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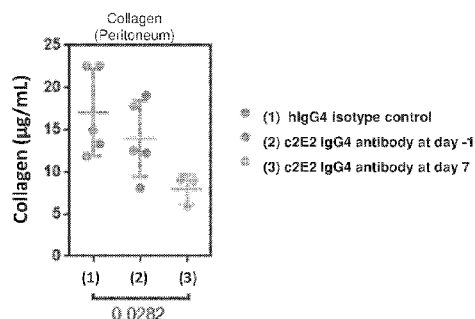
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(54) Title: METHODS AND COMPOSITIONS FOR TREATING FIBROTIC DISEASES

FIG. 7



(57) Abstract: The invention provides methods and compositions for the prevention or treatment of fibrotic diseases such as idiopathic pulmonary fibrosis and pre-fibrotic diseases such as chronic hypersensitivity pneumonitis. In particular, the invention provides methods for the prevention or treatment of fibrotic diseases and pre-fibrotic diseases through administration of antibodies or agonists that bind to human Siglec-8 or compositions comprising said antibodies or agonists. The invention also provides articles of manufacture or kits comprising antibodies or agonists that bind to human Siglec-8 for the prevention or treatment fibrotic diseases such as idiopathic pulmonary fibrosis and pre-fibrotic diseases such as chronic hypersensitivity pneumonitis.

METHODS AND COMPOSITIONS FOR TREATING FIBROTIC DISEASES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application Serial No. 62/181,146 filed on June 17, 2015, U.S. Provisional Application Serial No. 62/296,482 filed on February 17, 2016, and U.S. Provisional Application Serial No. 62/344,357 filed on June 1, 2016, the disclosures of which are incorporated herein by reference in their entirety.

SEQUENCE LISTING

[0002] The Sequence Listing is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0003] This invention relates to methods for preventing and/or treating fibrotic diseases and pre-fibrotic diseases by administration of antibodies or agonists that bind to human Siglec-8 or compositions comprising said antibodies or agonists.

BACKGROUND OF THE INVENTION

[0004] Fibrosis is a condition characterized by excess deposition of extracellular matrix components in an organ or tissue that leads to an accumulation of tough fibrous scar tissue. For example, pulmonary fibrosis is a major type of fibrotic disease that is characterized by an inflammatory response that includes macrophages, neutrophils, lymphocytes, and mast cells. Idiopathic Pulmonary Fibrosis (IPF) is a chronic lung disease with unknown etiology in which normal lung parenchyma is progressively replaced with fibrotic tissue, leading to dyspnea, cough, impaired lung function, and increased mortality. IPF has a poor prognosis, with a median survival of approximately three years from the time of diagnosis, and great associated morbidity, with wide-ranging negative effects on quality of life. Most patients with IPF succumb to respiratory failure. There are several pathogenic events that occur in the development of IPF and several of these events have been targeted by therapeutic agents for the purpose of treating IPF. Therapeutic approaches targeting macrophages, neutrophils or lymphocytes have failed to alter the course of IPF pathogenesis. See Ahluwalia et al., *Am J Respir Crit Care Med.*, 190(8):867-78, 2014 and Woodcock et al., *F1000Prime Rep.*, 6:16, 2014 for a review of IPF and available treatments for IPF. Therefore, there remains a need for therapies that can control the activity of immune cells involved in the pathogenesis of fibrotic disease.

[0005] The number of mast cells and mast cell mediators are significantly elevated in IPF. Mast cells are the main source of TGF- β , a cytokine that plays a role in the development of

fibrosis via stimulation of collagen production by fibroblasts and signaling for differentiation of fibroblasts into myofibroblasts. It has also been shown that mast cell-deficient mice are protected from bleomycin-induced pulmonary fibrosis. *See* Veerappan et al., *DNA Cell Biol.*, 32(4):206-18. Although mast cells appear to be involved in the pathogenesis of IPF, their exact role in the multitude of complex pathogenic events that underlay the development of fibrotic disease remains unclear.

[0006] Siglecs (sialic acid-binding immunoglobulin-like lectins) are single-pass transmembrane cell surface proteins found predominantly on leukocytes and are characterized by their specificity for sialic acids attached to cell-surface glycoconjugates. The Siglec family contains at least 15 members that are found in mammals (Pillai et al., *Annu Rev Immunol.*, 30:357-392, 2012). These members include sialoadhesion (Siglec-1), CD22 (Siglec-2), CD33 (Siglec-3), myelin associated glycoprotein (Siglec-4), Siglec-5, OBBP1 (Siglec-6), AIRM1 (Siglec-7), SAF-2 (Siglec-8), and CD329 (Siglec-9). Siglec-8 was first discovered as part of efforts to identify novel human eosinophil proteins. In addition to expression by eosinophils, it is also expressed by mast cells and basophils. Siglec-8 recognizes a sulfated glycan, *i.e.*, 6'-sulfo-sialyl Lewis X or 6'-sulfo-sialyl-N-acetyl-S-lactosamine, and contains an intracellular immunoreceptor tyrosine-based inhibitory motif (ITIM) domain shown to inhibit mast cell function. Siglec-8 has been shown to modulate cellular responses mediated by the IgE pathway but the effect of Siglec-8 activation on non-IgE mediated immune response pathways is unknown.

[0007] All references cited herein, including patent applications, patent publications, and scientific literature, are herein incorporated by reference in their entirety, as if each individual reference were specifically and individually indicated to be incorporated by reference.

