isolated anti-TSLP antibody constructs described above, the anti-TSLP antibody moiety is an scFv (“anti-TSLP scFv”). In some embodiments, the anti-TSLP scFv comprises the amino acid sequence of any one of SEQ ID NOs:106, 107, 191, and 192.

[0014] In some embodiments according to any of the isolated anti-TSLP antibody constructs described above, the anti-TSLP antibody moiety is a Fab (“anti-TSLP Fab”). In some embodiments, the anti-TSLP Fab comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO:109, and a second polypeptide comprising the amino acid sequence of SEQ ID NO:103.

[0015] In some embodiments according to any of the isolated anti-TSLP antibody constructs described above, the isolated anti-TSLP antibody construct is monospecific.

[0016] In some embodiments according to any of the isolated anti-TSLP antibody constructs described above, the isolated anti-TSLP antibody construct is a multispecific anti-TSLP antibody construct comprising a second antibody moiety specifically recognizing a second target antigen (e.g., IL-13). In some embodiments, the second antibody moiety is selected from the group consisting of a full-length antibody, a Fab, a Fab', a F(ab')₂, an sdAb, a diabody, and an scFv. In some embodiments, the second antibody moiety is an scFv. In some embodiments, the second antibody moiety is a Fab. In some embodiments, the second antibody moiety is a full-length antibody. In some embodiments, the anti-TSLP antibody moiety and the second antibody moiety are fused to each other via a linker, such as a linker comprising the amino acid sequence of any one of GG and SEQ ID NOs:14, 16-18, 97-99 and 163-172, e.g., any of GG and SEQ ID NOs:98-99.

[0017] Also provided are pharmaceutical compositions comprising any of the isolated anti-TSLP antibody constructs described herein, and a pharmaceutically acceptable carrier.

[0018] In another aspect of the present invention, there is provided a method of treating an inflammatory disease in an individual (e.g., human), comprising administering to the individual an effective amount of any of the isolated anti-TSLP antibody constructs described herein, or a pharmaceutical composition thereof (e.g., any of the pharmaceutical compositions described herein). In some embodiments, the inflammatory disease is asthma, atopic dermatitis, or chronic obstructive pulmonary disease (COPD).

[0019] Also provided are isolated nucleic acids encoding the polypeptide portion of any of the isolated anti-TSLP antibody constructs described above, vectors comprising such isolated nucleic acids, and host cells comprising such isolated nucleic acids or vectors.

[0020] Also provided are methods of making an anti-TSLP antibody construct, comprising: i) culturing a host cell comprising any of the isolated nucleic acids or vectors encoding the polypeptide portion of any of the anti-TSLP antibody constructs described above, or any of the host cells encoding any of the anti-TSLP antibody constructs described above, under a condition suitable for the expression of the anti-TSLP antibody construct; and ii) obtaining the expressed anti-TSLP antibody construct, e.g., from said host cell or from the cell culture (e.g., from the cell culture medium).

[0021] These and other aspects and advantages of the present invention will become apparent from the subsequent detailed description and the appended claims. 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 invention.

[0022] The disclosures of all publications, patents, patent applications, and published patent applications referred to herein are hereby incorporated herein by reference in their entirety.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] **FIG. 1** shows the percent inhibition of TSLP binding to the TSLP receptor complex expressed on the STAT5-HEK293 reporter cell when treated with exemplary anti-TSLP antibodies (*i.e.*, 51B2, 51A3, 51A4, 54A2, 54B3, and 54C4). The STAT5-HEK293 reporter cells were incubated with each antibody at varying concentrations and with 10 ng/mL TSLP. TSLP-induced luciferase production by the reporter cell acted as a readout for the TSLP binding activity, and IC₉₀ values for each antibody were calculated based on the antibody dose-dependent binding inhibition curve. Data were analyzed and presented using GraphPad. Ab, antibody; Conc., concentration.

[0024] **FIG. 2** shows 51B2 and a US-FDA approved anti-TSLP Ab (Reference antibody #1 or “Ref. Ab. #1”) binding to overlapping but different epitopes on TSLP in an ELISA competition experiment. Ab, antibody; OD, optical density.

[0025] **FIGs. 3A-3B** show 51B2 complete inhibition of TSLP-induced CCL17 secretion from PBMCs (**FIG. 3A**) or isolated dendritic cells (**FIG. 3B**) compared to a US-FDA approved anti-TSLP Ab (Ref. Ab. #1). **FIG. 3A** shows inhibition of TSLP-induced CCL17 secretion from human PBMCs that were seeded on 96-well plates and incubated with 50 ng/ml TSLP and with 51B2 or Ref. Ab. #1 at multiple concentrations. Cell culture supernatants collected at 48hr were analyzed for the level of secreted CCL17 by ELISA. **FIG. 3B** shows inhibition of TSLP-induced CCL17 secretion from isolated CD1c+ dendritic cells that were seeded on 96-well plates and incubated with 5 ng/ml TSLP and with 51B2 or Ref. Ab. #1 at multiple concentrations. Cell culture supernatants collected at 24hr were analyzed for the level of secreted CCL17 by ELISA. Data were analyzed and presented using GraphPad Prism. Ab, antibody; PBMC, peripheral blood mononuclear cell.

[0026] **FIG. 4** shows the resulting IC₅₀ (nM) and IC₉₀ (nM) values for each generated humanized 51B2 antibody compared to the parental Ch51B2 clone in human and cynomolgus monkey (“cyno”) TSLP inhibition assays using TSLP receptor complex-transfected HEK293 reporter cells. Humanized antibody clones bound by a box indicate clones selected for further analysis.

[0027] **FIG. 5A** shows the percent inhibition of TSLP binding to the TSLP receptor complex expressed on the STAT5-HEK293 reporter cell when treated with select humanized anti-TSLP antibodies (ch51B2 parental chimeric clone, hz51B2 L2H2, hz51B2 L2H9, and hz51B2 L3H9). The STAT5-HEK293 reporter cells were incubated with 10 ng/mL TSLP and with each antibody at multiple concentrations, and IC₉₀ values for each antibody were calculated based on the antibody dose-dependent binding inhibition curve. Data were analyzed and presented using GraphPad. Ab, antibody; Conc., concentration.

[0028] **FIG. 5B** shows the equilibrium binding analysis of hz51B2 L3H9 binding to human TSLP in solution.

DETAILED DESCRIPTION OF THE INVENTION

[0029] The present application provides novel anti-TSLP antibody constructs, including both monospecific and multispecific anti-TSLP antibody constructs. These novel anti-TSLP antibody constructs have several advantages. First, they demonstrate improved potency and ability to completely block TSLP signaling as compared to a US-FDA approved reference anti-TSLP antibody, which could only partially block TSLP signaling. For example, the anti-TSLP antibody constructs described herein have demonstrated activity in fully blocking CCL17 secretion by immune cells, such as peripheral blood mononuclear cells (PBMCs) and dendritic cells (DCs), indicating complete abrogation of TSLP signaling. Without being bound by theory, it is believed that the anti-TSLP antibody constructs described herein bind to a different epitope than the reference anti-TSLP antibody. Unlike the reference anti-TSLP antibody, which only blocks TSLP/TSLPR interaction, it is believed that the anti-TSLP antibody constructs described herein may block the interaction of TSLP with both TSLPR and IL-7R α , hence providing a stronger, more ideal TSLP blocking profile. Second, the anti-TSLP antibody constructs described herein displayed very strong binding affinity to human TSLP, comparable with and some even stronger than the US-FDA approved reference anti-TSLP antibody. Third, the anti-TSLP antibody constructs described herein have cross-reactivity and strong binding affinity to cynomolgus monkey TSLP, which can facilitate extrapolation of toxicity and efficacy study results from cynomolgus monkeys to human clinical studies.

[0030] Accordingly, in one aspect, the present invention provides an isolated antibody construct ("anti-TSLP antibody construct") comprising an antibody moiety that specifically recognizes TSLP ("anti-TSLP antibody moiety"). In some embodiments, the anti-TSLP

antibody moiety is a full-length antibody (“anti-TSLP full-length antibody”). In some embodiments, the anti-TSLP antibody moiety is an scFv (“anti-TSLP scFv”). In some embodiments, the anti-TSLP antibody moiety is a Fab (“anti-TSLP Fab”). In some embodiments, the isolated anti-TSLP antibody construct is monospecific. In some embodiments, the isolated anti-TSLP antibody construct is a multispecific anti-TSLP antibody construct further comprising a second antibody moiety that specifically recognizes a second target antigen (e.g., IL-13). Also provided are isolated anti-TSLP antibody constructs that can compete for binding to TSLP with any of the isolated anti-TSLP antibody constructs and/or anti-TSLP antibody moieties described herein.

[0031] Further provided are pharmaceutical compositions and kits comprising any of the isolated anti-TSLP antibody constructs described herein, and methods of use of any of the isolated anti-TSLP antibody constructs described herein or pharmaceutical compositions thereof, such as for treating inflammatory diseases, e.g., asthma, atopic dermatitis, or COPD.

I. Definitions

[0032] As used herein, the term “treatment” 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. For example, an individual is successfully “treated” if one or more symptoms associated with inflammatory diseases are mitigated or eliminated, including, but not limited to, reducing local or systemic inflammation, decreasing symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, etc.

[0033] As used herein, an “effective amount” refers to an amount of an agent or drug effective to treat a disease or disorder in a subject (such as an individual, e.g., a human). In the case of inflammatory disease, the effective amount of the agent may reduce the number of active immune cells; reduce the amount of pro-inflammatory cytokines; inhibit (*i.e.*, slow to some extent and preferably stop) inflammatory immune cell activity locally and/or systemically; and/or relieve to some extent one or more of the symptoms associated with the inflammatory disease. As is understood in the clinical context, an effective amount of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an “effective amount” may be considered in the context of administering one or more therapeutic agents, and a single

agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

[0034] As used herein, an “individual” or a “subject” refers to a mammal, including, but not limited to, human, bovine, horse, feline, canine, rodent, or non-human primate. In some embodiments, the individual is a human.

[0035] The term “antibody” is used in the broadest sense and specifically covers monoclonal antibodies (including full-length monoclonal antibodies), multispecific antibodies (*e.g.*, bispecific antibodies), and antibody fragments as long as they exhibit the desired biological activity or function. As used herein, the terms “immunoglobulin” (Ig) and “antibody” are used interchangeably.

[0036] The term “full-length antibody” is used herein to refer to an antibody in its substantially intact form, not antibody fragments as defined below. The term particularly refers to an antibody with heavy chains that contain an Fc region. Full-length antibodies are usually heterotetrameric glycoproteins of about 150,000 Daltons, composed of two identical light (L) chains and two identical heavy (H) chains.

[0037] The term “constant domain” refers to the portion of an immunoglobulin molecule having a more conserved amino acid sequence relative to the other portion of the immunoglobulin, the variable domain, which contains the antigen binding site. The constant domain contains the C_H1, C_H2 and C_H3 domains (collectively, CH) of the heavy chain and the C_HL (or CL) domain of the light chain.

[0038] 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 domain of the heavy chain may be referred to as “VH.” The variable domain of the light chain may be referred to as “VL.” These domains are generally the most variable parts of an antibody and contain the antigen-binding sites.

[0039] The term “variable” refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called hypervariable regions (HVRs, also referred to as CDRs) both in the light-chain and the heavy-chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FR). The variable domains of native heavy and light chains

each comprise four FRs, largely adopting a beta-sheet configuration, connected by three HVRs, which form loops connecting, and in some cases forming part of, the beta-sheet structure. The HVRs in each chain are held together in close proximity by the FRs and, with the HVRs from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat *et al.*, Sequences of Proteins of Immunological Interest, Fifth Edition, National Institute of Health, Bethesda, Md. (1991)). The constant domains are not involved directly in the binding of an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity. Unless otherwise indicated, the residues of the constant domains are described herein based on EU numbering.

[0040] The term “hypervariable region,” “HVR,” or “HV,” when used herein refers to the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops. Generally, antibodies comprise six HVRs; three in the VH (HVR-H1, HVR-H2, HVR-H3), and three in the VL (HVR-L1, HVR-L2, HVR-L3). In native antibodies, HVR-H3 and HVR-L3 display the most diversity of the six HVRs, and HVR-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, N.J., 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); Sheriff *et al.*, Nature Struct. Biol. 3:733-736 (1996). HVR is also referred to as “CDR” or “complementarity determining region”.

[0041] The structures and locations of immunoglobulin variable regions may be determined by reference to Kabat, E. A. *et al.*, Sequences of Proteins of Immunological Interest. 4th Edition. US Department of Health and Human Services. 1987, and updates thereof, now available on the Internet (immuno.bme.nwu.edu). Any immunoglobulin variable region definition scheme can be used, including but not limited to Kabat, Chothia, AbM, Contact, or IMGT.

[0042] As used herein, the term “CDR” or “complementarity determining region” is intended to mean the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. These particular regions have been described by Kabat *et al.*, J. Biol. Chem. 252:6609-6616 (1977); Kabat *et al.*, U.S. Dept. of Health and Human Services, “Sequences of proteins of immunological interest” (1991); Chothia *et al.*, J. Mol.

Biol. 196:901-917 (1987); Al-Lazikani B. et al., J. Mol. Biol., 273: 927-948 (1997); MacCallum et al., J. Mol. Biol. 262:732-745 (1996); Abhinandan and Martin, Mol. Immunol., 45: 3832-3839 (2008); Lefranc M.P. et al., Dev. Comp. Immunol., 27: 55-77 (2003); and Honegger and Plückthun, J. Mol. Biol., 309:657-670 (2001), where the definitions include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or grafted antibodies or variants thereof is intended to be within the scope of the term as defined and used herein. The amino acid residues which encompass the CDRs as defined by each of the above cited references are set forth below in **Table A** as a comparison. CDR prediction algorithms and interfaces are known in the art, including, for example, Abhinandan and Martin, Mol. Immunol., 45: 3832-3839 (2008); Ehrenmann F. et al., Nucleic Acids Res., 38: D301-D307 (2010); and Adolf-Bryfogle J. et al., Nucleic Acids Res., 43: D432-D438 (2015). The contents of the references cited in this paragraph are incorporated herein by reference in their entireties for use in the present application and for possible inclusion in one or more claims herein. Unless otherwise indicated, the amino acid residues of the CDRs provided herein are described herein based on Kabat.

Table A. CDR Definitions

	Kabat ¹	Chothia ²	MacCallum ³	IMGT ⁴	Aho ⁵
VH CDR1	31-35	26-32	30-35	27-38	25-40
VH CDR2	50-65	53-55	47-58	56-65	58-77
VH CDR3	95-102	96-101	93-101	105-117	109-137
VL CDR1	24-34	26-32	30-36	27-38	25-40
VL CDR2	50-56	50-52	46-55	56-65	58-77
VL CDR3	89-97	91-96	89-96	105-117	109-137

¹Residue numbering follows the nomenclature of Kabat *et al.*, *supra*
²Residue numbering follows the nomenclature of Chothia *et al.*, *supra*
³Residue numbering follows the nomenclature of MacCallum *et al.*, *supra*
⁴Residue numbering follows the nomenclature of Lefranc *et al.*, *supra*
⁵Residue numbering follows the nomenclature of Honegger and Plückthun, *supra*

[0043] “Framework” or “FR” residues are those variable domain residues other than the HVR or CDR residues as herein defined. Unless otherwise indicated, the amino acid residues of the FRs are described herein based on Kabat numbering.

[0044] The “light chains” of antibodies (immunoglobulins) derived from any mammalian species can be assigned to one of two clearly distinct types, called kappa (“κ”) and lambda (“λ”), based on the amino acid sequences of their constant domains.

[0045] The term IgG “isotype” or “subclass” as used herein is meant any of the subclasses of immunoglobulins defined by the chemical and antigenic characteristics of their constant regions.

[0046] Depending on the amino acid sequences of the constant domains of their heavy chains, antibodies (immunoglobulins) can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α , δ , ϵ , γ , and μ , respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known and described generally in, for example, Abbas *et al.* Cellular and Mol. Immunology, 4th ed. (W.B. Saunders, Co., 2000). An antibody may be part of a larger fusion molecule, formed by covalent or non-covalent association of the antibody with one or more other proteins or peptides.

[0047] “Antibody fragments” comprise a portion of an intact antibody, preferably comprising the antigen binding region thereof. In some embodiments, the antibody fragment described herein is an antigen binding fragment. Examples of antibody fragments or antigen binding fragments include Fab, Fab', F(ab')₂, and Fv fragments (such as single-chain variable fragment, scFv); diabodies; linear antibodies; single-chain antibody (sdAb) molecules; and multispecific antibodies formed from antibody fragments.

[0048] Papain digestion of antibodies produces two identical antigen-binding fragments, called “Fab” fragments, each with a single antigen-binding site, and a residual “Fc” fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields a F(ab')₂ fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

[0049] “Fv” is the minimum antibody fragment which contains a complete antigen-binding site. In one embodiment, a two-chain Fv species consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. In a single-chain Fv (scFv) species, one heavy- and one light-chain variable domain can be covalently linked by a flexible peptide linker such that the light and heavy chains can associate in a “dimeric” structure analogous to that in a two-chain Fv species. It is in this configuration that the three HVRs of each variable domain interact to define an antigen-binding site on the surface of the VH-VL dimer. Collectively, the six HVRs 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.

[0050] The Fab fragment has two polypeptide chains, containing the heavy- and light-chain variable domains (VH, VL), and also containing the constant domain of the light chain (CL) and the first constant domain (C_{H1}) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain 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.

[0051] "Single-chain Fv" or "scFv" antibody fragments comprise the VH and VL domains of antibody, wherein these domains are present in a single polypeptide chain. Generally, the scFv polypeptide further comprises a polypeptide linker between the VH and VL domains, which enables the scFv to form the desired structure for antigen binding. For a review of scFv, see, *e.g.*, Pluckthün, *The Pharmacology of Monoclonal Antibodies*. Springer Berlin Heidelberg, 1994. 269-315.

[0052] The "Fc" fragment comprises the carboxy-terminal portions of both heavy chains held together by di-sulfides. The effector functions of antibodies are determined by sequences in the Fc region, which is also the region recognized by Fc receptors (FcR) found on certain types of cells.

[0053] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *e.g.*, the individual antibodies comprising the population are identical except for possible mutations, *e.g.*, naturally occurring mutations, that may be present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies. In some embodiments, such a monoclonal antibody typically includes an antibody comprising a polypeptide sequence that binds a target, wherein the target-binding polypeptide sequence was obtained by a process that includes the selection of a single target binding polypeptide sequence from a plurality of polypeptide sequences. For example, the selection process can be the selection of a unique clone from a plurality of clones, such as a pool of hybridoma clones, phage clones, or recombinant DNA clones. It should be understood that a selected target binding sequence can be further altered, for example, to improve affinity for the target,

to humanize the target binding sequence, to improve its production in cell culture, to reduce its immunogenicity *in vivo*, to create a multispecific antibody, *etc.*, and that an antibody comprising the altered target binding sequence is also a monoclonal antibody of this invention. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. In addition to their specificity, monoclonal antibody preparations are advantageous in that they are typically uncontaminated by other immunoglobulins.

[0054] 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 invention may be made by a variety of techniques, including, for example, the hybridoma method (*e.g.*, Kohler and Milstein, *Nature* 256:495-97 (1975); Hongo *et al.*, *Hybridoma* 14 (3): 253-260 (1995), Harlow *et al.*, *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling *et al.*, *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981)), recombinant DNA methods (see, *e.g.*, U.S. Pat. No. 4,816,567), phage-display technologies (see, *e.g.*, Clackson *et al.*, *Nature* 352: 624-628 (1991); Marks *et al.*, *J. Mol. Biol.* 222: 581-597 (1992); Sidhu *et al.*, *J. Mol. Biol.* 338(2): 299-310 (2004); Lee *et al.*, *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee *et al.*, *J. Immunol. Methods* 284(1-2): 119-132 (2004)), 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 (see, *e.g.*, WO 1998/24893; WO 1996/34096; WO 1996/33735; WO 1991/10741; Jakobovits *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 2551 (1993); Jakobovits *et al.*, *Nature* 362: 255-258 (1993); Bruggemann *et al.*, *Year in Immunol.* 7:33 (1993); U.S. Pat. Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016; Marks *et al.*, *Bio/Technology* 10: 779-783 (1992); Lonberg *et al.*, *Nature* 368: 856-859 (1994); Morrison, *Nature* 368: 812-813 (1994); Fishwild *et al.*, *Nature Biotechnol.* 14: 845-851 (1996); Neuberger, *Nature Biotechnol.* 14: 826 (1996); and Lonberg and Huszar, *Intern. Rev. Immunol.* 13: 65-93 (1995)).

[0055] The monoclonal antibodies herein specifically include “chimeric” antibodies 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 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 (see, *e.g.*, U.S. Pat. No. 4,816,567; and Morrison *et al.*, Proc. Natl. Acad. Sci. USA 81:6851-6855 (1984)). Chimeric antibodies include PRIMATIZED® antibodies wherein the antigen-binding region of the antibody is derived from an antibody produced by, *e.g.*, immunizing macaque monkeys with the antigen of interest.

[0056] “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 nonhuman 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. 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, and all or substantially all of the FRs are those of a human immunoglobulin sequence. 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, *e.g.*, 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.

[0057] A “human antibody” is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art,

including phage-display libraries. Hoogenboom and Winter, J. Mol. Biol. 227:381 (1991); Marks *et al.*, J. Mol. Biol. 222:581 (1991). Also available for the preparation of human monoclonal antibodies are methods described in Cole *et al.*, Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, 77 (1985); Boerner *et al.*, J. Immunol. 147(1):86-95 (1991). See also van Dijk and van de Winkel, Curr. Opin. Pharmacol. 5: 368-74 (2001). Human antibodies can be prepared by administering the antigen to a transgenic animal that has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled, *e.g.*, immunized xenomice (see, *e.g.*, U.S. Pat. Nos. 6,075,181 and 6,150,584 regarding XENOMOUSE™ technology). See also, for example, Li *et al.*, Proc. Natl. Acad. Sci. USA 103:3557-3562 (2006) regarding human antibodies generated via a human B-cell hybridoma technology.

[0058] The term “covalently linked” as used herein, refers to a direct linkage through one or more chemical bonds or an indirect linkage through one or more linkers. Any suitable chemical bond can be used to create a direct linkage, including but not limited to, a covalent bond such as a peptide bond and a disulfide bond, or a non-covalent bond such as a hydrogen bond, a hydrophobic bond, an ionic bond, or a van der Waals bond.

[0059] “Covalent bond” as used herein refers to a stable bond between two atoms sharing one or more electrons. Examples of covalent bonds include, but are not limited to, peptide bonds and disulfide bonds. As used herein, “peptide bond” refers to a covalent bond formed between a carboxyl group of an amino acid and an amine group of an adjacent amino acid. A “disulfide bond” as used herein refers to a covalent bond formed between two sulfur atoms, such as a combination of a heavy chain fragment C_H1 and a light chain fragment CL by one or more disulfide bonds. One or more disulfide bonds may be formed between the two fragments by linking the thiol groups in the two fragments. In some embodiments, one or more disulfide bonds can be formed between one or more cysteines of the heavy chain fragment and the light chain fragment, respectively. Disulfide bonds can be formed by oxidation of two thiol groups. In some embodiments, the covalent linkage is directly linked by a covalent bond. In some embodiments, the covalent linkage is directly linked by a peptide bond or a disulfide bond.

[0060] As used herein, the term “binds,” “specifically binds to,” “specifically recognizes,” or is “specific for” refers to measurable and reproducible interactions such as binding between a target and an antibody, which is determinative of the presence of the target in the presence of a heterogeneous population of molecules including biological molecules. For example, an

antibody that binds to or specifically recognizes a target (which can be an epitope) is an antibody that binds this target with greater affinity, avidity, more readily, and/or with greater duration than it binds to other targets. In one embodiment, the extent of binding of an antibody to an unrelated target is less than about 10% of the binding of the antibody to the target as measured, *e.g.*, by a radioimmunoassay (RIA). In some embodiments, an antibody that specifically recognizes a target has a dissociation constant (K_d) of $\leq 1\mu\text{M}$, $\leq 100\text{ nM}$, $\leq 10\text{ nM}$, $\leq 1\text{ nM}$, or $\leq 0.1\text{ nM}$. In some embodiments, an antibody specifically recognizes an epitope on a protein that is conserved among the protein from different species. In another embodiment, specific binding can include, but does not require exclusive binding.

[0061] As used herein, “Percent (%) amino acid sequence identity” and “homology” with respect to a peptide, polypeptide, or antibody sequence are defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific peptide or 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 measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

[0062] An amino acid substitution may include but is not limited to the replacement of one amino acid in a polypeptide with another amino acid. Exemplary substitutions are shown in **Table 1**. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, *e.g.*, retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

Table 1. Exemplary amino acid substitutions.

Original Residue	Exemplary Substitutions
Ala (A)	Val; Leu; Ile
Arg (R)	Lys; Gln; Asn
Asn (N)	Gln; His; Asp, Lys; Arg
Asp (D)	Glu; Asn
Cys (C)	Ser; Ala
Gln (Q)	Asn; Glu
Glu (E)	Asp; Gln
Gly (G)	Ala
His (H)	Asn; Gln; Lys; Arg

Original Residue	Exemplary Substitutions
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe
Lys (K)	Arg; Gln; Asn
Met (M)	Leu; Phe; Ile
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr
Pro (P)	Ala
Ser (S)	Thr
Thr (T)	Val; Ser
Trp (W)	Tyr; Phe
Tyr (Y)	Trp; Phe; Thr; Ser
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine

[0063] Amino acids may be grouped according to 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; and (6) aromatic: Trp, Tyr, Phe. Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[0064] The term “multispecific” as used in conjunction with an antibody or antigen binding protein refers to an antibody or antigen binding protein having polyepitopic specificity (*i.e.*, is capable of specifically recognizing two, three, or more, different epitopes on one biological molecule or is capable of specifically recognizing epitopes on two, three, or more, different biological molecules). Unless otherwise indicated, the order in which the antigens bound by a multispecific antibody are listed in a multispecific antibody name is arbitrary.

[0065] The term “bispecific” as used in conjunction with an antibody or antigen binding protein refers to an antibody or antigen binding protein capable of specifically recognizing two different epitopes on one biological molecule, or capable of specifically recognizing epitopes on two different biological molecules. Unless otherwise indicated, the order in which the antigens bound by a bispecific antibody are listed in a bispecific antibody name is arbitrary.

[0066] The “knob-in-hole” strategy (see, e.g., PCT Intl. Publ. No. WO 2006/028936) has its plain and ordinary meaning as read in light of the specification and refers to a strategy that may be used to generate full-length bispecific antibodies. Briefly, selected amino acids forming the interface of the C_H3 domains in human IgG can be mutated at positions affecting C_H3 domain interactions to promote heterodimer formation. An amino acid with a small side chain (hole) is introduced into a heavy chain of an antibody specifically recognizing a first antigen and an amino acid with a large side chain (knob) is introduced into a heavy chain of an antibody specifically recognizing a second antigen. After co-expression of the two

antibodies, a heterodimer is formed as a result of the preferential interaction of the heavy chain with a “hole” with the heavy chain with a “knob”.

[0067] The term “vector,” as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as “expression vectors.”

[0068] It is understood that embodiments of the invention described herein include “consisting of” and/or “consisting essentially of” embodiments.

[0069] Reference to “about” a value or parameter herein includes (and describes) variations that are directed to that value or parameter per se. For example, description referring to “about X” includes description of “X”.

[0070] The terms “about” and “approximately” mean within 20%, within 15%, within 10%, within 9%, within 8%, within 7%, within 6%, within 5%, within 4%, within 3%, within 2%, within 1%, or less of a given value or range.

[0071] As used herein, reference to “not” a value or parameter generally means and describes “other than” a value or parameter. For example, the method is not used to treat an inflammatory disease of type X means the method is used to treat inflammatory diseases of types other than X.

[0072] The term “about X-Y” used herein has the same meaning as “about X to about Y.”

[0073] As used herein and in the appended claims, the singular forms “a,” “or,” and “the” include plural referents unless the context clearly dictates otherwise.

II. Isolated Anti-TSLP Antibody Constructs

[0074] TSLP is a cytokine that is produced by gut and lung epithelial cells, skin keratinocytes, and dendritic cells, although it can be produced also by (*e.g.*) airway smooth muscle cells, mast cells, monocytes, macrophages, granulocytes, synovial fibroblasts, *etc.* TSLP is involved in helper 2 T cell (Th2) inflammation and has been identified as a possible therapeutic target in the treatment of asthma. This antigen can be used as a biomarker for specific inflammatory disease (*e.g.*, allergy or autoimmune disease) development, as a

prognostic indicator, and as an immunotherapeutic target for antigen-expressing inflammatory diseases.

[0075] In one aspect, the present application provides an isolated antibody construct (anti-TSLP antibody construct) comprising (or consisting of, or consisting essentially of) an anti-TSLP antibody moiety, such as any of the anti-TSLP antibody moieties described herein. In some embodiments, the isolated anti-TSLP antibody construct consists of one or more anti-TSLP antibody moieties. Hence in some embodiments, the present application also provides anti-TSLP antibody moieties, such as any of the anti-TSLP antibody moieties described herein. In some embodiments, the isolated anti-TSLP antibody construct is monospecific. In some embodiments, the isolated anti-TSLP antibody construct is multispecific (e.g., bispecific). In some embodiments, there is provided a multispecific anti-TSLP antibody construct comprising one or more of any of the anti-TSLP antibody moieties (e.g., anti-TSLP full-length antibody, anti-TSLP scFv, or anti-TSLP Fab) described herein, and one or more additional antibody moieties specifically recognizing one or more additional target antigens or epitopes. Also provided are isolated anti-TSLP antibody constructs and isolated anti-TSLP antibody moieties that compete for binding to TSLP with any of the isolated anti-TSLP antibody constructs or isolated anti-TSLP antibody moieties described herein. In some embodiments, there is provided an isolated anti-TSLP antibody construct comprising a means for specifically recognizing TSLP.

[0076] In some embodiments, the isolated anti-TSLP antibody construct comprises (or consists of, or consists essentially of) an anti-TSLP full-length antibody, a Fab, a Fab', a Fab'-SH, a F(ab')₂, an Fv, an scFv, or a combination thereof. In some embodiments, the isolated anti-TSLP antibody construct comprises (or consists of) an anti-TSLP Fab fragment. In some embodiments, the isolated anti-TSLP antibody construct comprises (or consists of) an anti-TSLP scFv. In some embodiments, the isolated anti-TSLP antibody construct comprises (or consists of) an anti-TSLP full-length antibody.

[0077] In some embodiments, the isolated anti-TSLP antibody construct is monovalent. In some embodiments, the isolated anti-TSLP antibody construct is multivalent. In some embodiments, the isolated anti-TSLP antibody construct is multivalent and monospecific (i.e., comprising two or more antibody moieties binding to the same TSLP epitope). In some embodiments, the isolated anti-TSLP antibody construct is multivalent and multispecific.

[0078] In some embodiments, the isolated anti-TSLP antibody construct is selected from the group consisting of: a monospecific antibody construct, a multispecific antibody construct (e.g., bispecific antibody construct), and an antibody-detection tag conjugate.

[0079] The invention further provides fusion proteins comprising any of the anti-TSLP antibody moieties (e.g., full-length antibody, scFv, or Fab) described herein, and conjugates (e.g., detection tag conjugates) comprising any of the isolated anti-TSLP antibody constructs or isolated anti-TSLP antibody moieties described herein. Also provided are isolated anti-TSLP antibody constructs comprising any of the anti-TSLP antibody moieties (e.g., full-length antibody, scFv, or Fab) described herein, and a fusion partner (e.g., another protein, an expression tag, a purification tag, or a moiety for enhancing half-life).

[0080] In some embodiments, the N-terminus of the one or more polypeptides of the isolated anti-TSLP antibody construct is additionally fused with a signal peptide for better expression.

[0081] In some embodiments, the N-terminus and/or C-terminus of the one or more polypeptides of the isolated anti-TSLP antibody construct may comprise a histidine tag (HIS-tag) for protein purification. For example, the C-terminus of one or more polypeptides of an anti-TSLP full-length antibody may further comprise a histidine tag.

Anti-TSLP antibody moieties

[0082] Any of the anti-TSLP antibody moieties described herein can be used in the isolated anti-TSLP antibody constructs described herein.

[0083] TSLP antigens may be from various species, including human, non-human primate, mouse, rat, rabbit, or other mammals. In some embodiments, the TSLP molecule is human TSLP. In some embodiments, the TSLP molecule is mouse TSLP. In some embodiments, the TSLP molecule is cynomolgus monkey TSLP. In some embodiments, the TSLP molecule is wild-type. In some embodiments, the TSLP molecule is a mutant (e.g., comprises one or more insertions, deletions, and/or amino acid substitutions compared to a wild-type reference TSLP molecule).

[0084] In some embodiments, the anti-TSLP antibody moiety binds to a TSLP antigen with a $K_D \leq 1 \mu\text{M}$, such as $\leq 100 \text{ nM}$, preferably $\leq 10 \text{ nM}$, more preferably $\leq 1 \text{ nM}$. In some embodiments, the anti-TSLP antibody moiety binds to a human TSLP antigen and/or a monkey (e.g., cynomolgus monkey) TSLP antigen with a K_D of from about $1 \times 10^{-14} \text{ M}$ to about $1 \times 10^{-7} \text{ M}$, such as from about $1 \times 10^{-14} \text{ M}$ to about $1 \times 10^{-10} \text{ M}$, from about $1 \times 10^{-14} \text{ M}$ to about $1 \times 10^{-11} \text{ M}$, from about $1 \times 10^{-14} \text{ M}$ to about $1 \times 10^{-12} \text{ M}$, from about $1 \times 10^{-13} \text{ M}$ to about

1×10^{-10} M, from about 1×10^{-13} M to about 1×10^{-11} M, from about 1×10^{-13} M to about 1×10^{-12} M, from about 1×10^{-12} M to about 1×10^{-10} M, from about 1×10^{-11} M to about 1×10^{-10} M, from about 1×10^{-11} M to about 1×10^{-9} M, from about 1×10^{-11} M to about 1×10^{-8} M, from about 1×10^{-10} M to about 1×10^{-9} M, from about 1×10^{-10} M to about 1×10^{-8} M, from about 1×10^{-10} M to about 1×10^{-7} M, from about 1×10^{-9} M to about 1×10^{-7} M, from about 1×10^{-9} M to about 1×10^{-8} M, or from about 1×10^{-8} M to about 1×10^{-7} M. In some embodiments, the anti-TSLP antibody moiety binds to human TSLP with a K_D of from about 1×10^{-14} M to about 1×10^{-10} M. In some embodiments, the anti-TSLP antibody moiety binds to cynomolgus monkey TSLP with a K_D of from about 1×10^{-12} M to about 1×10^{-9} M.

[0085] In some embodiments, the anti-TSLP antibody moiety binds to a TSLP antigen (e.g., human TSLP and/or cyno TSLP) with a similar affinity (e.g., within 2-fold difference in K_D) compared to a reference anti-TSLP antibody, such as a US-FDA approved anti-TSLP reference antibody #1 (Ref. Ab. #1) described herein. In some embodiments, the anti-TSLP antibody moiety binds to a TSLP antigen (e.g., human TSLP and/or cyno TSLP) at least about any of 2-, 5-, 10-, 20-, 100-, 1000-fold, or more, stronger compared to a reference anti-TSLP antibody, such as a US-FDA approved anti-TSLP Ref. Ab. #1 described herein. For example, in some embodiments, the K_D of a reference anti-TSLP antibody (e.g., US-FDA approved anti-TSLP Ref. Ab. #1) in binding to a TSLP antigen (e.g., human TSLP and/or cyno TSLP) is at least about any of 2-, 5-, 10-, 20-, 100-, 1000-fold, or more, of the K_D of the anti-TSLP antibody moiety described herein.

[0086] In some embodiments, the anti-TSLP antibody moiety inhibits (e.g., at least about any of 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100%) TSLP-induced CCL17 secretion by pro-inflammatory cells that express TSLP receptors (e.g., PBMCs, or CD1c+ dendritic cells) in the presence of TSLP (e.g., human TSLP). In some embodiments, the inhibition of TSLP-induced CCL17 secretion by pro-inflammatory cells that express TSLP receptors (e.g., PBMCs, or CD1c+ dendritic cells) by an anti-TSLP antibody moiety described herein is at least about 1.2-fold (e.g., at least about any of 1.5-, 2-, 5-, 10-, 50-fold, or more) of that by a reference anti-TSLP antibody (e.g., US-FDA approved anti-TSLP Ref. Ab. #1).