[0007a] It is to be understood that if any prior art publication is referred to herein, such reference does not constitute an admission that the publication forms a part of the common general knowledge in the art in Australia or any other country.

SUMMARY OF THE INVENTION

[0008] Provided herein are methods of using antibodies or agonists that bind to human Siglec-8, or compositions comprising thereof, for the prevention or treatment of fibrotic diseases or pre-fibrotic diseases. Fibrotic diseases include, but are not limited to, pulmonary fibrosis (*e.g.*, idiopathic pulmonary fibrosis), hepatic fibrosis, renal fibrosis (*e.g.*, renal interstitial fibrosis), cardiac fibrosis, spleen fibrosis, ocular fibrosis, mechanical-induced fibrosis (*e.g.*, ventilator-induced pulmonary fibrosis), implant-induced fibrosis, radiation-induced fibrosis, drug-induced fibrosis (*e.g.*, bleomycin-induced pulmonary fibrosis), viral-induced fibrosis, cystic fibrosis,

cancer-associated fibrosis, atherosclerosis, bone marrow fibrosis (*e.g.*, myelofibrosis), scleroderma (*e.g.*, systemic sclerosis), mediastinal fibrosis and retroperitoneal cavity fibrosis. Pre-fibrotic diseases include, but are not limited to, bleomycin-induced pneumonitis, chronic hypersensitivity pneumonitis, polycythemia vera, essential thrombocythemia, age-related macular degeneration, diabetic retinopathy, neovascular glaucoma, retinopathy of prematurity, and proliferative vitreoretinopathy.

[0008a] A first aspect provides a method for treating fibrotic disease in an individual, comprising administering to the individual an effective amount of an antibody that binds to human Siglec-8; wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:6 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:16 or 21; wherein the antibody comprises a heavy chain Fc region comprising a human IgG Fc region; and wherein the fibrotic disease is pulmonary fibrosis, hepatic fibrosis, renal fibrosis, cardiac fibrosis, spleen fibrosis, ocular fibrosis, mechanical-induced fibrosis, implant-induced fibrosis, radiation-induced fibrosis, drug-induced fibrosis, viral-induced fibrosis, cystic fibrosis, cancer-associated fibrosis, atherosclerosis, bone marrow fibrosis, scleroderma, mediastinal fibrosis, retroperitoneal cavity fibrosis, or Dupuytren's contracture.

[0008b] A second aspect provides use of an antibody that binds to human Siglec-8 in the manufacture of a medicament for treating fibrotic disease in an individual; wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:6 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:16 or 21; wherein the antibody comprises a heavy chain Fc region comprising a human IgG Fc region; and wherein the fibrotic disease is pulmonary fibrosis, hepatic fibrosis, renal fibrosis, cardiac fibrosis, spleen fibrosis, ocular fibrosis, mechanical-induced fibrosis, implant-induced fibrosis, radiation-induced fibrosis, drug-induced fibrosis, viral-induced fibrosis, cystic fibrosis, cancer-associated fibrosis, atherosclerosis, bone marrow fibrosis, scleroderma, mediastinal fibrosis, retroperitoneal cavity fibrosis, or Dupuytren's contracture.

[0009] Also disclosed is a method for treating or preventing fibrotic disease in an individual comprising administering to the individual an effective amount of an antibody or an agonist that binds to human Siglec-8. In some embodiments, the individual has a fibrotic disease. In some embodiments, the individual has been diagnosed with a fibrotic disease or is at risk of developing the fibrotic disease. In some embodiments herein, the fibrotic disease is selected from the group consisting of: pulmonary fibrosis, hepatic fibrosis, renal fibrosis, cardiac fibrosis, spleen fibrosis, and ocular fibrosis. In a further embodiment, the pulmonary fibrosis is idiopathic pulmonary fibrosis. In some embodiments, the pulmonary fibrosis is associated with chronic obstructive pulmonary disease. In some embodiments herein, the fibrotic disease is selected from the group consisting of: mechanical-induced fibrosis, implant-induced fibrosis,

radiation-induced fibrosis, drug-induced fibrosis and viral-induced fibrosis. In a further embodiment, the mechanical induced fibrosis is ventilator-induced pulmonary fibrosis. In yet another further embodiment, the drug-induced fibrosis is bleomycin-induced pulmonary fibrosis. In some embodiments, the fibrotic disease is selected from the group consisting of: cystic fibrosis, cancer-associated fibrosis, atherosclerosis, bone marrow fibrosis, scleroderma, mediastinal fibrosis and retroperitoneal cavity fibrosis. In some embodiments, the scleroderma is systemic sclerosis. In some of the embodiments herein, one or more symptom in the individual with the fibrotic disease is reduced relative to baseline after administration of the antibody or the agonist that binds to human Siglec-8. In a further embodiment, the one or more symptom can be a symptom associated with a fibrotic disease disclosed herein, such as, but not limited to, shortness of breath, dry cough, weight loss, fatigue, malaise, finger or toe clubbing, muscle pain, or joint pain. In some embodiments herein, one or more pulmonary function in the individual with pulmonary fibrosis is increased by at least 5% relative to baseline after administration of the antibody or the agonist that binds to human Siglec-8. In a further embodiment, the one or more pulmonary function is selected from the group consisting of: vital capacity (VC), residual volume (V), forced expiratory volume (FEV₁), forced vital capacity (FVC), forced expiratory flow (FEF), peak expiratory flow rate (PEFR), inspiratory reserve volume (IRV), functional residual capacity (FRC), inspirator capacity (IC), total lung capacity (TLC), expiratory reserve volume (ERV), tidal volume (TV) and maximum voluntary ventilation (MVV). In some embodiments, one or more pathologic parameter in the individual with fibrotic disease is reduced by at least 5% relative to baseline after administration of the antibody or the agonist that binds to human Siglec-8. In a further embodiment, the one or more pathologic parameter is selected from the group consisting of: neutrophil influx, number of mast cells, cytokine release, collagen accumulation, fibroblast or myofibroblast infiltration and fibroblastic foci formation. In some embodiments, mast cell activation is reduced in the individual relative to baseline after administration of the antibody or the agonist that binds to human Siglec-8. In any of the embodiments herein, the individual can be a human. In any of the embodiments herein, the antibody can be in a pharmaceutical composition comprising the antibody and a pharmaceutically acceptable carrier. In any of the embodiments herein, the agonist can be in a pharmaceutical composition comprising the agonist and a pharmaceutically acceptable carrier.

[0010] Also disclosed is a method for treating or preventing pre-fibrotic disease in an individual comprising administering to the individual an effective amount of an antibody or an agonist that binds to human Siglec-8. In some embodiments, the individual has a pre-fibrotic disease. In some embodiments, the individual has been diagnosed with a pre-fibrotic disease or is at risk of developing the pre-fibrotic disease. In some embodiments, the pre-fibrotic disease is

selected from the group consisting of: bleomycin-induced pneumonitis, chronic hypersensitivity pneumonitis, polycythemia vera, essential thrombocythemia, age-related macular degeneration, diabetic retinopathy, neovascular glaucoma, retinopathy of prematurity, and proliferative vitreoretinopathy. In some of the embodiments herein, one or more symptom in the individual with pre-fibrotic disease is reduced relative to baseline after administration of the antibody or the agonist that binds to human Siglec-8. In a further embodiment, the one or more symptom can be a symptom associated with a pre-fibrotic disease disclosed herein, such as, but not limited to, shortness of breath, dry cough, weight loss, fatigue, malaise, finger or toe clubbing, muscle pain, or joint pain. In some embodiments, one or more pathologic parameter in the individual with pre-fibrotic disease is reduced by at least 5% relative to baseline after administration of the antibody or the agonist that binds to human Siglec-8. In a further embodiment, the one or more pathologic parameter is selected from the group consisting of: neutrophil influx, number of mast cells, cytokine release, collagen accumulation, fibroblast or myofibroblast infiltration and fibroblastic foci formation. In some embodiments, mast cell activation is reduced in the individual relative to baseline after administration of the antibody or the agonist that binds to human Siglec-8. In any of the embodiments herein, the individual can be a human. In any of the embodiments herein, the antibody can be in a pharmaceutical composition comprising the antibody and a pharmaceutically acceptable carrier. In any of the embodiments herein, the agonist can be in a pharmaceutical composition comprising the agonist and a pharmaceutically acceptable carrier.

[0011] Also disclosed is a method for reducing mast cell activation by an agent of a non-IgE mediated cell signaling pathway in an individual comprising administering to the individual an effective amount of an antibody or an agonist that binds to human Siglec-8. In one embodiment, the individual has a fibrotic disease. In some embodiments, the individual has been diagnosed with a fibrotic disease or is at risk of developing the fibrotic disease. In some embodiments herein, the fibrotic disease is selected from the group consisting of: pulmonary fibrosis, hepatic fibrosis, renal fibrosis, cardiac fibrosis, spleen fibrosis, and ocular fibrosis. In a further embodiment, the pulmonary fibrosis is idiopathic pulmonary fibrosis. In some embodiments, the pulmonary fibrosis is associated with chronic obstructive pulmonary disease. In some embodiments herein, the fibrotic disease is selected from the group consisting of: mechanical-induced fibrosis, implant-induced fibrosis, radiation-induced fibrosis, drug-induced fibrosis and viral-induced fibrosis. In a further embodiment, the mechanical induced fibrosis is ventilator-induced pulmonary fibrosis. In yet another further embodiment, the drug-induced fibrosis is bleomycin-induced pulmonary fibrosis. In some embodiments, the fibrotic disease is selected from the group consisting of: cystic fibrosis, cancer-associated fibrosis, atherosclerosis, bone