[0087] In some embodiments, the anti-TSLP antibody moiety specifically recognizes both human and non-human primate (such as cynomolgus monkey) TSLP. Isolated anti-TSLP antibody constructs comprising such anti-TSLP antibody moieties with cross-reactivity to monkey TSLP may facilitate toxicity studies in non-human primates, which can provide more relevant safety assessments for human clinical trial candidates, without having to perform

toxicity studies in chimpanzees or using surrogate molecules. In some embodiments, the anti-TSLP antibody moiety only binds to human TSLP. In some embodiments, the anti-TSLP antibody moiety binds to human TSLP stronger (such as at least about any of 1.5-, 2-, 5-, 10-, 20-, 50-, 100-, 1000-fold, or more, stronger) than non-human (*e.g.*, cynomolgus monkey) TSLP.

[0088] The anti-TSLP antibody moiety may fully or partially modulate, block, inhibit, reduce, antagonize, neutralize, or interfere with the functional activity of TSLP (*e.g.*, binding to TSLPR complex and/or inducing signaling). When the functional activity of TSLP is reduced by at least about 95% (such as at least about any of 96%, 97%, 98%, 99%, or 100%) in the presence of an anti-TSLP antibody moiety compared to when not bound by an anti-TSLP antibody moiety, the anti-TSLP antibody moiety is considered capable of fully modulating, blocking, inhibiting, reducing, antagonizing, neutralizing, or interfering with the functional activity of TSLP. When the functional activity of TSLP is reduced by at least about 50% (such as at least about any of 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90%) in the presence of an anti-TSLP antibody moiety compared to when not bound by anti-TSLP antibody moiety, the anti-TSLP antibody moiety is considered capable of significantly modulating, blocking, inhibiting, reducing, antagonizing, neutralizing, or interfering with the functional activity of TSLP. When the functional activity of TSLP is reduced by less than about 50% (such as reduced by less than about any of 10%, 15%, 20%, 25%, 30%, 35%, 40%, or 45%) in the presence of an anti-TSLP antibody moiety compared to when not bound by an anti-TSLP antibody moiety, the anti-TSLP antibody moiety is considered capable of partially modulating, blocking, inhibiting, reducing, antagonizing, neutralizing, or interfering with the functional activity of TSLP.

[0089] In some embodiments, inhibiting or reducing TSLP activity by the anti-TSLP antibody moiety comprises inhibiting or reducing (*e.g.*, at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more) one or more of: i) binding of TSLP to its receptor; ii) proliferation, activation, and/or differentiation of cells expressing TSLPR in the presence of TSLP; iii) Th2 cytokine (*i.e.*, cytokine expressed by Th2 cells; *e.g.*, IL-4, IL-5, IL-13, and IL-10) production in a polarization assay in the presence of TSLP; iv) dendritic cell activation and/or maturation in the presence of TSLP; and v) mast cell cytokine release in the presence of TSLP.

[0090] In some embodiments, the anti-TSLP antibody moiety comprises one or more variations in the sequence. In some embodiments, the amino acid variations (*e.g.*,

substitutions, such as conservative substitutions) in the variant sequences do not substantially reduce (*e.g.*, reducing at most about any of 50%, 40%, 30%, 20%, 10%, 5%, 1%, or less) the ability of the anti-TSLP antibody moiety to bind to TSLP. Also contemplated are modifications that substantially improve (*e.g.*, improving at least about any of 1.2-, 1.5-, 2-, 5-, 10-, 20-, 50-fold, or more) the binding affinity of the anti-TSLP antibody moiety to TSLP, or other properties, such as specificity, immunogenicity, and/or cross-reactivity with TSLP epitope variants.

[0091] In some embodiments, there is provided an anti-TSLP antibody moiety comprising a VH and a VL, wherein the VH comprises (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:2, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as conservative substitution(s)); (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:3, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as conservative substitution(s)); and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:4, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as conservative substitution(s)); and the VL comprises (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:6, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as conservative substitution(s)); (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:7, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as conservative substitution(s)); and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as conservative substitution(s)). In some embodiments, the anti-TSLP antibody moiety comprises a VH comprising (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:2; (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:3; and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:4; and a VL comprising (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:6; (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:7; and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:8. In some embodiments, the anti-TSLP antibody moiety comprises CDR-H1, CDR-H2, and CDR-H3 of a VH comprising the amino acid sequence of SEQ ID NO:1, and CDR-L1, CDR-L2, and

CDR-L3 of a VL comprising the amino acid sequence of SEQ ID NO:5. In some embodiments, the anti-TSLP antibody moiety comprises (a) a VH comprising an amino acid sequence having at least about 80% (such as at least about any of 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity with SEQ ID NO:1, and a VL comprising an amino acid sequence having at least about 80% (such as at least about any of 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity with SEQ ID NO:5; or (b) a VH comprising an amino acid sequence having at least about 80% (such as at least about any of 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity with SEQ ID NO:9, and a VL comprising an amino acid sequence having at least about 80% (such as at least about any of 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity with SEQ ID NO:10. In some embodiments, the anti-TSLP antibody moiety comprises: (a) a VH comprising the amino acid sequence of SEQ ID NO:1, and a VL comprising the amino acid sequence of SEQ ID NO:5; or (b) a VH comprising the amino acid sequence of SEQ ID NO:9, and a VL comprising the amino acid sequence of SEQ ID NO:10.

[0092] In some embodiments, there is provided an anti-TSLP antibody moiety comprising a VH and a VL, wherein the VH comprises (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:12, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as conservative substitution(s)); (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:13, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as conservative substitution(s)); and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:4, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as conservative substitution(s)); and the VL comprises (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:6, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as conservative substitution(s)); (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:7, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as conservative substitution(s)); and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as conservative substitution(s)). In some embodiments, the anti-TSLP antibody moiety comprises a VH comprising (i) a CDR-

H1 comprising the amino acid sequence of SEQ ID NO:12; (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:13; and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:4; and a VL comprising (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:6; (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:7; and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:8. In some embodiments, the anti-TSLP antibody moiety comprises CDR-H1, CDR-H2, and CDR-H3 of a VH comprising the amino acid sequence of SEQ ID NO:11, and CDR-L1, CDR-L2, and CDR-L3 of a VL comprising the amino acid sequence of SEQ ID NO:15. In some embodiments, the anti-TSLP antibody moiety comprises a VH comprising an amino acid sequence having at least about 80% (such as at least about any of 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity with SEQ ID NO:11, and a VL comprising an amino acid sequence having at least about 80% (such as at least about any of 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity with SEQ ID NO:15. In some embodiments, the anti-TSLP antibody moiety comprises a VH comprising the amino acid sequence of SEQ ID NO:11, and a VL comprising the amino acid sequence of SEQ ID NO:15.

[0093] In some embodiments, there is provided an anti-TSLP antibody moiety comprising a VH and a VL, wherein the VH comprises (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:28, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as conservative substitution(s)); (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:29, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as conservative substitution(s)); and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:30, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as conservative substitution(s)); and the VL comprises (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:32, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as conservative substitution(s)); (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:33, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as conservative substitution(s)); and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:34, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid

variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as conservative substitution(s)). In some embodiments, the anti-TSLP antibody moiety comprises a VH comprising (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:28; (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:29; and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:30; and a VL comprising (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:32; (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:33; and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:34. In some embodiments, the anti-TSLP antibody moiety comprises CDR-H1, CDR-H2, and CDR-H3 of a VH comprising the amino acid sequence of SEQ ID NO:27, and CDR-L1, CDR-L2, and CDR-L3 of a VL comprising the amino acid sequence of SEQ ID NO:31. In some embodiments, the anti-TSLP antibody moiety comprises a VH comprising an amino acid sequence having at least about 80% (such as at least about any of 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity with SEQ ID NO:27, and a VL comprising an amino acid sequence having at least about 80% (such as at least about any of 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity with SEQ ID NO:31. In some embodiments, the anti-TSLP antibody moiety comprises a VH comprising the amino acid sequence of SEQ ID NO:27, and a VL comprising the amino acid sequence of SEQ ID NO:31.

[0094] In some embodiments, there is provided an anti-TSLP antibody moiety comprising a VH and a VL, wherein the VH comprises (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:36, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as conservative substitution(s)); (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:37, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as conservative substitution(s)); and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:38, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as conservative substitution(s)); and the VL comprises (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:40, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as conservative substitution(s)); (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:41, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as

conservative substitution(s)); and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:42, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as conservative substitution(s)). In some embodiments, the anti-TSLP antibody moiety comprises a VH comprising (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:36; (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:37; and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:38; and a VL comprising (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:40; (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:41; and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:42. In some embodiments, the anti-TSLP antibody moiety comprises CDR-H1, CDR-H2, and CDR-H3 of a VH comprising the amino acid sequence of SEQ ID NO:35, and CDR-L1, CDR-L2, and CDR-L3 of a VL comprising the amino acid sequence of SEQ ID NO:39. In some embodiments, the anti-TSLP antibody moiety comprises a VH comprising an amino acid sequence having at least about 80% (such as at least about any of 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity with SEQ ID NO:35, and a VL comprising an amino acid sequence having at least about 80% (such as at least about any of 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity with SEQ ID NO:39. In some embodiments, the anti-TSLP antibody moiety comprises a VH comprising the amino acid sequence of SEQ ID NO:35, and a VL comprising the amino acid sequence of SEQ ID NO:39.

[0095] In some embodiments, there is provided an anti-TSLP antibody moiety comprising a VH and a VL, wherein the VH comprises (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:44, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as conservative substitution(s)); (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:45, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as conservative substitution(s)); and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:46, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as conservative substitution(s)); and the VL comprises (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:48, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as conservative substitution(s)); (ii) a CDR-L2 comprising the amino

acid sequence of SEQ ID NO:49, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as conservative substitution(s)); and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:50, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as conservative substitution(s)). In some embodiments, the anti-TSLP antibody moiety comprises a VH comprising (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:44; (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:45; and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:46; and a VL comprising (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:48; (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:49; and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:50. In some embodiments, the anti-TSLP antibody moiety comprises CDR-H1, CDR-H2, and CDR-H3 of a VH comprising the amino acid sequence of SEQ ID NO:43, and CDR-L1, CDR-L2, and CDR-L3 of a VL comprising the amino acid sequence of SEQ ID NO:47. In some embodiments, the anti-TSLP antibody moiety comprises a VH comprising an amino acid sequence having at least about 80% (such as at least about any of 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity with SEQ ID NO:43, and a VL comprising an amino acid sequence having at least about 80% (such as at least about any of 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity with SEQ ID NO:47. In some embodiments, the anti-TSLP antibody moiety comprises a VH comprising the amino acid sequence of SEQ ID NO:43, and a VL comprising the amino acid sequence of SEQ ID NO:47.

[0096] In some embodiments, the anti-TSLP antibody moiety comprises a VH and a VL, wherein the VH comprises (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:20, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as conservative substitution(s)); (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:21, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as conservative substitution(s)); and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:22, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as conservative substitution(s)); and the VL comprises (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:24, or a variant thereof comprising up to about 3 (*e.g.*, 1,

2, or 3) amino acid variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as conservative substitution(s)); (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:25, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as conservative substitution(s)); and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:26, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid variations (*e.g.*, insertion(s), deletion(s), and/or substitution(s), such as conservative substitution(s)). In some embodiments, the anti-TSLP antibody moiety comprises a VH comprising (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:20; (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:21; and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:22; and a VL comprising (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:24; (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:25; and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:26. In some embodiments, the anti-TSLP antibody moiety comprises CDR-H1, CDR-H2, and CDR-H3 of a VH comprising the amino acid sequence of SEQ ID NO:19, and CDR-L1, CDR-L2, and CDR-L3 of a VL comprising the amino acid sequence of SEQ ID NO:23. In some embodiments, the anti-TSLP antibody moiety comprises: (a) a VH comprising an amino acid sequence having at least about 80% (such as at least about any of 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity with SEQ ID NO:19, and a VL comprising an amino acid sequence having at least about 80% (such as at least about any of 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity with SEQ ID NO:23; (b) a VH comprising an amino acid sequence having at least about 80% (such as at least about any of 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity with SEQ ID NO:63, and a VL comprising an amino acid sequence having at least about 80% (such as at least about any of 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity with SEQ ID NO:53; (c) a VH comprising an amino acid sequence having at least about 80% (such as at least about any of 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity with SEQ ID NO:63, and a VL comprising an amino acid sequence having at least about 80% (such as at least about any of 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity with SEQ ID NO:54; (d) a VH comprising an amino acid sequence having at least about 80% (such as at least about any of 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity with SEQ ID NO:56, and a VL comprising an amino acid sequence having at least about 80% (such as at least about any of 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity with SEQ ID NO:52; (e) a VH comprising an amino acid sequence

[illegible]

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least about any of 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity with SEQ ID NO:64, and a VL comprising an amino acid sequence having at least about 80% (such as at least about any of 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity with SEQ ID NO:54; (v) a VH comprising an amino acid sequence having at least about 80% (such as at least about any of 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity with SEQ ID NO:63, and a VL comprising an amino acid sequence having at least about 80% (such as at least about any of 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity with SEQ ID NO:52; (w) a VH comprising an amino acid sequence having at least about 80% (such as at least about any of 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity with SEQ ID NO:188, and a VL comprising an amino acid sequence having at least about 80% (such as at least about any of 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity with SEQ ID NO:189; or (x) a VH comprising an amino acid sequence having at least about 80% (such as at least about any of 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity with SEQ ID NO:188, and a VL comprising an amino acid sequence having at least about 80% (such as at least about any of 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity with SEQ ID NO:190. In some embodiments, the anti-TSLP antibody moiety comprises: (a) a VH comprising the amino acid sequence of SEQ ID NO:19, and a VL comprising the amino acid sequence of SEQ ID NO:23; (b) a VH comprising the amino acid sequence of SEQ ID NO:63, and a VL comprising the amino acid sequence of SEQ ID NO:53; (c) a VH comprising the amino acid sequence of SEQ ID NO:63, and a VL comprising the amino acid sequence of SEQ ID NO:54; (d) a VH comprising the amino acid sequence of SEQ ID NO:56, and a VL comprising the amino acid sequence of SEQ ID NO:52; (e) a VH comprising the amino acid sequence of SEQ ID NO:55, and a VL comprising the amino acid sequence of SEQ ID NO:51; (f) a VH comprising the amino acid sequence of SEQ ID NO:56, and a VL comprising the amino acid sequence of SEQ ID NO:51; (g) a VH comprising the amino acid sequence of SEQ ID NO:63, and a VL comprising the amino acid sequence of SEQ ID NO:51; (h) a VH comprising the amino acid sequence of SEQ ID NO:64, and a VL comprising the amino acid sequence of SEQ ID NO:51; (i) a VH comprising the amino acid sequence of SEQ ID NO:55, and a VL comprising the amino acid sequence of SEQ ID NO:52; (j) a VH comprising the amino acid sequence of SEQ ID NO:58, and a VL comprising the amino acid sequence of SEQ ID NO:52; (k) a VH comprising the amino acid sequence of SEQ ID NO:60, and a VL comprising the amino acid sequence of SEQ ID NO:52; (l) a VH comprising the amino acid sequence of SEQ ID NO:64, and a VL

comprising the amino acid sequence of SEQ ID NO:52; (m) a VH comprising the amino acid sequence of SEQ ID NO:55, and a VL comprising the amino acid sequence of SEQ ID NO:53; (n) a VH comprising the amino acid sequence of SEQ ID NO:57, and a VL comprising the amino acid sequence of SEQ ID NO:53; (o) a VH comprising the amino acid sequence of SEQ ID NO:58, and a VL comprising the amino acid sequence of SEQ ID NO:53; (p) a VH comprising the amino acid sequence of SEQ ID NO:62, and a VL comprising the amino acid sequence of SEQ ID NO:53; (q) a VH comprising the amino acid sequence of SEQ ID NO:64, and a VL comprising the amino acid sequence of SEQ ID NO:53; (r) a VH comprising the amino acid sequence of SEQ ID NO:55, and a VL comprising the amino acid sequence of SEQ ID NO:54; (s) a VH comprising the amino acid sequence of SEQ ID NO:61, and a VL comprising the amino acid sequence of SEQ ID NO:54; (t) a VH comprising the amino acid sequence of SEQ ID NO:62, and a VL comprising the amino acid sequence of SEQ ID NO:54; (u) a VH comprising the amino acid sequence of SEQ ID NO:64, and a VL comprising the amino acid sequence of SEQ ID NO:54; (v) a VH comprising the amino acid sequence of SEQ ID NO:63, and a VL comprising the amino acid sequence of SEQ ID NO:52; (w) a VH comprising the amino acid sequence of SEQ ID NO:188, and a VL comprising the amino acid sequence of SEQ ID NO:189; or (x) a VH comprising the amino acid sequence of SEQ ID NO:188, and a VL comprising the amino acid sequence of SEQ ID NO:190.

[0097] In some embodiments, there is provided an anti-TSLP antibody moiety that competes for binding (e.g., competes for binding by at least about 50% or more, such as at least about any of 55%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or more) to TSLP against a reference anti-TSLP antibody comprising: (a) a reference VH comprising the amino acid sequence of SEQ ID NO:1, and a reference VL comprising the amino acid sequence of SEQ ID NO:5; (b) a reference VH comprising the amino acid sequence of SEQ ID NO:9, and a reference VL comprising the amino acid sequence of SEQ ID NO:10; (c) a reference VH comprising the amino acid sequence of SEQ ID NO:11, and a reference VL comprising the amino acid sequence of SEQ ID NO:15; (d) a reference VH comprising the amino acid sequence of SEQ ID NO:19, and a reference VL comprising the amino acid sequence of SEQ ID NO:23; (e) a reference VH comprising the amino acid sequence of SEQ ID NO:27, and a reference VL comprising the amino acid sequence of SEQ ID NO:31; (f) a reference VH comprising the amino acid sequence of SEQ ID NO:35, and a reference VL comprising the amino acid sequence of SEQ ID NO:39; (g) a reference VH comprising the amino acid

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amino acid sequence of SEQ ID NO:54; (bb) a reference VH comprising the amino acid sequence of SEQ ID NO:63, and a reference VL comprising the amino acid sequence of SEQ ID NO:52; (cc) a reference VH comprising the amino acid sequence of SEQ ID NO:188, and a reference VL comprising the amino acid sequence of SEQ ID NO:189; or (dd) a reference VH comprising the amino acid sequence of SEQ ID NO:188, and a reference VL comprising the amino acid sequence of SEQ ID NO:190.

[0098] In some embodiments, the anti-TSLP antibody moiety is selected from the group consisting of a full-length antibody, a Fab, a Fab', a F(ab')₂, a diabody, and an scFv.