marrow fibrosis, scleroderma, mediastinal fibrosis and retroperitoneal cavity fibrosis. In some embodiments, the scleroderma is systemic sclerosis. In some of the embodiments herein, one or more symptom in the individual with the fibrotic disease is reduced relative to baseline after administration of the antibody or the agonist that binds to human Siglec-8. In a further embodiment, the one or more symptom can be a symptom associated with a fibrotic disease disclosed herein, such as, but not limited to, shortness of breath, dry cough, weight loss, fatigue, malaise, finger or toe clubbing, muscle pain, or joint pain. In some embodiments herein, one or more pulmonary function in the individual with pulmonary fibrosis is increased by at least 5% relative to baseline after administration of the antibody or the agonist that binds to human Siglec-8. In a further embodiment, the one or more pulmonary function is selected from the group consisting of: vital capacity (VC), residual volume (V), forced expiratory volume (FEV₁), forced vital capacity (FVC), forced expiratory flow (FEF), peak expiratory flow rate (PEFR), inspiratory reserve volume (IRV), functional residual capacity (FRC), inspirator capacity (IC), total lung capacity (TLC), expiratory reserve volume (ERV), tidal volume (TV) and maximum voluntary ventilation (MVV). In some embodiments, one or more pathologic parameter in the individual with fibrotic disease is reduced by at least 5% relative to baseline after administration of the antibody or the agonist that binds to human Siglec-8. In a further embodiment, the one or more pathologic parameter is selected from the group consisting of: neutrophil influx, number of mast cells, cytokine release, collagen accumulation, fibroblast or myofibroblast infiltration and fibroblastic foci formation. In some of the embodiments herein, the agent of the non-IgE mediated cell signaling pathway is selected from the group consisting of: thymic stromal lymphopoietin (TSLP), Stem cell factor (SCF), Toll-like Receptor 3 (TLR3), Interleukin 33 (IL-33), and complement proteins. In some embodiments, mast cell activation is reduced in the individual relative to baseline after administration of the antibody or the agonist that binds to human Siglec-8. In any of the embodiments herein, the individual can be a human. In any of the embodiments herein, the antibody can be in a pharmaceutical composition comprising the antibody and a pharmaceutically acceptable carrier. In any of the embodiments herein, the agonist can be in a pharmaceutical composition comprising the agonist and a pharmaceutically acceptable carrier.

[0012] Also disclosed is a method for reducing mast cell activation by an agent of a non-IgE mediated cell signaling pathway in an individual comprising administering to the individual an effective amount of an antibody or an agonist that binds to human Siglec-8. In one embodiment, the individual has a pre-fibrotic disease. In some embodiments, the individual has been diagnosed with a pre-fibrotic disease or is at risk of developing the pre-fibrotic disease. In some embodiments, the pre-fibrotic disease is selected from the group consisting of: bleomycin-

induced pneumonitis, chronic hypersensitivity pneumonitis, polycythemia vera, essential thrombocythemia, age-related macular degeneration, diabetic retinopathy, neovascular glaucoma, retinopathy of prematurity, and proliferative vitreoretinopathy. In some of the embodiments herein, one or more symptom in the individual with pre-fibrotic disease is reduced relative to baseline after administration of the antibody or the agonist that binds to human Siglec-8. In a further embodiment, the one or more symptom can be a symptom associated with a pre-fibrotic disease disclosed herein, such as, but not limited to, shortness of breath, dry cough, weight loss, fatigue, malaise, finger or toe clubbing, muscle pain, or joint pain. In some embodiments, one or more pathologic parameter in the individual with pre-fibrotic disease is reduced by at least 5% relative to baseline after administration of the antibody or the agonist that binds to human Siglec-8. In a further embodiment, the one or more pathologic parameter is selected from the group consisting of: neutrophil influx, number of mast cells, cytokine release, collagen accumulation, fibroblast or myofibroblast infiltration and fibroblastic foci formation. In some of the embodiments herein, the agent of the non-IgE mediated cell signaling pathway is selected from the group consisting of: thymic stromal lymphopoietin (TSLP), Stem cell factor (SCF), Toll-like Receptor 3 (TLR3), Interleukin 33 (IL-33), and complement proteins. In some embodiments, mast cell activation is reduced in the individual relative to baseline after administration of the antibody or the agonist that binds to human Siglec-8. In any of the embodiments herein, the individual can be a human. In any of the embodiments herein, the antibody can be in a pharmaceutical composition comprising the antibody and a pharmaceutically acceptable carrier. In any of the embodiments herein, the agonist can be in a pharmaceutical composition comprising the agonist and a pharmaceutically acceptable carrier.

[0013] Also disclosed is a composition comprising an antibody or an agonist that binds to human Siglec-8 for use in treating or preventing fibrotic disease in an individual. In some embodiments, the individual has a fibrotic disease. In some embodiments, the individual has been diagnosed with a fibrotic disease or is at risk of developing the fibrotic disease. In some embodiments, the antibody comprises a Fc region and N-glycoside-linked carbohydrate chains linked to the Fc region, wherein less than 50% of the N-glycoside-linked carbohydrate chains contain a fucose residue. In a further embodiment, substantially none of the N-glycoside-linked carbohydrate chains contain a fucose residue. In some embodiments herein, the fibrotic disease is selected from the group consisting of: pulmonary fibrosis, hepatic fibrosis, renal fibrosis, cardiac fibrosis, spleen fibrosis, and ocular fibrosis. In a further embodiment, the pulmonary fibrosis is idiopathic pulmonary fibrosis. In some embodiments, the pulmonary fibrosis is associated with chronic obstructive pulmonary disease. In some embodiments herein, the fibrotic disease is selected from the group consisting of: mechanical-induced fibrosis, implant-