[0099] In some embodiments, the anti-TSLP antibody moiety is a full-length antibody (anti-TSLP full-length antibody). In some embodiments, the anti-TSLP full-length antibody comprises a human kappa CL or a human lambda CL, such as a CL comprising the amino acid sequence of any one of SEQ ID NOs:73-75. In some embodiments, the anti-TSLP full-length antibody comprises an Fc domain derived from a human IgG, such as human IgG1, IgG2, IgG3, or IgG4, e.g., human IgG1. Any Fc domains described in the "Fc domains" subsection below can be used herein. In some embodiments, the Fc domain comprises: i) L234A+L235A ("LALA") mutations, ii) M252Y+S254T+T256E ("YTE") mutations, and/or iii) M428L+N434S ("LS") mutations, according to EU numbering. In some embodiments, the Fc domain further comprises knob-in-hole mutations, such as a T366W "knob" mutation in one Fc subunit, and T366S+L368A+Y407V "hole" mutations in another Fc subunit. In some embodiments, i) a first subunit and a second subunit of the Fc domain each comprises the amino acid sequence of any of SEQ ID NOs:76-79; ii) a first subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO: 80, and a second subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO: 81; or iii) a first subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO: 81, and a second subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO: 80. In some embodiments, the first subunit and the second subunit of the Fc domain each comprises the amino acid sequence of SEQ ID NO:77 or 79. In some embodiments, the anti-TSLP full-length antibody comprises: i) two heavy chains each comprising the amino acid sequence of SEQ ID NO:104, or a variant thereof having at least about 80% (such as at least about any one of 85%, 90%, 95%, 99%, or more) sequence identity to the amino acid sequence of SEQ ID NO:104, and two light chains each comprising the amino acid sequence of SEQ ID NO:103, or a variant thereof having at least about 80% (such as at least about any one of 85%, 90%, 95%, 99%, or more) sequence identity to the amino acid sequence of SEQ ID NO:103; ii) two heavy chains

each comprising the amino acid sequence of SEQ ID NO:105, or a variant thereof having at least about 80% (such as at least about any one of 85%, 90%, 95%, 99%, or more) sequence identity to the amino acid sequence of SEQ ID NO:105, and two light chains each comprising the amino acid sequence of SEQ ID NO:103, or a variant thereof having at least about 80% (such as at least about any one of 85%, 90%, 95%, 99%, or more) sequence identity to the amino acid sequence of SEQ ID NO:103; or iii) a first heavy chain comprising the amino acid sequence of SEQ ID NO:136 (or a variant thereof having at least about 80% (such as at least about any one of 85%, 90%, 95%, 99%, or more) sequence identity to the amino acid sequence of SEQ ID NO:136), a second heavy chain comprising the amino acid sequence of SEQ ID NO:137 (or a variant thereof having at least about 80% (such as at least about any one of 85%, 90%, 95%, 99%, or more) sequence identity to the amino acid sequence of SEQ ID NO:137), and two light chains each comprising the amino acid sequence of SEQ ID NO:103 (or a variant thereof having at least about 80% (such as at least about any one of 85%, 90%, 95%, 99%, or more) sequence identity to the amino acid sequence of SEQ ID NO:103). In some embodiments, the anti-TSLP full-length antibody comprises: i) two heavy chains each comprising the amino acid sequence of SEQ ID NO:104, and two light chains each comprising the amino acid sequence of SEQ ID NO:103; ii) two heavy chains each comprising the amino acid sequence of SEQ ID NO:105, and two light chains each comprising the amino acid sequence of SEQ ID NO:103; or iii) a first heavy chain comprising the amino acid sequence of SEQ ID NO:136, a second heavy chain comprising the amino acid sequence of SEQ ID NO:137, and two light chains each comprising the amino acid sequence of SEQ ID NO:103.

[0100] In some embodiments, the anti-TLSP antibody moiety is an scFv (anti-TSLP scFv). In some embodiments, the anti-TSLP scFv comprises a VH and a VL fused to each other via an optional linker. In some embodiments, the anti-TSLP scFv comprises from N-terminus to C-terminus: VH-optional linker-VL, or VL-optional linker-VH. Any linker in the “Linkers” subsection below can be used for linking VH and VL of the anti-TSLP scFv. In some embodiments, the optional linker comprises an amino acid sequence selected from the group consisting of GG and SEQ ID NOs:14, 16-18, 97-99 and 163-172, such as any of GG and SEQ ID NOs:98-99. In some embodiments, the anti-TSLP scFv comprises the amino acid sequence of any one of SEQ ID NOs:106, 107, 191, and 192.

[0101] In some embodiments, the anti-TSLP antibody moiety is a Fab fragment (anti-TSLP Fab fragment), comprising a first polypeptide comprising a VH and a CH1, and a second

polypeptide comprising a VL and a CL. In some embodiments, the configuration of the variable and constant regions within the anti-TSLP Fab fragment may be different from what is found in a native anti-TSLP Fab fragment. For example, in some embodiments, the anti-TSLP Fab fragment comprises a first polypeptide comprising a VH and a CL, and a second polypeptide comprising a VL and a CH1 (see, for example, Shaefer et al. (2011), PNAS, 108:111870-92, the content of which is incorporated herein by reference in its entirety).

[0102] In some embodiments, the CH1 and VH heterodimerize with the VL and CL in the anti-TSLP Fab and are covalently linked by a disulfide bond between the heavy and light chain constant regions. In some embodiments, the anti-TSLP Fab fragment has the basic structure $\text{NH}_2\text{-VL-CL-S-S-CH1-VH-NH}_2$. In some embodiments, the CH1 and the CL of the anti-TSLP Fab fragment are connected by one or more disulfide bonds. In some embodiments, the number of disulfide bonds between CH1 and CL of the anti-TSLP Fab fragment is at least one, such as 2, 3, 4, 5, or more. In some embodiments, cysteine residues are engineered in the anti-TSLP Fab fragment (such as in the CH1 and CL regions) to introduce disulfide bonds.

[0103] In some embodiments, the anti-TSLP Fab fragment does not comprise a disulfide bond at the C-terminus. For example, the heavy and light chains of the anti-TSLP Fab fragment may be engineered in such a way so as to stably interact without the need for disulfide bonds. For example, the heavy and light chains may be engineered in such a way so as to stably interact without the need for disulfide bonds. In some embodiments, the heavy chain or light chain can be engineered to remove a cysteine residue, and wherein the heavy and light chains still stably interact and function as a Fab. In some embodiments, mutations are made to facilitate stable interactions between the heavy and light chains. For example, a “knob-in-hole” engineering strategy can be used to facilitate dimerization between the heavy and light chains of a Fab (see *e.g.*, 1996 Protein Engineering, 9:617 – 621). Also contemplated for use herein are variant Fab fragments designed for a particular purpose, for example, amino acid changes in the constant domains of CH1 and/or CL, and removal of a disulfide bond or addition of tags for purification, etc. These mutations are described in more detail in the “Constant and Fc Domains” subsection below.

[0104] In some embodiments, the CH1 and the CL of the anti-TSLP Fab fragment are connected by at least 1 or 2 disulfide bonds. In some embodiments, the anti-TSLP Fab fragment comprises a human immunoglobulin CH1, *e.g.*, comprising the amino acid sequence of SEQ ID NO:158. In some embodiments, the anti-TSLP Fab fragment comprises

a human lambda light chain constant region, *e.g.*, comprising the amino acid sequence of SEQ ID NO:74 or 75. In some embodiments, the anti-TSLP Fab fragment comprises a human kappa light chain constant region, *e.g.*, comprising the amino acid sequence of SEQ ID NO:73.

[0105] In some embodiments, the anti-TSLP Fab fragment comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO:109, and a second polypeptide comprising the amino acid sequence of SEQ ID NO:103.

Multispecific Anti-TSLP Antibody Constructs

[0106] In some embodiments, the isolated anti-TSLP antibody construct described herein is a multispecific anti-TSLP antibody construct comprising an anti-TSLP antibody moiety (*e.g.*, any of the anti-TSLP antibody moieties described herein, such as anti-TSLP full-length antibody, anti-TSLP Fab, and/or anti-TSLP scFv), and a second antibody moiety specifically recognizing a second target antigen (*e.g.*, TSLP or IL-13). In some embodiments, the isolated anti-TSLP antibody construct comprises one or more anti-TSLP antibody moieties (*e.g.*, any of the anti-TSLP antibody moieties described herein), and one or more antibody moieties specifically recognizing one or more target antigens (*e.g.*, TSLP or IL-13). In some embodiments, the isolated anti-TSLP antibody construct is multivalent but monospecific, *i.e.*, all antibody moieties contained therein specifically recognize the same TSLP epitope. In some embodiments, the isolated anti-TSLP antibody construct is multivalent and multispecific (*e.g.*, bispecific). In some embodiments, the isolated anti-TSLP antibody construct specifically recognizes two or more epitopes of TSLP. In some embodiments, the isolated anti-TSLP antibody construct specifically recognizes TSLP via its one or more anti-TSLP antibody moieties (*e.g.*, any of the anti-TSLP antibody moieties described herein), and one or more target antigens that are not TSLP. In some embodiments, the second target antigen is IL-13. In some embodiments, the two or more anti-TSLP antibody moieties (*e.g.*, scFv, Fab) have the same amino acid sequence. In some embodiments, the two or more anti-TSLP antibody moieties (*e.g.*, scFv, Fab) have different amino acid sequences. In some embodiments, the two or more anti-TSLP antibody moieties bind to the same TSLP epitope. In some embodiments, the two or more anti-TSLP antibody moieties bind to different TSLP epitopes. In some embodiments, the second antibody moiety is selected from the group consisting of a full-length antibody, a Fab, a Fab', a F(ab')₂, an sdAb, a diabody, and an scFv. In some embodiments, the second antibody moiety is an scFv (*e.g.*, anti-IL-13 scFv). In some embodiments, the second antibody moiety is a Fab (*e.g.*, anti-IL-13 Fab). In some

embodiments, the second antibody moiety is a full-length antibody (e.g., anti-IL-13 full-length antibody). In some embodiments, the one or more anti-TSLP antibody moieties (e.g., any of the anti-TSLP antibody moieties described herein), and one or more antibody moieties specifically recognizing one or more target antigens (e.g., TSLP or IL-13) are fused to each other via a linker. Any linker described in the “Linkers” subsection below can be used herein. In some embodiments, the linker comprises an amino acid sequence selected from any one of GG and SEQ ID NOs:14, 16-18, 97-99, and 163-172, such as any one of GG and SEQ ID NOs:98-99. When the isolated anti-TSLP antibody construct comprises an Fc domain, any Fc domain described in the “Fc domains” subsection below can be used herein.

Fc Domains

[0107] Further described herein are FC domains (e.g., each of two Fc subunits) and variants thereof that may be included in any of the isolated anti-TSLP antibody constructs described herein (e.g., anti-TSLP full-length antibody).

[0108] The Fc (Fragment, crystallizable) domain is the tail region of an antibody that interacts with cell surface receptors called Fc receptors and some proteins of the complement system. The Fc-mediated effector functions of antibodies include antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and complement-dependent cytotoxicity (CDC). This property is key for antibodies to activate the immune system. This Fc domain is composed of two or three constant domains attached to the CH1 domain on each of two heavy chains, depending on the class of the antibody. Mutations in the CH3 subunits of the Fc domain on both heavy chains, termed “knob-in-hole” mutations, can further improve heterodimerization when assembling two antibody polypeptides. For example, the CH3 domain of the Fc domain on a first heavy chain can comprise the “knob” mutation (e.g., T366W), when the following “hole” substitutions are introduced at positions 366, 368, and 407: T366S, L368A, and Y407V in the CH3 domain of the Fc domain on a second heavy chain. The location of the knob vs. hole mutations can be switched, e.g., the knob mutation can be on the second heavy chain, and the hole mutations can be on the first heavy chain. Additional Fc domain mutations, such as the LALA mutations (e.g., L234A and L235A substitutions in the CH2 domain of the Fc domain), can be introduced to reduce FcγR activation and Fc-mediated toxicity that could induce side effects such as cytokine release syndrome in individuals being administered the antibody. Furthermore, Fc domain mutations, such as the LS mutations (e.g., M428L and N434S substitutions in the CH3 domain of the Fc domain), can be introduced to extend antibody half-life without impacting potency. FcRn

affinity-enhancing Fc mutant can also be used herein, such as the M252Y/S254T/T256E (YTE) mutation which, when incorporated into the Fc domain, is able to extend serum half-life. Accordingly, the described mutations can be used singly or in combination for improved antibody function and/or reduced toxicity to the patient. In some embodiments, the Fc domain comprises the LALA mutations. In some embodiments, the Fc domain comprises the LS or YTE mutations. In some embodiments, the Fc domain comprises the LALA mutations and the LS mutations. In some embodiments, the Fc domain comprises the LALA mutations and the YTE mutations. In some embodiments, i) a first subunit and a second subunit of the Fc domain each comprises the amino acid sequence of any of SEQ ID NOs:76-79; ii) a first subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO:80, and a second subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO:81; or iii) a first subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO:81, and a second subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO:80.

Linkers

[0109] The isolated anti-TSLP antibody constructs (e.g., multispecific anti-TSLP antibody construct, anti-TSLP scFv) described herein may comprise one or more linkers (such as a peptide linker) connecting two or more domains or moieties contained within, for example, between the VH and the VL domain (e.g., within an scFv), between the anti-TSLP antibody moiety and its fusion partner or a second antibody moiety (e.g., scFv, Fab, or full-length antibody) specifically recognizing a second target antigen, or between two or more anti-TSLP antibody moieties. In some embodiments, the two or more linkers are the same. In some embodiments, the two or more linkers are different. In some embodiments, the isolated anti-TSLP antibody construct does not comprise a linker, i.e., two or more domains or moieties contained therein are connected to each other directly.

[0110] The linkers can be peptide linkers of any length. In some embodiments, the peptide linker is from about 1 to about 10 amino acids (aa) long, from about 2 to about 15 aa long, from about 3 to about 12 aa long, from about 4 to about 10 aa long, from about 5 to about 9 aa long, from about 6 to about 8 aa long, from about 1 to about 20 aa long, from about 21 to about 30 aa long, from about 1 to about 30 aa long, from about 10 to about 30 aa long, or from about 2 to about 5 aa long. In some embodiments, the peptide linker is any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids long. In some

embodiments, the peptide linker is any of 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 amino acids long. In some embodiments, the peptide linker is about 2 to about 15 amino acids long.

[0111] A peptide linker can have a naturally occurring sequence or a non-naturally occurring sequence. For example, a sequence derived from the hinge region of a heavy chain only antibody can be used as a linker. See, for example, WO1996/34103. In some embodiments, the peptide linker is a human IgG1 or IgG4 hinge. In some embodiments, the linker is a flexible linker. Exemplary flexible linkers include glycine polymers (G)_n (e.g., GG), glycine-serine polymers (including, for example, (GS)_n, (GSGGS)_n (SEQ ID NO:14), (GGGS)_n (SEQ ID NO:16), or (GGGGS)_n (SEQ ID NO:17), where n is an integer of at least one), glycine-alanine polymers, alanine-serine polymers, and other flexible linkers known in the art. Glycine and glycine-serine polymers are relatively unstructured, and therefore may be able to serve as a neutral tether between components. Glycine accesses significantly more phi-psi space than even alanine and is much less restricted than residues with longer side chains (see Scheraga, Rev. Computational Chem. 11 173-142 (1992)). In some embodiments, the linker comprises amino acid residues selected from the group consisting of glycine, serine, arginine, and alanine. Exemplary flexible linkers include, but are not limited to Gly-Gly, GGSG (SEQ ID NO:18), GGSGG (SEQ ID NO:163), GSGSG (SEQ ID NO:164), GSGGG (SEQ ID NO:165), GGGSG (SEQ ID NO:166), GSSSG (SEQ ID NO:167), GGSGGS (SEQ ID NO:168), SGGGGS (SEQ ID NO:169), GRAGGGGAGGGG (SEQ ID NO:170), GRAGGG (SEQ ID NO:171), GGGSGGGGSGGGGS (SEQ ID NO:99), GGGG (SEQ ID NO: 98), GGGGS (SEQ ID NO:172), and the like. In some embodiments, the linker between the VH and the VL of an scFv comprises the sequence of SEQ ID NO:99. The ordinarily skilled artisan will recognize that design of an isolated anti-TSLP antibody construct or anti-TSLP antibody moiety can include linkers that are all or partially flexible, such that the linker can include a flexible linker portion as well as one or more portions that confer less flexible structure to provide a desired anti-TSLP antibody construct structure.

[0112] In some embodiments, the linker between the anti-TSLP antibody moiety and the second antibody moiety, or the linker within an anti-TSLP antibody moiety, is a stable linker (not cleavable by proteases, especially MMPs).

[0113] In some embodiments, the linker is a cleavable linker. Substrate sequences that can be cleaved by MMPs have been extensively studied. For example, the sequence of PLGLAG (SEQ ID NO:97) can be cleaved by most MMPs.

III. Methods of Preparation

[0114] Also provided are methods of making any of the isolated anti-TSLP antibody constructs or anti-TSLP antibody moieties described herein.

[0115] The isolated anti-TSLP antibody constructs or anti-TSLP antibody moieties described herein may be prepared by any of the known protein expression and purification methods in the art. DNA sequence(s) encoding the isolated anti-TSLP antibody constructs or anti-TSLP antibody moieties can be fully synthesized. After obtaining such sequence, it is cloned into a suitable expression vector, then transfected into a suitable host cell. The transfected host cells are cultured, and the supernatant is harvested and purified to obtain the anti-TSLP antibody constructs or anti-TSLP antibody moieties described herein. In some embodiments, the host cell is lysed to obtain the expressed anti-TSLP antibody constructs or anti-TSLP antibody moieties.

[0116] In some embodiments, the present application provides isolated nucleic acids encoding one or more of the polypeptides of any one of the isolated anti-TSLP antibody constructs described herein. The isolated nucleic acids may be DNA or RNA.

[0117] In some embodiments, the isolated nucleic acid is inserted into a vector, such as an expression vector, a viral vector, or a cloning vector. Hence also provided are vectors comprising any of the isolated nucleic acids described herein. For expression of the nucleic acids, the vector may be introduced into a host cell to allow expression of the nucleic acids within the host cell. The expression vectors may contain a variety of elements for controlling expression, including, without limitation, promoter sequences, transcription initiation sequences, enhancer sequences, selectable markers, and signal sequences. These elements may be selected as appropriate by a person of ordinary skill in the art. For example, the promoter sequence(s) may be selected to promote the transcription of the polynucleotide in the vector. Suitable promoter sequences include, without limitation, T7 promoter, T3 promoter, SP6 promoter, beta-actin promoter, EF1a promoter, CMV promoter, and SV40 promoter. Enhancer sequences may be selected to enhance the transcription of the nucleic acids. Selectable markers may be selected to allow selection of the host cells inserted with the vector from those cells lacking the vector, for example, the selectable markers may be genes that confer antibiotic resistance. Signal sequences may be selected to allow the expressed polypeptide to be transported outside of the host cell.