induced fibrosis, radiation-induced fibrosis, drug-induced fibrosis and viral-induced fibrosis. In a further embodiment, the mechanical induced fibrosis is ventilator-induced pulmonary fibrosis. In yet another further embodiment, the drug-induced fibrosis is bleomycin-induced pulmonary fibrosis. In some embodiments, the fibrotic disease is selected from the group consisting of: cystic fibrosis, cancer-associated fibrosis, atherosclerosis, bone marrow fibrosis, scleroderma, mediastinal fibrosis and retroperitoneal cavity fibrosis. In some embodiments, the scleroderma is systemic sclerosis. In some of the embodiments herein, one or more symptom in the individual with the fibrotic disease is reduced relative to baseline after administration of the composition comprising the antibody or the agonist that binds to human Siglec-8. In a further embodiment, the one or more symptom can be a symptom associated with a fibrotic disease disclosed herein, such as, but not limited to, shortness of breath, dry cough, weight loss, fatigue, malaise, finger or toe clubbing, muscle pain, or joint pain. In some embodiments herein, one or more pulmonary function in the individual with pulmonary fibrosis is increased by at least 5% relative to baseline after administration of the composition comprising the antibody or the agonist that binds to human Siglec-8. In a further embodiment, the one or more pulmonary function is selected from the group consisting of: vital capacity (VC), residual volume (V), forced expiratory volume (FEV₁), forced vital capacity (FVC), forced expiratory flow (FEF), peak expiratory flow rate (PEFR), inspiratory reserve volume (IRV), functional residual capacity (FRC), inspirator capacity (IC), total lung capacity (TLC), expiratory reserve volume (ERV), tidal volume (TV) and maximum voluntary ventilation (MVV). In some embodiments, one or more pathologic parameter in the individual with fibrotic disease is reduced by at least 5% relative to baseline after administration of the composition comprising the antibody or the agonist that binds to human Siglec-8. In a further embodiment, the one or more pathologic parameter is selected from the group consisting of: neutrophil influx, number of mast cells, cytokine release, collagen accumulation, fibroblast or myofibroblast infiltration and fibroblastic foci formation. In some embodiments, mast cell activation is reduced in the individual relative to baseline after administration of the composition comprising the antibody or the agonist that binds to human Siglec-8. In any of the embodiments herein, the individual can be a human. In any of the embodiments herein, the composition can further comprise a pharmaceutically acceptable carrier.

[0014] Also disclosed is a composition comprising an antibody or an agonist that binds to human Siglec-8 for use in treating or preventing pre-fibrotic disease in an individual. In some embodiments, the individual has a pre-fibrotic disease. In some embodiments, the individual has been diagnosed with a pre-fibrotic disease or is at risk of developing the pre-fibrotic disease. In some embodiments, the antibody comprises a Fc region and N-glycoside-linked carbohydrate

chains linked to the Fc region, wherein less than 50% of the N-glycoside-linked carbohydrate chains contain a fucose residue. In a further embodiment, substantially none of the N-glycoside-linked carbohydrate chains contain a fucose residue. In some embodiments, the pre-fibrotic disease is selected from the group consisting of: bleomycin-induced pneumonitis, chronic hypersensitivity pneumonitis, polycythemia vera, essential thrombocythemia, age-related macular degeneration, diabetic retinopathy, neovascular glaucoma, retinopathy of prematurity, and proliferative vitreoretinopathy. In some of the embodiments herein, one or more symptom in the individual with pre-fibrotic disease is reduced relative to baseline after administration of the composition comprising the antibody or the agonist that binds to human Siglec-8. In a further embodiment, the one or more symptom can be a symptom associated with a pre-fibrotic disease disclosed herein, such as, but not limited to, shortness of breath, dry cough, weight loss, fatigue, malaise, finger or toe clubbing, muscle pain, or joint pain. In some embodiments, one or more pathologic parameter in the individual with pre-fibrotic disease is reduced by at least 5% relative to baseline after administration of the composition comprising the antibody or the agonist that binds to human Siglec-8. In a further embodiment, the one or more pathologic parameter is selected from the group consisting of: neutrophil influx, number of mast cells, cytokine release, collagen accumulation, fibroblast or myofibroblast infiltration and fibroblastic foci formation. In some embodiments, mast cell activation is reduced in the individual relative to baseline after administration of the composition comprising the antibody or the agonist that binds to human Siglec-8. In any of the embodiments herein, the individual can be a human. In any of the embodiments herein, the composition can further comprise a pharmaceutically acceptable carrier.

[0015] Also disclosed is a composition comprising an antibody or an agonist that binds to human Siglec-8 for use in reducing mast cell activation by an agent of a non-IgE mediated cell signaling pathway in an individual comprising administering to the individual the composition. In some embodiments, the antibody comprises a Fc region and N-glycoside-linked carbohydrate chains linked to the Fc region, wherein less than 50% of the N-glycoside-linked carbohydrate chains contain a fucose residue. In a further embodiment, substantially none of the N-glycoside-linked carbohydrate chains contain a fucose residue. In some embodiments, the individual has a fibrotic disease. In some embodiments, the individual has been diagnosed with a fibrotic disease or is at risk of developing the fibrotic disease. In some embodiments herein, the fibrotic disease is selected from the group consisting of: pulmonary fibrosis, hepatic fibrosis, renal fibrosis, cardiac fibrosis, spleen fibrosis, and ocular fibrosis. In a further embodiment, the pulmonary fibrosis is idiopathic pulmonary fibrosis. In some embodiments, the pulmonary fibrosis is associated with chronic obstructive pulmonary disease. In some embodiments herein, the

fibrotic disease is selected from the group consisting of: mechanical-induced fibrosis, implant-induced fibrosis, radiation-induced fibrosis, drug-induced fibrosis and viral-induced fibrosis. In a further embodiment, the mechanical induced fibrosis is ventilator-induced pulmonary fibrosis. In yet another further embodiment, the drug-induced fibrosis is bleomycin-induced pulmonary fibrosis. In some embodiments, the fibrotic disease is selected from the group consisting of: cystic fibrosis, cancer-associated fibrosis, atherosclerosis, bone marrow fibrosis, scleroderma, mediastinal fibrosis and retroperitoneal cavity fibrosis. In some embodiments, the scleroderma is systemic sclerosis. In some of the embodiments herein, one or more symptom in the individual with the fibrotic disease is reduced relative to baseline after administration of the composition comprising the antibody or the agonist that binds to human Siglec-8. In a further embodiment, the one or more symptom can be a symptom associated with a fibrotic disease disclosed herein, such as, but not limited to, shortness of breath, dry cough, weight loss, fatigue, malaise, finger or toe clubbing, muscle pain, or joint pain. In some embodiments herein, one or more pulmonary function in the individual with pulmonary fibrosis is increased by at least 5% relative to baseline after administration of the composition comprising the antibody or the agonist that binds to human Siglec-8. In a further embodiment, the one or more pulmonary function is selected from the group consisting of: vital capacity (VC), residual volume (V), forced expiratory volume (FEV₁), forced vital capacity (FVC), forced expiratory flow (FEF), peak expiratory flow rate (PEFR), inspiratory reserve volume (IRV), functional residual capacity (FRC), inspirator capacity (IC), total lung capacity (TLC), expiratory reserve volume (ERV), tidal volume (TV) and maximum voluntary ventilation (MVV). In some embodiments, one or more pathologic parameter in the individual with fibrotic disease is reduced by at least 5% relative to baseline after administration of the composition comprising the antibody or the agonist that binds to human Siglec-8. In a further embodiment, the one or more pathologic parameter is selected from the group consisting of: neutrophil influx, number of mast cells, cytokine release, collagen accumulation, fibroblast or myofibroblast infiltration and fibroblastic foci formation. In some of the embodiments herein, the agent of the non-IgE mediated cell signaling pathway is selected from the group consisting of: thymic stromal lymphopoietin (TSLP), Stem cell factor (SCF), Toll-like Receptor 3 (TLR3), Interleukin 33 (IL-33), and complement proteins. In some embodiments, mast cell activation is reduced in the individual relative to baseline after administration of the composition comprising the antibody or the agonist that binds to human Siglec-8. In any of the embodiments herein, the individual can be a human. In any of the embodiments herein, the composition can further comprise a pharmaceutically acceptable carrier.

[0016] Also disclosed is a composition comprising an antibody or an agonist that binds to human Siglec-8 for use in reducing mast cell activation by an agent of a non-IgE mediated cell signaling pathway in an individual comprising administering to the individual the composition. In some embodiments, the antibody comprises a Fc region and N-glycoside-linked carbohydrate chains linked to the Fc region, wherein less than 50% of the N-glycoside-linked carbohydrate chains contain a fucose residue. In a further embodiment, substantially none of the N-glycoside-linked carbohydrate chains contain a fucose residue. In some embodiments, the individual has a pre-fibrotic disease. In some embodiments, the individual has been diagnosed with a pre-fibrotic disease or is at risk of developing the pre-fibrotic disease. In some embodiments, the pre-fibrotic disease is selected from the group consisting of: bleomycin-induced pneumonitis, chronic hypersensitivity pneumonitis, polycythemia vera, essential thrombocythemia, age-related macular degeneration, diabetic retinopathy, neovascular glaucoma, retinopathy of prematurity, and proliferative vitreoretinopathy. In some of the embodiments herein, one or more symptom in the individual with pre-fibrotic disease is reduced relative to baseline after administration of the composition comprising the antibody or the agonist that binds to human Siglec-8. In a further embodiment, the one or more symptom can be a symptom associated with a pre-fibrotic disease disclosed herein, such as, but not limited to, shortness of breath, dry cough, weight loss, fatigue, malaise, finger or toe clubbing, muscle pain, or joint pain. In some embodiments, one or more pathologic parameter in the individual with pre-fibrotic disease is reduced by at least 5% relative to baseline after administration of the composition comprising the antibody or the agonist that binds to human Siglec-8. In a further embodiment, the one or more pathologic parameter is selected from the group consisting of: neutrophil influx, number of mast cells, cytokine release, collagen accumulation, fibroblast or myofibroblast infiltration and fibroblastic foci formation. In some of the embodiments herein, the agent of the non-IgE mediated cell signaling pathway is selected from the group consisting of: thymic stromal lymphopoietin (TSLP), Stem cell factor (SCF), Toll-like Receptor 3 (TLR3), Interleukin 33 (IL-33), and complement proteins. In some embodiments, mast cell activation is reduced in the individual relative to baseline after administration of the composition comprising the antibody or the agonist that binds to human Siglec-8. In any of the embodiments herein, the individual can be a human. In any of the embodiments herein, the composition can further comprise a pharmaceutically acceptable carrier.