[0118] In some embodiments, there is provided an isolated host cell comprising any of the isolated nucleic acids or vectors encoding the polypeptide portion of any of the isolated anti-TSLP antibody constructs described herein. In some embodiments, there is provided an isolated host cell expressing any of the isolated anti-TSLP antibody constructs described herein. In some embodiments, two or more polypeptides of any of the isolated anti-TSLP antibody constructs (*e.g.*, full-length antibody, Fab fragment, or multispecific anti-TSLP antibody construct) described herein are encoded by a single vector. In some embodiments, two or more polypeptides of any of the isolated anti-TSLP antibody constructs (*e.g.*, full-length antibody, Fab fragment, or multispecific isolated anti-TSLP antibody construct) described herein are encoded by two or more vectors. The host cells containing the vector may be useful in expression or cloning of the isolated nucleic acids. Suitable host cells can include, without limitation, prokaryotic cells, fungal cells, yeast cells, or higher eukaryotic cells such as mammalian cells. The expression of antibodies and antigen-binding fragments in prokaryotic cells such as *E. coli* is well established in the art. For a review, see for example Pluckthun, A. *BioTechnology* 9: 545-551 (1991). Expression in eukaryotic cells in culture is also available to those skilled in the art as an option for production of antibodies or antigen-binding fragments thereof, see reviews, for example Ref, M. E. (1993) *Curr. Opinion Biotech.* 4: 573-576; Trill J. J. *et al.* (1995) *Curr. Opinion Biotech* 6: 553-560. Higher eukaryotic cells, in particular those derived from multicellular organisms, can be used for expression of glycosylated polypeptides. Suitable higher eukaryotic cells include, without limitation, invertebrate cells and insect cells, and vertebrate cells. In some embodiments, the host cell is *E. coli*. In some embodiments, the host cell is a Chinese hamster ovary (CHO) cell or a HEK293 cell.

[0119] The vector can be introduced to the host cell using any suitable methods known in the art, including, but not limited to, DEAE-dextran mediated delivery, calcium phosphate precipitate method, cationic lipids mediated delivery, liposome mediated transfection, electroporation, microprojectile bombardment, receptor-mediated gene delivery, delivery mediated by polylysine, histone, chitosan, and peptides. Standard methods for transfection and transformation of cells for expression of a vector of interest are well known in the art. In some embodiments, the host cells comprise two or more vectors each encoding a polypeptide of any of the isolated anti-TSLP antibody constructs described herein. The two or more vectors can be introduced into the host cells at a same ratio, or at different ratios. In some embodiments, the host cells comprise a single vector comprising isolated nucleic acids

encoding two or more polypeptides of any of the isolated anti-TSLP antibody constructs described herein. The two or more nucleic acids encoding the two or more polypeptides of the isolated anti-TSLP antibody construct can be under the same promoter control, or different promoter controls. For example, two or more nucleic acids under the same promoter control can be connected via an IRES sequence, or a sequence encoding a self-cleaving peptide (e.g., P2A, T2A).

[0120] In some embodiments, the present application provides methods of making any of the isolated anti-TSLP antibody constructs described herein, comprising: i) culturing an isolated host cell comprising any of the isolated nucleic acids described herein or any of the vectors described herein, or any of the isolated host cells described herein (e.g., a host cell comprising any of the isolated nucleic acids or vectors described herein), under a condition suitable for the expression of the anti-TSLP antibody construct, and ii) obtaining the expressed anti-TSLP antibody constructs from said host cell (e.g., from the cell culture, or by lysing the host cell). The isolated host cells are cultured under conditions that allow expression of the nucleic acids inserted in the vectors. Suitable conditions for expression of polynucleotides may include, without limitation, suitable medium, suitable density of host cells in the culture medium, presence of necessary nutrients, presence of supplemental factors, suitable temperatures and humidity, and absence of microorganism contaminants. A person with ordinary skill in the art can select the suitable conditions as appropriate for the purpose of the expression. In some embodiments, the methods of making further comprise purifying any of the obtained anti-TSLP antibody constructs.

[0121] In some embodiments, the polypeptides expressed by the host cell can assemble together (e.g., form a polypeptide complex such as a dimer) and produce any of the isolated anti-TSLP antibody constructs described herein. In some embodiments, the polypeptide complex may be formed inside the host cell. For example, the polypeptide complex may be formed inside the host cell with the aid of relevant enzymes and/or cofactors. In some embodiments, the polypeptide complex may be secreted out of the cell. In some embodiments, individual polypeptides may be secreted out of the host cell then form a polypeptide complex (such as any of the isolated anti-TSLP antibody constructs described herein) outside of the host cell.

[0122] In some embodiments, two or more polypeptides of any of the isolated anti-TSLP antibody constructs described herein may be separately expressed and allowed to form (e.g., dimerize) the isolated anti-TSLP antibody construct under suitable conditions. For example,

the first polypeptide and the second polypeptide may be combined in a suitable buffer to allow the first protein monomer and the second protein monomer to dimerize through appropriate interactions such as hydrophobic interactions. In some embodiments, the first polypeptide and the second polypeptide may be combined in a suitable buffer containing an enzyme and/or a cofactor which can promote the dimerization of the first polypeptide and the second polypeptide. In some embodiments, the first polypeptide and the second polypeptide may be combined in a suitable vehicle allowing them to react with each other in the presence of a suitable reagent and/or catalyst.

[0123] The expressed polypeptide(s) and/or the polypeptide complex can be collected using any suitable methods. The polypeptide(s) and/or the polypeptide complex can be expressed intracellularly, in the periplasmic space, or secreted outside of the cell into the culture medium. If the polypeptide and/or the polypeptide complex are expressed intracellularly, the host cells containing the polypeptide and/or the polypeptide complex may be lysed, and the polypeptide and/or the polypeptide complex may be isolated from the lysate by removing the unwanted debris by centrifugation or ultrafiltration. If the polypeptide and/or the polypeptide complex is secreted into periplasmic space of *E. coli*, the cell paste may be thawed in the presence of agents such as sodium acetate (pH 3.5), EDTA, and phenylmethylsulfonylfluoride (PMSF) for about 30 min, and cell debris can be removed by centrifugation (Carter *et al.*, BioTechnology 10:163-167 (1992)). If the polypeptide and/or the polypeptide complex is secreted into the medium, the supernatant of the cell culture may be collected and concentrated using a commercially available protein concentration filter, for example, an Amincon or Millipore Pellicon ultrafiltration unit. A protease inhibitor and/or an antibiotic may be included in the collection and concentration steps to inhibit protein degradation and/or growth of contaminating microorganisms.

[0124] The expressed polypeptide(s) and/or the polypeptide complex can be further purified by a suitable method, such as, without limitation, affinity chromatography, hydroxylapatite chromatography, size exclusion chromatography, gel electrophoresis, dialysis, ion exchange 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 (*see*, for review, Bonner, P. L., Protein purification, published by Taylor & Francis. 2007; Janson, J. C., *et al.*, Protein purification: principles, high resolution methods and applications, published by Wiley-VCH, 1998).

[0125] In some embodiments, the polypeptides and/or polypeptide complexes can be purified by affinity chromatography. In some embodiments, protein A chromatography or protein A/G (fusion protein of protein A and protein G) chromatography can be useful for purification of polypeptides and/or polypeptide complexes comprising a component derived from antibody CH2 domain and/or CH3 domain (Lindmark *et al.*, J. Immunol. Meth. 62:1-13 (1983)); Zettlitz, K. A., Antibody Engineering, Part V, 531-535, 2010). In some embodiments, protein G chromatography can be useful for purification of polypeptides and/or polypeptide complexes comprising IgG γ 3 heavy chain (Guss *et al.*, EMBO J. 5:1567-1575 (1986)). In some embodiments, protein L chromatography can be useful for purification of polypeptides and/or polypeptide complexes comprising κ light chain (Sudhir, P., Antigen engineering protocols, Chapter 26, published by Humana Press, 1995; Nilson, B. H. K. *et al.*, J. Biol. Chem., 267, 2234-2239 (1992)). The matrix to which the affinity ligand is attached is most often 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 any of the isolated anti-TSLP antibody constructs comprises an additional CH3 domain, the Bakerbond ABX resin (J. T. Baker, Phillipsburg, N.J.) is useful for purification.

IV. Pharmaceutical Compositions, Unit Dosages, Articles of Manufacture, and Kits

[0126] Further provided by the present application are pharmaceutical compositions comprising an isolated anti-TSLP antibody construct of the present disclosure and optionally a pharmaceutically acceptable carrier.

[0127] The isolated anti-TSLP antibody constructs or pharmaceutical compositions thereof may be suitable for a variety of modes of administration described herein, including for example systemic or localized administration. In some embodiments, the pharmaceutical composition is formulated for intravenous administration. In some embodiments, the pharmaceutical composition is formulated for subcutaneous injection.

[0128] "Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers which 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. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium

chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose, or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™, or polyethylene glycol (PEG).

[0129] In some embodiments, the pharmaceutical composition is formulated to have a pH in the range of about 4.5 to about 9.0, including for example pH ranges of any one of about 5.0 to about 8.0, about 6.5 to about 7.5, about 6.5 to about 7.0, or about 7.0 to about 7.5. In some embodiments, the pharmaceutical composition can also be made to be isotonic with blood by the addition of a suitable tonicity modifier, such as glycerol.

[0130] The pharmaceutical compositions to be used for *in vivo* administration are generally formulated as sterile, substantially isotonic, and in full compliance with all Good Manufacturing Practice (GMP) regulations of the U.S. Food and Drug Administration. Sterility is readily accomplished by filtration through sterile filtration membranes. In some embodiments, the composition is free of any pathogens. For injection, the pharmaceutical composition can be in the form of liquid solutions, for example in physiologically compatible buffers such as Hank's solution or Ringer's solution. In addition, the pharmaceutical composition can be in a solid form and re-dissolved or suspended immediately prior to use. Lyophilized compositions are also included.

[0131] In some embodiments, the pharmaceutical composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for parenteral (*e.g.*, intravenous, intramuscular, or subcutaneous) administration. Typically, compositions for injection are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lidocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline.

Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0132] In some embodiments, the pharmaceutical composition is suitable for administration to a mammal, such as a human. In some embodiments, the pharmaceutical composition is suitable for administration to a rodent (*e.g.*, mice, rats) or non-human primates (*e.g.*, *Cynomolgus* monkey). In some embodiments, the pharmaceutical composition is contained in a single-use vial, such as a single-use sealed vial. In some embodiments, the pharmaceutical composition is contained in a multi-use vial. In some embodiments, the pharmaceutical composition is contained in bulk in a container. In some embodiments, the pharmaceutical composition is cryopreserved.

[0133] Also provided are unit dosage forms of any of the isolated anti-TSLP antibody constructs described herein, or compositions (such as pharmaceutical compositions) thereof. The term “unit dosage form” refers to a physically discrete unit suitable as unitary dosages for an individual (*e.g.*, human), each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical carrier, diluent, or excipient. These unit dosage forms can be stored in a suitable packaging in single or multiple unit dosages and may also be further sterilized and sealed.

[0134] The present application further provides articles of manufacture comprising the compositions (such as pharmaceutical compositions) described herein in suitable packaging. Suitable packaging for compositions (such as pharmaceutical compositions) described herein are known in the art, and include, for example, vials (such as sealed vials), vessels, ampoules, bottles, jars, flexible packaging (*e.g.*, sealed Mylar or plastic bags), and the like. These articles of manufacture may further be sterilized and/or sealed.

[0135] The present application also provides kits comprising compositions (such as pharmaceutical compositions) described herein and may further comprise instruction(s) on methods of using the composition, such as uses described herein. The kits described herein may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and/or applicators and package inserts with instructions for performing any methods described herein.

V. Methods of Treating an Inflammatory Disease

[0136] Also provided are methods of treating an inflammatory disease in an individual (*e.g.*, human), comprising administering (*e.g.*, intravenously or subcutaneously) to the individual an effective amount of any of the isolated anti-TSLP antibody constructs or pharmaceutical compositions described herein. In some embodiments, the inflammatory disease is asthma (*e.g.*, severe asthma), atopic dermatitis (*e.g.*, moderate to severe atopic dermatitis), or chronic obstructive pulmonary disease (COPD). In some embodiments, the method further comprises administration of an effective amount of a corticosteroid, either simultaneously or sequentially with the isolated anti-TSLP antibody construct or pharmaceutical composition thereof.

[0137] In some embodiments, the inflammatory disease is a TSLP-associated disease. An inflammatory disease associated with TSLP can be a disease caused by, or associated with, the expression of TSLP, *e.g.*, over-expression compared to a healthy state, or mis-expression at a location different from a healthy state (*e.g.*, on a different cell or tissue).

[0138] In some embodiments, the inflammatory disease is an autoimmune disease or autoimmune condition, such as atopic dermatitis, Netherton Syndrome, lupus, Sjogren's syndrome, multiple sclerosis, myasthenia gravis, psoriasis, psoriatic arthritis, rheumatoid arthritis, sarcoidosis, ulcerative colitis, inflammatory bowel disease, *etc.* In some embodiments, the inflammatory disease is a disease wherein immune function is implicated (*e.g.*, asthma, endometriosis, fibrosis, Hodgkin's lymphoma). In some embodiments, the inflammatory disease is an allergic reaction, such as allergic asthma, allergic rhinitis, allergic rhinosinusitis, allergic conjunctivitis, atopic dermatitis, eosinophilic esophagitis, *etc.* In some embodiments, the inflammatory disease is fibrosis, inflammatory bowel disease, or COPD. In some embodiments, the inflammatory disease is asthma or atopic dermatitis, such as any severity of asthma or atopic dermatitis.

[0139] In some embodiments, there is provided a method of treating an inflammatory disease (*e.g.*, atopic dermatitis, COPD, or asthma) in an individual (*e.g.*, human), comprising administering (*e.g.*, intravenously or subcutaneously) to the individual an effective amount of an isolated anti-TSLP antibody construct (or a pharmaceutical composition thereof), wherein the isolated anti-TSLP antibody construct comprises an anti-TSLP Fab, wherein the anti-TSLP Fab comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO:109, and a second polypeptide comprising the amino acid sequence of SEQ ID NO:103.

[0140] In some embodiments, there is provided a method of treating an inflammatory disease (e.g., atopic dermatitis, COPD, or asthma) in an individual (*e.g.*, human), comprising administering (e.g., intravenously or subcutaneously) to the individual an effective amount of an isolated anti-TSLP antibody construct (or a pharmaceutical composition thereof), wherein the isolated anti-TSLP antibody construct comprises an anti-TSLP full-length antibody, wherein the anti-TSLP full-length antibody comprises: i) two heavy chains each comprising the amino acid sequence of SEQ ID NO:104, and two light chains each comprising the amino acid sequence of SEQ ID NO:103; ii) two heavy chains each comprising the amino acid sequence of SEQ ID NO:105, and two light chains each comprising the amino acid sequence of SEQ ID NO:103; or iii) a first heavy chain comprising the amino acid sequence of SEQ ID NO:136, a second heavy chain comprising the amino acid sequence of SEQ ID NO:137, and two light chains each comprising the amino acid sequence of SEQ ID NO:103.

[0141] In some embodiments, there is provided a method of treating an inflammatory disease (e.g., atopic dermatitis, COPD, or asthma) in an individual (*e.g.*, human), comprising administering (e.g., intravenously or subcutaneously) to the individual an effective amount of an isolated anti-TSLP antibody construct (or a pharmaceutical composition thereof), wherein the isolated anti-TSLP antibody construct comprises an anti-TSLP scFv comprising the amino acid sequence of any of SEQ ID NOs:106, 107, 191, and 192.

[0142] In some embodiments, the method of treating an inflammatory disease can achieve one or more of the following biological activities: (1) inhibiting (*e.g.*, by at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%) immune cell maturation and/or activation (*e.g.*, B cell, mast cell, T-helper 2 (Th2) cell, or APC (*e.g.*, dendritic cell)); (2) inhibiting (*e.g.*, by at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%) proliferation of immune cells; (3) reducing (*e.g.*, by at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%) systemic or local inflammation levels; (4) alleviating (*e.g.*, by at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%) one or more symptoms in an individual having the inflammatory disease; (5) reducing (*e.g.*, by at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%) systemic or local cytokine levels (such as Th2 cytokine levels, *e.g.*, IL-13, IL-4, IL-5); (6) reducing (*e.g.*, by at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%) the presentation, incidence, or burden of the inflammatory disease; (7) prolonging duration between inflammatory immune flares, such as prolonging the time after symptoms of the inflammatory disease subside until symptoms reappear in the

individual by at least about any of 1, 2, 4, 6, 12, 18, 20, or more hours, 1, 2, 3, 4, 5, 6, 7, or more days, 1, 2, 3, 4, or more weeks, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 18, or 24 months, or more; (8) preventing the reappearance of symptoms of the inflammatory disease by at least about any of 1, 2, 4, 6, 12, 18, 20, or more hours, 1, 2, 3, 4, 5, 6, 7, or more days, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, or more; and (9) preventing, inhibiting, or reducing (*e.g.*, by at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%) the likelihood of the recurrence of an inflammatory disease.

[0143] The methods provided herein may be practiced in a primary therapeutic setting, *i.e.*, wherein the methods being carried out are the primary/definitive therapy. In some embodiments, the method may be carried out before or in conjunction with the primary/definitive therapy. In some embodiments, the method is used to treat an individual (such as a human) who has previously been treated. Any of the methods of treatment provided herein may be used to treat an individual (such as a human) who has not previously been treated. In some embodiments, the method is used as a first line therapy. In some embodiments, the method is used as a second line therapy.

[0144] The methods described herein are suitable for treating a variety of inflammatory diseases. The methods are applicable to inflammatory diseases of all stages, including shortly after onset of symptoms or for an inflammatory disease that is in remission. The methods described herein may be used as a first therapy, second therapy, third therapy, or combination therapy with other types of therapies known in the art, such as anti-inflammatory agents (*e.g.*, corticosteroids), gene therapy, immunotherapy, bone marrow transplantation, stem cell transplantation, targeted therapy, nutritional therapy, or the like. In some embodiments, the inflammatory disease has been unresponsive to prior therapy.

[0145] Exemplary routes of administration of any of the isolated anti-TSLP antibody constructs described herein (or pharmaceutical compositions thereof) include, but are not limited to, intravenous, intracavitary, intraarterial, intramuscular, subcutaneous, parenteral, transdermal, or intraperitoneal routes, or can be delivered into lymph glands, body spaces, organs, or tissues affected by the inflammatory disease. In some embodiments, the isolated anti-TSLP antibody constructs or pharmaceutical compositions thereof are administered intravenously, such as by infusion.