[0017] Also disclosed is an article of manufacture comprising a medicament comprising an antibody or an agonist that binds to human Siglec-8 and a package insert comprising instructions for administration of the medicament in an individual in need thereof to treat or prevent fibrotic disease. In some embodiments, the individual has a fibrotic disease. In some embodiments, the

individual has been diagnosed with a fibrotic disease or is at risk of developing the fibrotic disease. In some embodiments herein, the fibrotic disease is selected from the group consisting of: pulmonary fibrosis, hepatic fibrosis, renal fibrosis, cardiac fibrosis, spleen fibrosis, and ocular fibrosis. In a further embodiment, the pulmonary fibrosis is idiopathic pulmonary fibrosis. In some embodiments, the pulmonary fibrosis is associated with chronic obstructive pulmonary disease. In some embodiments herein, the fibrotic disease is selected from the group consisting of: mechanical-induced fibrosis, implant-induced fibrosis, radiation-induced fibrosis, drug-induced fibrosis and viral-induced fibrosis. In a further embodiment, the mechanical induced fibrosis is ventilator-induced pulmonary fibrosis. In yet another further embodiment, the drug-induced fibrosis is bleomycin-induced pulmonary fibrosis. In some embodiments, the fibrotic disease is selected from the group consisting of: cystic fibrosis, cancer-associated fibrosis, atherosclerosis, bone marrow fibrosis, scleroderma, mediastinal fibrosis and retroperitoneal cavity fibrosis. In some embodiments, the scleroderma is systemic sclerosis. In some of the embodiments herein, the package insert further indicates that the treatment is effective in reducing one or more symptom in the individual with the fibrotic disease relative to baseline after administration of the antibody or the agonist that binds to human Siglec-8. In a further embodiment, the one or more symptom can be a symptom associated with a fibrotic disease disclosed herein, such as, but not limited to, shortness of breath, dry cough, weight loss, fatigue, malaise, finger or toe clubbing, muscle pain, or joint pain. In some embodiments herein, one or more pulmonary function in the individual with pulmonary fibrosis is increased by at least 5% relative to baseline after administration of the antibody or the agonist that binds to human Siglec-8. In a further embodiment, the one or more pulmonary function is selected from the group consisting of: vital capacity (VC), residual volume (V), forced expiratory volume (FEV₁), forced vital capacity (FVC), forced expiratory flow (FEF), peak expiratory flow rate (PEFR), inspiratory reserve volume (IRV), functional residual capacity (FRC), inspirator capacity (IC), total lung capacity (TLC), expiratory reserve volume (ERV), tidal volume (TV) and maximum voluntary ventilation (MVV). In some embodiments, one or more pathologic parameter in the individual with fibrotic disease is reduced by at least 5% relative to baseline after administration of the antibody or the agonist that binds to human Siglec-8. In a further embodiment, the one or more pathologic parameter is selected from the group consisting of: neutrophil influx, number of mast cells, cytokine release, collagen accumulation, fibroblast or myofibroblast infiltration and fibroblastic foci formation. In some embodiments, mast cell activation is reduced in the individual relative to baseline after administration of the antibody or the agonist that binds to human Siglec-8. In any of the embodiments herein, the individual can be a human. In any of the embodiments herein, the antibody can be in a pharmaceutical

composition comprising the antibody and a pharmaceutically acceptable carrier. In any of the embodiments herein, the agonist can be in a pharmaceutical composition comprising the agonist and a pharmaceutically acceptable carrier.

[0018] Also disclosed is an article of manufacture comprising a medicament comprising an antibody or an agonist that binds to human Siglec-8 and a package insert comprising instructions for administration of the medicament in an individual in need thereof to treat or prevent pre-fibrotic disease. In some embodiments, the individual has a pre-fibrotic disease. In some embodiments, the individual has been diagnosed with a pre-fibrotic disease or is at risk of developing the pre-fibrotic disease. In some embodiments, the pre-fibrotic disease is selected from the group consisting of: bleomycin-induced pneumonitis, chronic hypersensitivity pneumonitis, polycythemia vera, essential thrombocythemia, age-related macular degeneration, diabetic retinopathy, neovascular glaucoma, retinopathy of prematurity, and proliferative vitreoretinopathy. In some of the embodiments herein, the package insert further indicates that the treatment is effective in reducing one or more symptom in the individual with pre-fibrotic disease relative to baseline after administration of the antibody or the agonist that binds to human Siglec-8. In a further embodiment, the one or more symptom can be a symptom associated with a pre-fibrotic disease disclosed herein, such as, but not limited to, shortness of breath, dry cough, weight loss, fatigue, malaise, finger or toe clubbing, muscle pain, or joint pain. In some embodiments, one or more pathologic parameter in the individual with pre-fibrotic disease is reduced by at least 5% relative to baseline after administration of the antibody or the agonist that binds to human Siglec-8. In a further embodiment, the one or more pathologic parameter is selected from the group consisting of: neutrophil influx, number of mast cells, cytokine release, collagen accumulation, fibroblast or myofibroblast infiltration and fibroblastic foci formation. In some embodiments, mast cell activation is reduced in the individual relative to baseline after administration of the antibody or the agonist that binds to human Siglec-8. In any of the embodiments herein, the individual can be a human. In any of the embodiments herein, the antibody can be in a pharmaceutical composition comprising the antibody and a pharmaceutically acceptable carrier. In any of the embodiments herein, the agonist can be in a pharmaceutical composition comprising the agonist and a pharmaceutically acceptable carrier.

[0019] Also disclosed is a method for treating or preventing fibrotic disease in an individual comprising administering to the individual an effective amount of an agonist that binds to human Siglec-8. In some embodiments, the individual has a fibrotic disease. In some embodiments, the individual has been diagnosed with a fibrotic disease or is at risk of developing the fibrotic disease. In some embodiments herein, the agonist is a 6'-sulfo-sLe^X-containing agonist selected

from the group consisting of: a 6'-sulfo-sLe^X-containing ligand, a 6'-sulfo-sLe^X-containing oligosaccharide, a 6'-sulfo-sLe^X-containing polypeptide, and a 6'-sulfo-sLe^X-containing glycoprotein. In some embodiments, the agonist is an agonist antibody that binds to human Siglec-8.