[0146] In some embodiments, the isolated anti-TSLP antibody constructs described herein (or pharmaceutical compositions thereof) are administered by intravenous infusion at any suitable rate.

[0147] The dosing regimen of the isolated anti-TSLP antibody constructs described herein, or pharmaceutical compositions thereof, administered to the individual (such as human) may vary with the particular composition, the method of administration, and the particular type of inflammatory disease being treated. In some embodiments, the effective amount of the isolated anti-TSLP antibody constructs is below the level that induces a toxicological effect (*i.e.*, an effect above a clinically acceptable level of toxicity) or is at a level where a potential side effect can be controlled or tolerated when the composition is administered to the individual.

[0148] The effective amount of the isolated anti-TSLP antibody constructs described herein (or pharmaceutical compositions thereof) may be administered in a single dose or in multiple doses. For methods that comprise administration of the isolated anti-TSLP antibody constructs (or pharmaceutical compositions thereof) in multiple doses, exemplary dosing frequencies include, but are not limited to, hourly, daily, daily without break, weekly, weekly without break, weekly for two out of three weeks, weekly for three out of four weeks, once every three weeks, once every two weeks, monthly, every six months, yearly, *etc.* In some embodiments, the isolated anti-TSLP antibody constructs (or pharmaceutical compositions thereof) are administered about once every 2 weeks, once every 3 weeks, once every 4 weeks, once every 6 weeks, or once every 8 weeks. In some embodiments, the isolated anti-TSLP antibody constructs (or pharmaceutical compositions thereof) are administered at least about any of 1×, 2×, 3×, 4×, 5×, 6×, or 7× (*i.e.*, daily) a week. In some embodiments, the intervals between each administration are less than about any of 3 years, 2 years, 12 months, 11 months, 10 months, 9 months, 8 months, 7 months, 6 months, 5 months, 4 months, 3 months, 2 months, 1 month, 4 weeks, 3 weeks, 2 weeks, 1 week, 6 days, 5 days, 4 days, 3 days, 2 days, or 1 day. In some embodiments, the intervals between each administration are more than about any of 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 2 weeks, 3 weeks, 4 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 2 years, or 3 years. In some embodiments, there is no break in the dosing schedule.

[0149] The administration of the isolated anti-TSLP antibody constructs described herein (or pharmaceutical compositions thereof) can be extended over an extended period of time, such

as from 1 day to about a week, from about a week to about a month, from about a month to about a year, from about a year to about several years. In some embodiments, the isolated anti-TSLP antibody constructs (or pharmaceutical compositions thereof) are administered over a period of at least about any of 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 1 year, 2 years, 3 years, 4 years, or more.

EXAMPLES

[0150] The examples below are intended to be purely exemplary of the invention and should therefore not be considered to limit the invention in any way. The following examples and detailed description are offered by way of illustration and not by way of limitation. For the embodiments in which details of the experimental methods are not described, such methods are carried out according to conventional conditions such as those described in Sambrook *et al.* *Molecular Cloning: A Laboratory Manual* (New York: Cold Spring Harbor Laboratory Press, 1989), or as suggested by the manufacturers.

Example 1: Generation of Exemplary Anti-TSLP Antibodies.

[0151] Anti-TSLP antibodies were generated using human TSLP and cynomolgus monkey (hereafter referred to as “cyno”) TSLP as immunogens. Single cell suspensions of lymphocytes were obtained from the spleens and lymph nodes of immunized mice after the individual animals had been determined to have suitable antibody titers. Lymphocytes were fused with murine myeloma cells by standard methods to generate hybridomas. Hybridoma supernatants were screened by ELISA for binding to human TSLP and cyno TSLP. Hybridomas that showed binding to human and cyno TSLP were further screened by reporter cell assay for anti-TSLP blocking antibodies.

[0152] *TSLP reporter cell assay:* The TSLP reporter cell assay determined the degree of blockage of TSLP by anti-TSLP antibodies in STAT5-luciferase HEK-293 reporter cells co-expressing TSLPR/IL-7R α , wherein increased STAT5 signaling induces an increase in luciferase production. Binding of TSLP to the TSLP receptor complex is known to activate the STAT5 signaling pathway. Thus, by measuring luciferase activity to monitor activation of the STAT5 signaling pathway, the degree of TSLP blockage can be determined. The STAT5-luciferase HEK-293 reporter cells with TSLPR/IL-7R α co-expression were simultaneously incubated with 10 ng/mL human TSLP and the anti-TSLP antibodies described herein with a

series of dilutions at 37°C for 5 hrs. After 5 hrs of co-incubation, the bioluminescence signal was measured. **FIG. 1** shows the inhibition percentage of luciferase (IC₉₀) for the reporter cells as analyzed using GraphPad Prism, demonstrating that all exemplary anti-TSLP antibody clones were effective at blocking the binding of free TSLP to TSLP receptor complex.

Example 2: Measurements of Binding Affinities for Exemplary Anti-TSLP Antibodies.

[0153] Recombinant chimeric anti-TSLP monoclonal antibodies (mAbs) comprising human constant domains were constructed based on the exemplary mouse anti-TSLP mAbs. Both exemplary mouse anti-TSLP mAbs and chimeric anti-TSLP mAbs were purified.

[0154] Surface plasmon resonance (SPR) measurement of the binding affinities of exemplary hybridoma antibodies targeting TSLP were performed using Biacore.

[0155] In brief, purified antibodies were captured with an anti-Fc antibody chip (i.e., anti-human Fc for recombinant antibodies with human Fc, and anti-mouse Fc for hybridoma or recombinant mouse antibodies). Antigen was flown over the chip at different concentrations (dilution series at 1:3 starting from 48 nM to 0.2 nM). Captured antigens were disassociated from the chip for 900 to 1200 sec. Sensorgrams were fitted to 1:1 binding model. Results are summarized in **Tables 2-4** below.

[0156] As can be seen from **Tables 2-4**, all tested exemplary mouse anti-TSLP mAbs demonstrated very strong binding to both human and cyno TSLP (higher binding towards human TSLP). Further, chimerization did not greatly affect the binding affinity of anti-TSLP antibodies (e.g., compare 51A4 and Ch51A4).

Table 2. Anti-TSLP binding affinity results for purified antibodies from mouse hybridoma.

Clone name	human ka (1/Ms)	human kd (1/s)	human KD (M)	cyno ka (1/Ms) ⁻¹	cyno kd (1/s) ⁻¹	cyno KD (M)
51B2	1.07E+07	2.05E-05	1.92E-12	8.44E+06	1.03E-03	1.22E-10
51A4	1.19E+07	6.34E-05	5.31E-12	9.37E+06	1.54E-03	1.65E-10
51A1	1.19E+07	9.98E-05	8.38E-12	9.48E+06	2.39E-03	2.52E-10
51A6	1.48E+07	1.52E-04	1.02E-11	1.10E+07	2.21E-03	2.01E-10
51A3	1.26E+07	1.70E-04	1.35E-11	1.05E+07	2.11E-03	2.02E-10

Table 3. Human TSLP binding affinity results for purified recombinant chimeric antibodies.

Name	ka (1/Ms)	kd (1/s)	KD (M)
Ch54A2 mAb	8.63E+06	3.50E-05	4.05E-12
Ch54C4 mAb	4.89E+06	2.61E-05	5.35E-12
Ch51A4 mAb	4.50E+06	5.78E-04	1.28E-10

Table 4. Cynomolgus monkey TSLP binding affinity results for purified recombinant chimeric antibodies.

Capture 1 Solution	ka (1/Ms)	kd (1/s)	KD (M)
Ch51A4 mAb	1.13E+07	1.73E-03	1.53E-10
Ch54A2 mAb	6.32E+06	1.87E-02	2.97E-09
Ch54C4 mAb	4.62E+05	3.29E-03	7.13E-09

[0157] *Determination of 51B2 binding epitope and potency compared to reference anti-TSLP antibody:* Varying concentrations of 51B2 and a US-FDA approved anti-TSLP reference antibody #1 (“Ref. Ab. #1”) were pre-incubated with biotinylated TSLP for 2 hours before being added onto an ELISA plate pre-coated with Ref. Ab. #1 to assess the cross-competition between Ref. Ab. #1 and 51B2. An inert negative control antibody (Ab) was used as a non-competing Ab. After brief incubation and washing of the ELISA plates, biotin-TSLP captured on the plate was detected by HRP-streptavidin. The results in **FIG. 2** show that the inert negative control Ab did not bind to TSLP and that Ref. Ab. #1 completely competed with itself for TSLP binding. **FIG. 2** demonstrates that 51B2 does not completely compete with and prevent Ref. Ab. #1 from binding to TSLP. These results show that 51B2 has a unique binding epitope different from Ref. Ab. #1.

[0158] The potency of 51B2 to inhibit TSLP signaling in primary human cells was tested in human PBMCs and in isolated CD1c+ dendritic cells. In brief, human PBMCs and isolated CD1c+ dendritic cells were seeded in 96-well plates and incubated with either 50 ng/ml or 5 ng/ml TSLP respectively, in the presence of 51B2 or Ref. Ab #1 at multiple concentrations. Cell culture supernatants were collected either 48 hours (PBMC) or 24 hours (dendritic cells) later and analyzed for the secretion of TSLP-induced CCL17 using a commercially available ELISA kit as per manufacturer’s protocol. Increased TSLP signaling induces increased levels of secreted CCL17, so the reduction or inhibition of CCL17 secretion by pro-inflammatory cells that express the TSLP receptor when treated with TSLP cytokine acts as a readout for inhibition of TSLP binding to the TSLPR complex. Data were analyzed and presented using GraphPad Prism. As shown in **FIG. 3A** (human PBMCs) and in **FIG. 3B** (CD1c+ dendritic cells), 51B2 displayed much stronger inhibition of CCL17 both in human PBMCs and in isolated CD1c+ dendritic cells across every tested antibody concentration compared to Ref. Ab. #1. Thus, these results demonstrate that 51B2 is much more potent and effective in blocking TSLP signaling in pro-inflammatory immune cells than the US-FDA approved Ref. Ab. #1.

[0159] *Humanization of 51B2 and humanized 51B2 variants:* The parental clone, 51B2, was humanized by grafting the CDRs from the heavy chain (HC) and light chain (LC) onto human HC and LC framework sequences. Briefly, HC and LC CDRs of chimeric 51B2 were grafted onto the closest HC and LC human germlines. Sequences were visually inspected to identify framework residues that are important for CDR binding and structure stability. These framework residues were mutated singly or in combination. See SEQ ID NOs:51-54 for VL sequences and SEQ ID NOs:55-64 for VH sequences. Humanized variants that retained binding and stability were selected as the final lead clones.

[0160] Humanized 51B2 antibodies were tested for binding affinity using Biacore and ranked by activity (similar to the TSLP-reporter assay described in Example 1) and expression quality/purification behavior. Based on Biacore binding results (see **Tables 5-6**) and results from the activity assay (see **FIG. 4** and **FIG. 5A**), the humanized 51B2 antibody, L3H9 (i.e., with VL-3 (SEQ ID NO:53) and VH-9 (SEQ ID NO:63) pairing), was selected as the lead humanized 51B2 clone.

[0161] *Humanized 51B2 variants binding to huTSLP in Biacore:* Purified humanized or chimeric antibodies were captured with an anti-human Fc antibody chip, and antigen (human TSLP) at different concentrations (dilution series at 1:3 starting from 48 nM to 0.2 nM) was flown over the chip with the captured antibodies, as described above. Captured antigens then were disassociated from the chip for 1200 sec. Sensorgrams were fitted to 1:1 binding model and summarized in **Table 5** below.

[0162] As shown in **Table 5**, almost all humanized 51B2 variants retained similar or even exhibited stronger binding affinity to human TSLP, except for L1H2 and L1H9 variants.

Table 5. Humanized 51B2 variant binding affinities to human TSLP.

LC	HC	ka (1/Ms)	kd (1/s)	KD (M)
Ch51B2	Ch51B2	4.37E+07	2.41E-04	5.52E-12
L1	H1	1.20E+07	3.33E-04	2.78E-11
L1	H2	7.05E+06	2.64E-01	3.75E-08
L1	H9	1.05E+07	2.98E-01	2.85E-08
L1	H10	5.11E+08	1.78E-03	3.49E-12
L2	H1	2.76E+11	1.21E+00	4.38E-12
L2	H2	4.35E+07	3.31E-03	7.62E-11
L2	H9	5.27E+07	6.51E-04	1.24E-11
L3	H3	5.81E+07	1.40E-02	2.41E-10
L3	H4	8.68E+07	1.20E-03	1.39E-11
L3	H8	1.80E+08	1.04E-03	5.78E-12
L3	H9	8.67E+07	1.07E-04	1.24E-12
L3	H10	5.13E+08	3.36E-04	6.54E-13
L4	H1	8.36E+06	2.38E-04	2.85E-11
L4	H7	7.81E+06	2.78E-04	3.55E-11

L4	H8	5.41E+06	1.68E-06	3.11E-13
L4	H9	5.41E+07	4.23E-04	7.82E-12
L4	H10	4.27E+07	1.88E-04	4.41E-12

[0163] *Activity characterization of anti-TSLP antibody hz51B2 L3H9*: The binding affinity of hz51B2-L3H9 for human and cyno TSLP were measured on Biacore T200 using an anti-human Fc capture chip for antibody capture and purified TSLP at different concentrations as the analyte. The binding sensorgrams were fitted to a 1:1 binding kinetic model. The binding constants of on-rate, off-rate, and Equilibrium binding KD are summarized in **Table 6** below.

Table 6. Binding affinity of hz51B2 L3H9 antibody to human and cynomolgus monkey TSLP.

Antibody	Antigen	ka (1/Ms)	kd (1/s)	KD (M)
hz51B2 L3H9	huTSLP	8.67E+07	1.07E-04	1.24E-12*
hz51B2 L3H9	cyno TSLP	1.20E+08*	1.04E-02	8.66E-11

[0164] The results summarized in **Table 6** demonstrate strong binding of humanized (hz)51B2 L3H9 antibody both to human TSLP and to cynomolgus monkey TSLP, which is beneficial for extrapolating the results from toxicity and efficacy studies performed in cynomolgus monkeys to human clinical studies for evaluating the anti-TSLP antibodies or constructs comprising thereof (such as any of the multispecific anti-TSLP antibody constructs described herein).

[0165] The binding of hz51B2 L3H9 to human TSLP was characterized by measuring the concentration of free-TSLP in the equilibrium binding reaction mixture using a BiacoreT200 instrument. Briefly, 1 nM human-TSLP was incubated overnight at room temperature with 3× serial dilutions of hz51B2 L3H9 solutions ranging from 96 nM to 44 pM of Fab arms in a PBS solution containing 0.1 mg/ml BSA and 0.005% polysorbate 20. Unbound TSLP in each binding reaction was measured by injecting binding solutions over the amine-immobilized hz51B2 L3H9 surface. The percentage of free TSLP concentration in each reaction mixture was fitted to a 1:1 equilibrium Ab-Ag binding model using GraphPad, and KD was derived from the curve fit (**FIG. 5B**).

[0166] To summarize, the lead 51B2 antibodies (e.g., hz51B2 Abs, such as hz51B2 L3H9) are tight binders for human TSLP with a KD of < 10 pM. Without being bound by theory, such a property enables the complete neutralization of TSLP-induced CCL17 in the PBMC and dendritic cell assays (see **FIGs. 3A-3B**), compared to the incomplete inhibition of TSLP-induced CCL17 by the US-FDA approved Ref. Ab. #1.

[0167] All references mentioned in the present invention are incorporated herein by reference as if each of those references has been incorporated by reference individually. Although the description referred to particular embodiments, it will be clear to a person skilled in the art that the present invention may be practiced with variation of these specific details. Hence this invention should not be construed as limited to the embodiments set forth herein.

SEQUENCE TABLE

SEQ ID NO:	Description	Sequence
1	Anti-TSLP 51A1 VH	<u>QVQLQQSGAELVRPGASVKLSCKASGYMFTDYAMHWVKQTPVHGLEWIGTIDPETGGTAYTQKFKGKATLTADISSSTAYVELRSLTSEDSAVYYCTREGSYGGYVDYWGQGTTLSVSS</u>
2	Anti-TSLP 51A1/51A4 CDR-H1	DYAMH
3	Anti-TSLP 51A1/51A4 CDR-H2	TIDPETGGTAYTQKFKG
4	Anti-TSLP 51A1/51A4/51A3/51A6 CDR-H3	EGSYGGYVDY
5	Anti-TSLP 51A1 VL	<u>DIVMTQSQKFMSTSVGDRVNITCKASQNVHTAVAWYQQKPGQSPKTLIFLASNRHTGVPDRFTGSGSGTDFLTISNVQSEDLADYFCLQLWNYPYTFGGGTKLEIK</u>
6	Anti-TSLP 51A1/51A4/51A3/51A6 CDR-L1	KASQNVHTAVA
7	Anti-TSLP 51A1/51A4/51A3/51A6 CDR-L2	LASNRHT
8	Anti-TSLP 51A1/51A4/51A3/51A6 CDR-L3	LQLWNYPYT
9	Anti-TSLP 51A4 VH	<u>QVQLQQSGAELVRPGASVKLSCKASGYMFTDYAMHWVKQTPVHGLEWIGTIDPETGGTAYTQKFKGKATLTADISSNTAYVELRSLTSEDSAVYYCTREGSYGGYVDYWGQGTTLSVSS</u>
10	Anti-TSLP 51A4 VL	<u>DIVMTQSQKFMSTSVGDRVSITCKASQNVHTAVAWYQQKPGQSPKTLIFLASNRHTGVPDRFTGSGSGTDFLTISNVQSEDLADYFCLQLWNYPYTFGGGTKLEIK</u>
11	Anti-TSLP 51A6 VH Anti-TSLP 51A3 VH	<u>QVQLQQSGAELVRPGASVKLSCKASGYTFTDYAMHWVKQTLVHGLEWVGTIDPETGGTAYTQKFOGKATLTADVSSSTAYVELRSLTSEDSAVYYCTREGSYGGYVDYWGQGTTLSVSS</u>
12	Anti-TSLP 51A3/51A6 CDR-H1	DYAMH
13	Anti-TSLP 51A3/51A6 CDR-H2	TIDPETGGTAYTQKFQG
14	Linker, wherein n is an integer of at least 1	(GSGGS) _n
15	Anti-TSLP 51A6 VL Anti-TSLP 51A3 VL	<u>DIVMTQSQKFMSTSEGDRVSITCKASQNVHTAVAWYQQKPGQSPKTLIFLASNRHTGVPDRFTGSGSGTDFLTISNVQSEDLADYFCLQLWNYPYTFGGGTKLEIK</u>
16	Linker, wherein n is an integer of at least 1	(GGGS) _n
17	Linker, wherein n is an integer of at least 1	(GGGGS) _n
18	Linker	GGSG

SEQ ID NO:	Description	Sequence
19	Anti-TSLP 51B2 VH	<u>QVQLLP</u> PGTELVKPGASVKLSCKASGYTFTSYLMHWVKQRPGQGLEWIGEIDP <u>SDSYTDYNQNFRG</u> KATLTVDMSSTAYMQLSSLTSEDSAVYYCALT <u>TGTINYFD</u> <u>YWGQG</u> TTLTVSS
20	Anti-TSLP 51B2 CDR-H1	SYLMH
21	Anti-TSLP 51B2 CDR-H2	EIDPSDSYTDYNQNFRG
22	Anti-TSLP 51B2 CDR-H3	TGTINYFDY
23	Anti-TSLP 51B2 VL	<u>DIVMTQSHKFMSTSVGDRVNITCKASQDVVTAVAWYQQKPGQSPKLLIY</u> WAS <u>MRHTGVPDRFTGSGSGTDFLT</u> ISNVQSEDLADYFCQQYSSFTFTFGSGTKLEIK
24	Anti-TSLP 51B2 CDR-L1	KASQDVVTAVA
25	Anti-TSLP 51B2 CDR-L2	WASMRHT
26	Anti-TSLP 51B2 CDR-L3	QQYSSFTFT
27	Anti-TSLP 54A2 VH	<u>QVQLRESGPGLVAPSQSL</u> SITCTVSGFSLTSYGVNWVRQPPGKGLEWLGVWID <u>DGTINYHSAFISRLSITKDNSKSQVFLK</u> NSLQTDDTATYYCAK <u>GSIIWYFDV</u> <u>WGAGTT</u> TVTVSS
28	Anti-TSLP 54A2 CDR-H1	SYGVN
29	Anti-TSLP 54A2 CDR-H2	VIWDDGTTNYHSAFIS
30	Anti-TSLP 54A2 CDR-H3	GSIIWYFDV
31	Anti-TSLP 54A2 VL	<u>DIVLTQSPASLAVSLGQRATISCKASQSV</u> DYEGDSHMN <u>WYQQKPGQSPKLLIY</u> <u>VASNLES</u> GIPARFSGSGSGTDFLTNIHPVEEEDAATYYCQQSNEVPFTFGSGTKL EIK
32	Anti-TSLP 54A2 CDR-L1	KASQSVDYEGDSHMN
33	Anti-TSLP 54A2 CDR-L2	VASNLES
34	Anti-TSLP 54A2 CDR-L3	QQSNEVPFT
35	Anti-TSLP 54B3 VH	<u>QVQLQQSGAELVRPGASVKLSCKASGYTFTDYEIH</u> WVRQTPVHGLEWIGLIDP <u>ETGGTAHNQKFKA</u> KATLTVDKSSITAYMELRSLTSEDSAVYYCT <u>REGQLGLSL</u> <u>DYWGQ</u> GTTTLTVSS
36	Anti-TSLP 54B3 CDR-H1	DYEIH
37	Anti-TSLP 54B3 CDR-H2	LIDPETGGTAHNQKFKA
38	Anti-TSLP 54B3 CDR-H3	EGQLGLSLDY
39	Anti-TSLP 54B3 VL	<u>DIVMTQSHKFMSTSVGERVSITCKASQDVGTTVAWYQQKPGQSPKLLIY</u> WTST <u>RHTGVPDRFTGSGSGTDFLT</u> ISNVQSEDLADYFCQQYSTYPLTFGSGTKLEIQ
40	Anti-TSLP 54B3 CDR-L1	KASQDVGTTVA
41	Anti-TSLP 54B3 CDR-L2	WTSTRHT
42	Anti-TSLP 54B3 CDR-L3	QQYSTYPLT
43	Anti-TSLP 54C4 VH	<u>EVQLQQSGPVLVKPGASVKMSCKASGYTFTDYYMN</u> WVKQSHGKSLEWIGVIN <u>PYNADTKFNQRFKDKATLT</u> VESSSTAYMELNSLTSEDSAVYYCARG <u>DDY</u> AEE <u>SFAYW</u> GQGTTLTVSAA

SEQ ID NO:	Description	Sequence
44	Anti-TSLP 54C4 CDR-H1	DYYMN
45	Anti-TSLP 54C4 CDR-H2	VINPYNADTKFNQRFKD
46	Anti-TSLP 54C4 CDR-H3	GDDYAEESFAY
47	Anti-TSLP 54C4 VL	QIVLTQSPSIMSASLGEEITLTC <u>SASSSVSYMH</u> WYQQKSGTSPKLLIYFTSNLASG VPSRFSGSGSGTFTYSLTISSVEAEDAADYYCHQWSSYPTFGGGTKLEIK
48	Anti-TSLP 54C4 CDR-L1	SASSSVSYMH
49	Anti-TSLP 54C4 CDR-L2	FTSNLAS
50	Anti-TSLP 54C4 CDR-L3	HQWSSYPT
51	Anti-TSLP hz51B2 VL-1 (CDRs underlined)	DIQLTQSPSFLSASVGDRTTITCKASQDVVTAVAWYQQKPGKAPKLLIYWASMRHTGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQYSSFTFTFGGQTKLEIK
52	Anti-TSLP hz51B2 VL-2 (CDRs underlined)	DIQLTQSPSFLSASVGDRTTITCKASQDVVTAVAWYQQKPGKAPKLLIYWASMRHTGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQYSSFTFTFGGQTKLEIK
53	Anti-TSLP hz51B2 VL-3 (CDRs underlined)	DIQMTQSPSFLSASVGDRTTITCKASQDVVTAVAWYQQKPGKAPKLLIYWASMRHTGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQYSSFTFTFGGQTKLEIK
54	Anti-TSLP hz51B2 VL-4 (CDRs underlined)	DIQMTQSPSFLSASVGDRTTITCKASQDVVTAVAWYQQKPGKAPKLLIYWASMRHTGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQYSSFTFTFGGQTKLEIK
55	Anti-TSLP hz51B2 VH-1 (CDRs underlined)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYLMHWVRQAPGQGLEWMGEIDPDSYTDYNQNFGRVMTMTDSTSTVYMESSLRSEDATVYYCALTGTINYFDYWGQGTLLTVSS
56	Anti-TSLP hz51B2 VH-2 (CDRs underlined)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYLMHWVRQAPGQGLEWMGEIDPDSYTDYNQNFGRVMTMTDSTSTVYMESSLRSEDATVYYCALTGTINYFDYWGQGTLLTVSS
57	Anti-TSLP hz51B2 VH-3 (CDRs underlined)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYLMHWVRQAPGQGLEWIGEIDPDSYTDYNQNFGRVMTMTDSTSTVYMESSLRSEDATVYYCALTGTINYFDYWGQGTLLTVSS
58	Anti-TSLP hz51B2 VH-4 (CDRs underlined)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYLMHWVRQAPGQGLEWMGEIDPDSYTDYNQNFGRVTLTVDMSTSTVYMESSLRSEDATVYYCALTGTINYFDYWGQGTLLTVSS
59	Anti-TSLP hz51B2 VH-5 (CDRs underlined)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYLMHWVRQAPGQGLEWIGEIDPDSYTDYNQNFGRVTLTVDMSTSTVYMESSLRSEDATVYYCALTGTINYFDYWGQGTLLTVSS
60	Anti-TSLP hz51B2 VH-6 (CDRs underlined)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYLMHWVRQAPGQGLEWMGEIDPDSYTDYNQNFGRATMTVDMSTSTVYMESSLRSEDATVYYCALTGTINYFDYWGQGTLLTVSS
61	Anti-TSLP hz51B2 VH-7 (CDRs underlined)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYLMHWVRQAPGQGLEWIGEIDPDSYTDYNQNFGRATMTVDMSTSTVYMESSLRSEDATVYYCALTGTINYFDYWGQGTLLTVSS
62	Anti-TSLP hz51B2 VH-8 (CDRs underlined)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYLMHWVRQAPGQGLEWIGEIDPDSYTDYNQNFGRATLTVDMSTSTVYMESSLRSEDATVYYCALTGTINYFDYWGQGTLLTVSS
63	Anti-TSLP hz51B2 VH-9 (CDRs underlined)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYLMHWVRQAPGQRLWWMGEIDPDSYTDYNQNFGRVTTITVDMSTSTVYMESSLRSEDATVYYCALTGTINYFDYWGQGTLLTVSS

SEQ ID NO:	Description	Sequence
64	Anti-TSLP hz51B2 VH-10 (CDRs underlined)	QVQLVQSGAEVKKPGASVKVSKASGYTFTSYLMHWVRQAPGQRLEWIGEID PSDSYTDYNQNFGRVRTITVDMASASTAYMELSSLRSEDTAVYYCALTGTINYFD YWGQGTLVTVSS
73	CL kappa wt	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKDSYSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
74	CL lambda-2 wt	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVE TTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
75	CL lambda-1 wt	GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADGSPVKAGV ETT K PSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
76	Human IgG1 Fc	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKF NWFYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHN HYTQKSLSLSPGK
77	IgG1 Fc-LALA	DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVK FNWFYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEAL HNHYTQKSLSLSPGK
78	IgG1 Fc-LALA-LS	DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVK FNWFYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVLHEALH SHYTQKSLSLSPGK
79	IgG1 Fc-LALA-YTE	DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLYITREPEVTCVVDVSHEDPEVK FNWFYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEAL HNHYTQKSLSLSPGK
80	IgG1 Fc-LALA-LS-hole	DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVK FNWFYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLSCAVKGFYPSDIAVE WESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVLHEALH SHYTQKSLSLSPGK
81	IgG1 Fc-LALA-LS-knob	DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVK FNWFYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLWCLVKGFYPSDIAV EWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVLHEAL HSHYTQKSLSLSPGK
82	Human IgG1	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWFYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA PIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYT QKSLSLSPGK*
83	IgG1 LALA	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT CPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWFYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA PIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYT QKSLSLSPGK
84	IgG1 LALA-LS	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT CPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWFYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA

SEQ ID NO:	Description	Sequence
		PIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCSVLHEALHSHYTQKSLSLSPGK
85	IgG1 LALA-LS-knob	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCSVLHEALHSHYTQKSLSLSPGK
86	IgG1 LALA-LS-hole	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCSVLHEALHSHYTQKSLSLSPGK
95	IgG1 Fc-LALA-h	DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCSVLHEALHSHYTQKSLSLSPGK
96	IgG1 Fc-LALA-k	DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCSVLHEALHSHYTQKSLSLSPGK
97	Linker	PLGLAG
98	Linker 1	GGGG
99	Linker 2	GGGGSGGGGSGGGGS
103	Anti-TSLP hz51B2-LC3 (CL kappa wt) (VL italicized and underlined)	<u>DIQMTQSPSFLSASVGDRTITCKASQDVVTAVAWYQOKPGKAPKLLIYWASMRHTGVPSRFGSGSGTEFTLTIS</u> <u>LOPEDFATYYCOOYSSFTFTFGOGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC</u>
104	Anti-TSLP hz51B2-HC9 (IgG1 LALA) (VH italicized and underlined)	<u>QVQLVQSGAEVKKPGASVKVSCKASGYFTSYLMHWVRQAPGORLEWMGEIDPSD</u> <u>SYTDYNQNFGRVITITVDM</u> <u>SASTAYMELSSLRSEDTAVYYCALTGTIN</u> <u>YFDYWGOGLVT</u> <u>VSS</u> ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCSVLHEALHSHYTQKSLSLSPGK
105	Anti-TSLP hz51B2-HC9 (IgG1 LALA-YTE) (VH italicized and underlined)	<u>QVQLVQSGAEVKKPGASVKVSCKASGYFTSYLMHWVRQAPGORLEWMGEIDPSD</u> <u>SYTDYNQNFGRVITITVDM</u> <u>SASTAYMELSSLRSEDTAVYYCALTGTIN</u> <u>YFDYWGOGLVT</u> <u>VSS</u> ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCSVLHEALHSHYTQKSLSLSPGK
106	Anti-TSLP hz51B2-HC9LC3 scFv-stab (linker bolded)	QVQLVQSGAEVKKPGASVKVSCKASGYFTSYLMHWVRQAPGQCLEWMGEIDPSDSYTDYNQNFGRVITITVDM SASTAYMELSSLRSEDTAVYYCALTGTIN YFDYWGOGLVT VSSGGGGSGGGGSGGGGSD IQMTQSPSFLSASVGDRTITCKASQDVVTAVAWYQOKPGKAPKLLIYWASMRHTGVPSRFGSGSGTEFTLTISLQPEDFATYYCQOYSSFTFTFGCGTKLEIK

SEQ ID NO:	Description	Sequence
107	Anti-TSLP hz51B2-HC9 LC4 scFv-stab (linker bolded)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYLMHWVRQAPGQ C LEWMGEI DPSDSYTDYNQNFGRVTTITVDMASASTAYMELSSLRSEDTAVYYCALTGTINYF DYWGQGTLLTVSS GGGGSGGGGSGGGG SDIQMTQSPSFLSASVGDRVTTITCK ASQDVVTAVAWYQQKPGKAPKLLIYWASMRHTGVPSPRFSGSGSGTEFTLTIS LQPEDFATYFCQQYSSFTFTFG C GTKLEIK
109	Anti-TSLP hz51B2-HC9 VH-CH1 (VH italicized and underlined)	<u><i>QVOLVQSGAEVKKPGASVKVSCKASGYTFTSYLMHWVRQAPGORLEWMGEIDPSD</i></u> <u><i>SYTDYNQNFGRVTTITVDMASASTAYMELSSLRSEDTAVYYCALTGTINYFDYWGGQGT</i></u> <u><i>LVTVSS</i></u> ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
136	Anti-TSLP hz51B2-HC9-IgG1 Fc LALA-LS-knob (VH underlined and italicized; mutations underlined and bolded)	<u><i>QVOLVQSGAEVKKPGASVKVSCKASGYTFTSYLMHWVRQAPGORLEWMGEIDPSD</i></u> <u><i>SYTDYNQNFGRVTTITVDMASASTAYMELSSLRSEDTAVYYCALTGTINYFDYWGGQGT</i></u> <u><i>LVTVSS</i></u> ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD KTHTCPPCPAPE AA GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTIKAKGQPREPQVYTLPPSREEMTKNQVSL W CLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLY S SKLTVDKSRWQQGNVFCSCSV L HEALH S HYTQKSLSLSPGK
137	Anti-TSLP hz51B2-HC9-IgG1 Fc LALA-LS-hole (VH underlined and italicized; mutations underlined and bolded)	<u><i>QVOLVQSGAEVKKPGASVKVSCKASGYTFTSYLMHWVRQAPGORLEWMGEIDPSD</i></u> <u><i>SYTDYNQNFGRVTTITVDMASASTAYMELSSLRSEDTAVYYCALTGTINYFDYWGGQGT</i></u> <u><i>LVTVSS</i></u> ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD KTHTCPPCPAPE AA GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTIKAKGQPREPQVYTLPPSREEMTKNQVSL SCA VKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFL Y SKLTVDKSRWQQGNVFCSCSV L HEALH S HYTQKSLSLSPGK
158	Human IgG1 CH1 domain	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
163	Linker	GGSGG
164	Linker	GSGSG
165	Linker	GSGGG
166	Linker	GGGSG
167	Linker	GSSSG
168	Linker	GSGGS
169	Linker	SGGGGS
170	Linker	GRAGGGGAGGGG
171	Linker	GRAGGG
172	Linker	GGGGS
188	Anti-TSLP HC9-stab. mut. VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYLMHWVRQAPGQ C LEWMGEI DPSDSYTDYNQNFGRVTTITVDMASASTAYMELSSLRSEDTAVYYCALTGTINYF DYWGQGTLLTVSS
189	Anti-TSLP LC3-stab. mut. VL	DIQMTQSPSFLSASVGDRVTTITCKASQDVVTAVAWYQQKPGKAPKLLIYWASMRHTGVPSPRFSGSGSGTEFTLTISLQPEDFATYFCQQYSSFTFTFGCGTKLEIK
190	Anti-TSLP LC4-stab. mut. VL	DIQMTQSPSFLSASVGDRVTTITCKASQDVVTAVAWYQQKPGKAPKLLIYWASMRHTGVPSPRFSGSGSGTEFTLTISLQPEDFATYFCQQYSSFTFTFGCGTKLEIK
191	Anti-TSLP hz51B2-HC9 LC3 scFv no SS stabilization (linker bolded)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYLMHWVRQAPGQ R LEWMGEI DPSDSYTDYNQNFGRVTTITVDMASASTAYMELSSLRSEDTAVYYCALTGTINYF DYWGQGTLLTVSS GGGGSGGGGSGGGG SDIQMTQSPSFLSASVGDRVTTITCK ASQDVVTAVAWYQQKPGKAPKLLIYWASMRHTGVPSPRFSGSGSGTEFTLTIS LQPEDFATYFCQQYSSFTFTFG C GTKLEIK
192	Anti-TSLP hz51B2-HC9 LC4 scFv no SS stabilization (linker bolded)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYLMHWVRQAPGQ R LEWMGEI DPSDSYTDYNQNFGRVTTITVDMASASTAYMELSSLRSEDTAVYYCALTGTINYF DYWGQGTLLTVSS GGGGSGGGGSGGGG SDIQMTQSPSFLSASVGDRVTTITCK

SEQ ID NO:	Description	Sequence
		ASQDVVTAVAWYQQKPGKAPKLLIYWASMRHTGVPSRFSGSGSGTEFTLTSS LQPEDFATYFCQQYSSFTFTFGQGTKLEIK

CLAIMS

What is claimed is:

1. An isolated antibody construct (“anti-TSLP antibody construct”) comprising an antibody moiety that specifically recognizes thymic stromal lymphopoietin (TSLP; “anti-TSLP antibody moiety”), wherein the anti-TSLP antibody moiety comprises a heavy chain variable region (VH) and a light chain variable region (VL), and wherein:
 - (a) the VH comprises (i) a CDR-H1 comprising an amino acid sequence of SEQ ID NO:2, or a variant thereof comprising up to 3 amino acid variations; (ii) a CDR-H2 comprising an amino acid sequence of SEQ ID NO:3, or a variant thereof comprising up to 3 amino acid variations; and (iii) a CDR-H3 comprising an amino acid sequence of SEQ ID NO:4, or a variant thereof comprising up to 3 amino acid variations; and the VL comprises (i) a CDR-L1 comprising an amino acid sequence of SEQ ID NO:6, or a variant thereof comprising up to 3 amino acid variations; (ii) a CDR-L2 comprising an amino acid sequence of SEQ ID NO:7, or a variant thereof comprising up to 3 amino acid variations; and (iii) a CDR-L3 comprising an amino acid sequence of SEQ ID NO:8, or a variant thereof comprising up to 3 amino acid variations;
 - (b) the VH comprises (i) a CDR-H1 comprising an amino acid sequence of SEQ ID NO:12, or a variant thereof comprising up to 3 amino acid variations; (ii) a CDR-H2 comprising an amino acid sequence of SEQ ID NO:13, or a variant thereof comprising up to 3 amino acid variations; and (iii) a CDR-H3 comprising an amino acid sequence of SEQ ID NO:4, or a variant thereof comprising up to 3 amino acid variations; and the VL comprises (i) a CDR-L1 comprising an amino acid sequence of SEQ ID NO:6, or a variant thereof comprising up to 3 amino acid variations; (ii) a CDR-L2 comprising an amino acid sequence of SEQ ID NO:7, or a variant thereof comprising up to 3 amino acid variations; and (iii) a CDR-L3 comprising an amino acid sequence of SEQ ID NO:8, or a variant thereof comprising up to 3 amino acid variations;
 - (c) the VH comprises (i) a CDR-H1 comprising an amino acid sequence of SEQ ID NO:20, or a variant thereof comprising up to 3 amino acid variations; (ii) a CDR-H2 comprising an amino acid sequence of SEQ ID NO:21, or a variant thereof comprising up to 3 amino acid variations; and (iii) a CDR-H3 comprising an amino acid sequence of SEQ ID NO:22, or a variant thereof comprising up to 3 amino acid variations; and the VL comprises (i) a CDR-L1 comprising an amino acid sequence of SEQ ID NO:24, or a variant thereof comprising up to 3 amino acid variations; (ii) a CDR-L2 comprising an amino acid sequence

of SEQ ID NO:25, or a variant thereof comprising up to 3 amino acid variations; and (iii) a CDR-L3 comprising an amino acid sequence of SEQ ID NO:26, or a variant thereof comprising up to 3 amino acid variations;

(d) the VH comprises (i) a CDR-H1 comprising an amino acid sequence of SEQ ID NO:28, or a variant thereof comprising up to 3 amino acid variations; (ii) a CDR-H2 comprising an amino acid sequence of SEQ ID NO:29, or a variant thereof comprising up to 3 amino acid variations; and (iii) a CDR-H3 comprising an amino acid sequence of SEQ ID NO:30, or a variant thereof comprising up to 3 amino acid variations; and the VL comprises (i) a CDR-L1 comprising an amino acid sequence of SEQ ID NO:32, or a variant thereof comprising up to 3 amino acid variations; (ii) a CDR-L2 comprising an amino acid sequence of SEQ ID NO:33, or a variant thereof comprising up to 3 amino acid variations; and (iii) a CDR-L3 comprising an amino acid sequence of SEQ ID NO:34, or a variant thereof comprising up to 3 amino acid variations;

(e) the VH comprises (i) a CDR-H1 comprising an amino acid sequence of SEQ ID NO:36, or a variant thereof comprising up to 3 amino acid variations; (ii) a CDR-H2 comprising an amino acid sequence of SEQ ID NO:37, or a variant thereof comprising up to 3 amino acid variations; and (iii) a CDR-H3 comprising an amino acid sequence of SEQ ID NO:38, or a variant thereof comprising up to 3 amino acid variations; and the VL comprises (i) a CDR-L1 comprising an amino acid sequence of SEQ ID NO:40, or a variant thereof comprising up to 3 amino acid variations; (ii) a CDR-L2 comprising an amino acid sequence of SEQ ID NO:41, or a variant thereof comprising up to 3 amino acid variations; and (iii) a CDR-L3 comprising an amino acid sequence of SEQ ID NO:42, or a variant thereof comprising up to 3 amino acid variations; or

(f) the VH comprises (i) a CDR-H1 comprising an amino acid sequence of SEQ ID NO:44, or a variant thereof comprising up to 3 amino acid variations; (ii) a CDR-H2 comprising an amino acid sequence of SEQ ID NO:45, or a variant thereof comprising up to 3 amino acid variations; and (iii) a CDR-H3 comprising an amino acid sequence of SEQ ID NO:46, or a variant thereof comprising up to 3 amino acid variations; and the VL comprises (i) a CDR-L1 comprising an amino acid sequence of SEQ ID NO:48, or a variant thereof comprising up to 3 amino acid variations; (ii) a CDR-L2 comprising an amino acid sequence of SEQ ID NO:49, or a variant thereof comprising up to 3 amino acid variations; and (iii) a CDR-L3 comprising an amino acid sequence of SEQ ID NO:50, or a variant thereof comprising up to 3 amino acid variations.

2. The isolated anti-TSLP antibody construct of claim 1, wherein:

(a) the VH comprises (i) a CDR-H1 comprising an amino acid sequence of SEQ ID NO:2; (ii) a CDR-H2 comprising an amino acid sequence of SEQ ID NO:3; and (iii) a CDR-H3 comprising an amino acid sequence of SEQ ID NO:4; and the VL comprises (i) a CDR-L1 comprising an amino acid sequence of SEQ ID NO:6; (ii) a CDR-L2 comprising an amino acid sequence of SEQ ID NO:7; and (iii) a CDR-L3 comprising an amino acid sequence of SEQ ID NO:8;

(b) the VH comprises (i) a CDR-H1 comprising an amino acid sequence of SEQ ID NO:12; (ii) a CDR-H2 comprising an amino acid sequence of SEQ ID NO:13; and (iii) a CDR-H3 comprising an amino acid sequence of SEQ ID NO:4; and the VL comprises (i) a CDR-L1 comprising an amino acid sequence of SEQ ID NO:6; (ii) a CDR-L2 comprising an amino acid sequence of SEQ ID NO:7; and (iii) a CDR-L3 comprising an amino acid sequence of SEQ ID NO:8;

(c) the VH comprises (i) a CDR-H1 comprising an amino acid sequence of SEQ ID NO:20; (ii) a CDR-H2 comprising an amino acid sequence of SEQ ID NO:21; and (iii) a CDR-H3 comprising an amino acid sequence of SEQ ID NO:22; and the VL comprises (i) a CDR-L1 comprising an amino acid sequence of SEQ ID NO:24; (ii) a CDR-L2 comprising an amino acid sequence of SEQ ID NO:25; and (iii) a CDR-L3 comprising an amino acid sequence of SEQ ID NO:26;

(d) the VH comprises (i) a CDR-H1 comprising an amino acid sequence of SEQ ID NO:28; (ii) a CDR-H2 comprising an amino acid sequence of SEQ ID NO:29; and (iii) a CDR-H3 comprising an amino acid sequence of SEQ ID NO:30; and the VL comprises (i) a CDR-L1 comprising an amino acid sequence of SEQ ID NO:32; (ii) a CDR-L2 comprising an amino acid sequence of SEQ ID NO:33; and (iii) a CDR-L3 comprising an amino acid sequence of SEQ ID NO:34;

(e) the VH comprises (i) a CDR-H1 comprising an amino acid sequence of SEQ ID NO:36; (ii) a CDR-H2 comprising an amino acid sequence of SEQ ID NO:37; and (iii) a CDR-H3 comprising an amino acid sequence of SEQ ID NO:38; and the VL comprises (i) a CDR-L1 comprising an amino acid sequence of SEQ ID NO:40; (ii) a CDR-L2 comprising an amino acid sequence of SEQ ID NO:41; and (iii) a CDR-L3 comprising an amino acid sequence of SEQ ID NO:42; or

(f) the VH comprises (i) a CDR-H1 comprising an amino acid sequence of SEQ ID NO:44; (ii) a CDR-H2 comprising an amino acid sequence of SEQ ID NO:45; and (iii) a CDR-H3 comprising an amino acid sequence of SEQ ID NO:46; and the VL comprises (i) a

CDR-L1 comprising an amino acid sequence of SEQ ID NO:48; (ii) a CDR-L2 comprising an amino acid sequence of SEQ ID NO:49; and (iii) a CDR-L3 comprising an amino acid sequence of SEQ ID NO:50.

3. The isolated anti-TSLP antibody construct of claim 1 or 2, wherein:

(a) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:1, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:5;

(b) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:9, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:10;

(c) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:11, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:15;

(d) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:19, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:23;

(e) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:27, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:31;

(f) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:35, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:39;

(g) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:43, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:47;

(h) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:63, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:53;

(i) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:63, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:54;

(j) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:56, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:52;

(k) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:55, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:51;

(l) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:56, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:51;

(m) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:63, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:51;

(n) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:64, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:51;

(o) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:55, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:52;

(p) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:58, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:52;

(q) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:60, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:52;

(r) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:64, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:52;

(s) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:55, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:53;

(t) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:57, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:53;

(u) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:58, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:53;

(v) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:62, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:53;

(w) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:64, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:53;

(x) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:55, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:54;

(y) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:61, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:54;

(z) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:62, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:54;

(aa) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:64, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:54;

(bb) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:63, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:52;

(cc) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:188, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:189; or

(dd) the VH comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:188, and the VL comprises an amino acid sequence having at least about 80% sequence identity with SEQ ID NO:190.

4. The isolated anti-TSLP antibody construct of any one of claims 1-3, wherein:

(a) the VH comprises an amino acid sequence of SEQ ID NO:1, and the VL comprises an amino acid sequence of SEQ ID NO:5;

(b) the VH comprises an amino acid sequence of SEQ ID NO:9, and the VL comprises an amino acid sequence of SEQ ID NO:10;

(c) the VH comprises an amino acid sequence of SEQ ID NO:11, and the VL comprises an amino acid sequence of SEQ ID NO:15;

(d) the VH comprises an amino acid sequence of SEQ ID NO:19, and the VL comprises an amino acid sequence of SEQ ID NO:23;

(e) the VH comprises an amino acid sequence of SEQ ID NO:27, and the VL comprises an amino acid sequence of SEQ ID NO:31;

(f) the VH comprises an amino acid sequence of SEQ ID NO:35, and the VL comprises an amino acid sequence of SEQ ID NO:39;

(g) the VH comprises an amino acid sequence of SEQ ID NO:43, and the VL comprises an amino acid sequence of SEQ ID NO:47;

(h) the VH comprises an amino acid sequence of SEQ ID NO:63, and the VL comprises an amino acid sequence of SEQ ID NO:53;

(i) the VH comprises an amino acid sequence of SEQ ID NO:63, and the VL comprises an amino acid sequence of SEQ ID NO:54;

(j) the VH comprises an amino acid sequence of SEQ ID NO:56, and the VL comprises an amino acid sequence of SEQ ID NO:52;

(k) the VH comprises an amino acid sequence of SEQ ID NO:55, and the VL comprises an amino acid sequence of SEQ ID NO:51;

(l) the VH comprises an amino acid sequence of SEQ ID NO:56, and the VL comprises an amino acid sequence of SEQ ID NO:51;

(m) the VH comprises an amino acid sequence of SEQ ID NO:63, and the VL comprises an amino acid sequence of SEQ ID NO:51;

(n) the VH comprises an amino acid sequence of SEQ ID NO:64, and the VL comprises an amino acid sequence of SEQ ID NO:51;

(o) the VH comprises an amino acid sequence of SEQ ID NO:55, and the VL comprises an amino acid sequence of SEQ ID NO:52;

(p) the VH comprises an amino acid sequence of SEQ ID NO:58, and the VL comprises an amino acid sequence of SEQ ID NO:52;

(q) the VH comprises an amino acid sequence of SEQ ID NO:60, and the VL comprises an amino acid sequence of SEQ ID NO:52;

(r) the VH comprises an amino acid sequence of SEQ ID NO:64, and the VL comprises an amino acid sequence of SEQ ID NO:52;

(s) the VH comprises an amino acid sequence of SEQ ID NO:55, and the VL comprises an amino acid sequence of SEQ ID NO:53;

(t) the VH comprises an amino acid sequence of SEQ ID NO:57, and the VL comprises an amino acid sequence of SEQ ID NO:53;

(u) the VH comprises an amino acid sequence of SEQ ID NO:58, and the VL comprises an amino acid sequence of SEQ ID NO:53;

(v) the VH comprises an amino acid sequence of SEQ ID NO:62, and the VL comprises an amino acid sequence of SEQ ID NO:53;

(w) the VH comprises an amino acid sequence of SEQ ID NO:64, and the VL comprises an amino acid sequence of SEQ ID NO:53;

(x) the VH comprises an amino acid sequence of SEQ ID NO:55, and the VL comprises an amino acid sequence of SEQ ID NO:54;

(y) the VH comprises an amino acid sequence of SEQ ID NO:61, and the VL comprises an amino acid sequence of SEQ ID NO:54;

(z) the VH comprises an amino acid sequence of SEQ ID NO:62, and the VL comprises an amino acid sequence of SEQ ID NO:54;

(aa) the VH comprises an amino acid sequence of SEQ ID NO:64, and the VL comprises an amino acid sequence of SEQ ID NO:54;

(bb) the VH comprises an amino acid sequence of SEQ ID NO:63, and the VL comprises an amino acid sequence of SEQ ID NO:52;

(cc) the VH comprises an amino acid sequence of SEQ ID NO:188, and the VL comprises an amino acid sequence of SEQ ID NO:189; or

(dd) the VH comprises an amino acid sequence of SEQ ID NO:188, and the VL comprises an amino acid sequence of SEQ ID NO:190.

5. The isolated anti-TSLP antibody construct of any one of claims 1-4, wherein the anti-TSLP antibody moiety is selected from the group consisting of a full-length antibody, a Fab, a Fab', a F(ab')₂, a diabody, and an scFv.

6. The isolated anti-TSLP antibody construct of any one of claims 1-5, wherein the anti-TSLP antibody moiety is a full-length antibody ("anti-TSLP full-length antibody").

7. The isolated anti-TSLP antibody construct of claim 6, wherein the anti-TSLP full-length antibody comprises a CL comprising the amino acid sequence of any one of SEQ ID NOs:73-75.
8. The isolated anti-TSLP antibody construct of claim 6 or 7, wherein the anti-TSLP full-length antibody comprises an Fc domain derived from human IgG1, IgG2, or IgG4.
9. The isolated anti-TSLP antibody construct of claim 8, wherein the Fc domain is derived from human IgG1, and wherein:
- i) a first subunit and a second subunit of the Fc domain each comprises the amino acid sequence of any one of SEQ ID NOs:76-79;
 - ii) a first subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO:80, and a second subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO:81;
 - iii) a first subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO:81, and a second subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO:80;
 - iv) a first subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO:95, and a second subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO:96; or
 - v) a first subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO:96, and a second subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO:95.
10. The isolated anti-TSLP antibody construct of claim 8 or 9, wherein the first subunit and the second subunit of the Fc domain each comprises the amino acid sequence of SEQ ID NO:77 or 79.
11. The isolated anti-TSLP antibody construct of any one of claims 6-10, wherein the anti-TSLP full-length antibody comprises:
- i) two heavy chains each comprising the amino acid sequence of SEQ ID NO:104, and two light chains each comprising the amino acid sequence of SEQ ID NO:103;
 - ii) two heavy chains each comprising the amino acid sequence of SEQ ID NO:105, and two light chains each comprising the amino acid sequence of SEQ ID NO:103; or

iii) a first heavy chain comprising the amino acid sequence of SEQ ID NO:136, a second heavy chain comprising the amino acid sequence of SEQ ID NO:137, and two light chains each comprising the amino acid sequence of SEQ ID NO:103.

12. The isolated anti-TSLP antibody construct of any one of claims 1-5, wherein the anti-TSLP antibody moiety is an scFv ("anti-TSLP scFv").

13. The isolated anti-TSLP antibody construct of claim 12, wherein the anti-TSLP scFv comprises the amino acid sequence of any one of SEQ ID NOs:106, 107, 191, and 192.

14. The isolated anti-TSLP antibody construct of any one of claims 1-5, wherein the anti-TSLP antibody moiety is a Fab ("anti-TSLP Fab").

15. The isolated anti-TSLP antibody construct of claim 14, wherein the anti-TSLP Fab comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO:109, and a second polypeptide comprising the amino acid sequence of SEQ ID NO:103.

16. The isolated anti-TSLP antibody construct of any one of claims 1-15, wherein the isolated anti-TSLP antibody construct is monospecific.

17. The isolated anti-TSLP antibody construct of any one of claims 1-15, wherein the isolated anti-TSLP antibody construct is a multispecific anti-TSLP antibody construct comprising a second antibody moiety specifically recognizing a second target antigen.

18. The isolated anti-TSLP antibody construct of claim 17, wherein the second antibody moiety is selected from the group consisting of a full-length antibody, a Fab, a Fab', a F(ab')₂, an sdAb, a diabody, and an scFv.

19. The isolated anti-TSLP antibody construct of claim 18, wherein the second antibody moiety is an scFv.

20. The isolated anti-TSLP antibody construct of claim 18, wherein the second antibody moiety is a Fab.

21. The isolated anti-TSLP antibody construct of claim 18, wherein the second antibody moiety is a full-length antibody.
22. The isolated anti-TSLP antibody construct of any one of claims 17-21, wherein the anti-TSLP antibody moiety and the second antibody moiety are fused to each other via a linker.
23. The isolated anti-TSLP antibody construct of claim 22, wherein the linker comprises an amino acid sequence selected from the group consisting of GG and SEQ ID NOs:14, 16-18, 97-99, and 163-172.
24. A pharmaceutical composition comprising the isolated anti-TSLP antibody construct of any one of claims 1-23 and a pharmaceutically acceptable carrier.
25. An isolated nucleic acid encoding the polypeptide portion of the isolated anti-TSLP antibody construct of any one of claims 1-23.
26. A vector comprising the isolated nucleic acid of claim 25.
27. A host cell comprising the isolated nucleic acid of claim 25, or the vector of claim 26.
28. A method of treating an inflammatory disease in an individual, comprising administering to the individual an effective amount of the isolated anti-TSLP antibody construct of any one of claims 1-23, or the pharmaceutical composition of claim 24.
29. The method of claim 28, wherein the inflammatory disease is asthma, atopic dermatitis, or chronic obstructive pulmonary disease.
30. The method of claim 28 or 29, wherein the individual is a human.
31. A method of producing an anti-TSLP antibody construct, comprising:
 - i) culturing a host cell comprising the isolated nucleic acid of claim 25 or the vector of claim 26, or the host cell of claim 27, under a condition suitable for the expression of the anti-TSLP antibody construct; and

ii) obtaining the expressed anti-TSLP antibody construct.