[0020] Also disclosed is a method for treating or preventing pre-fibrotic disease in an individual comprising administering to the individual an effective amount of an agonist that binds to human Siglec-8. In some embodiments, the individual has a pre-fibrotic disease. In some embodiments, the individual has been diagnosed with a pre-fibrotic disease or is at risk of developing the pre-fibrotic disease. In some embodiments herein, the agonist is a 6'-sulfo-sLe^X-containing agonist selected from the group consisting of: a 6'-sulfo-sLe^X-containing ligand, a 6'-sulfo-sLe^X-containing oligosaccharide, a 6'-sulfo-sLe^X-containing polypeptide, and a 6'-sulfo-sLe^X-containing glycoprotein. In some embodiments, the agonist is an agonist antibody that binds to human Siglec-8.

[0021] Also disclosed is a method for reducing mast cell activation by an agent of a non-IgE mediated cell signaling pathway in an individual comprising administering to the individual an effective amount of an agonist that binds to human Siglec-8. In some embodiments, the individual has a fibrotic disease. In some embodiments, the individual has been diagnosed with a fibrotic disease or is at risk of developing the fibrotic disease. In some embodiments, the individual has a pre-fibrotic disease. In some embodiments, the individual has been diagnosed with a pre-fibrotic disease or is at risk of developing the pre-fibrotic disease. In some embodiments herein, the agonist is a 6'-sulfo-sLe^X-containing agonist selected from the group consisting of: a 6'-sulfo-sLe^X-containing ligand, a 6'-sulfo-sLe^X-containing oligosaccharide, a 6'-sulfo-sLe^X-containing polypeptide, and a 6'-sulfo-sLe^X-containing glycoprotein. In some embodiments, the agonist is an agonist antibody that binds to human Siglec-8.

[0022] In any of the embodiments of the methods and compositions for use therein, the antibody can be a monoclonal antibody. In any of the embodiments of the methods and compositions for use therein, the antibody can be an IgG1 antibody. In any of the embodiments of the methods and compositions for use therein, the antibody can be engineered to improve antibody-dependent cell-mediated cytotoxicity (ADCC) activity. In a further embodiment, the antibody comprises at least one amino acid substitution in the Fc region that improves ADCC activity. In any of the embodiments of the methods and compositions for use therein, one or two of the heavy chains of the antibody can be non-fucosylated. In any of the embodiments of the methods and compositions for use therein, the antibody can be a human antibody, a humanized antibody or a chimeric antibody. In any of the embodiments of the methods and compositions for use therein, the antibody can be a murine antibody. In any of the embodiments of the

methods and compositions for use therein, the antibody can be an antibody fragment selected from the group consisting of Fab, Fab'-SH, Fv, scFv, and (Fab')₂ fragments. In some embodiments, the antibody comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:61, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:62, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:63; and/or wherein the light chain variable region comprises (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:64, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:65, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:66. In some embodiments, the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:6; and/or a light chain variable region comprising the amino acid sequence selected from SEQ ID NOs:16 or 21. In some embodiments, the antibody comprises a heavy chain Fc region comprising a human IgG Fc region. In a further embodiment, the human IgG Fc region comprises a human IgG1 or a human IgG4. In a further embodiment, the human IgG4 comprises the amino acid substitution S228P, and wherein the amino acid residues are numbered according to the EU index as in Kabat. In some embodiments, the human IgG1 comprises the amino acid sequence of SEQ ID NO:78. In some embodiments, the human IgG4 comprises the amino acid sequence of SEQ ID NO:79. In some embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:75; and/or a light chain comprising the amino acid sequence SEQ ID NOs:76 or 77. In some embodiments, the antibody comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:61, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:62, and (iii) HVR-H3 comprising the amino acid sequence selected from SEQ ID NOs:67-70; and/or wherein the light chain variable region comprises (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:64, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:65, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:71. In some embodiments, the antibody comprises a heavy chain variable region comprising the amino acid sequence selected from SEQ ID NOs:11-14; and/or a light chain variable region comprising the amino acid sequence selected from SEQ ID NOs:23-24. In some embodiments, the antibody comprises a heavy chain variable region comprising the amino acid sequence selected from SEQ ID NOs:2-14; and/or a light chain variable region comprising the amino acid sequence selected from SEQ ID NOs:16-24. In some embodiments, the antibody comprises a heavy chain variable region comprising the amino acid sequence selected from SEQ ID NOs:2-10; and/or a light chain variable region comprising the amino acid sequence selected from SEQ ID NOs:16-22. In some embodiments, the antibody

comprises: (a) heavy chain variable region comprising: (1) an HC-FR1 comprising the amino acid sequence selected from SEQ ID NOs:26-29; (2) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:61; (3) an HC-FR2 comprising the amino acid sequence selected from SEQ ID NOs:31-36; (4) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:62; (5) an HC-FR3 comprising the amino acid sequence selected from SEQ ID NOs:38-43; (6) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:63; and (7) an HC-FR4 comprising the amino acid sequence selected from SEQ ID NOs:45-46, and/or (b) a light chain variable region comprising: (1) an LC-FR1 comprising the amino acid sequence selected from SEQ ID NOs:48-49; (2) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:64; (3) an LC-FR2 comprising the amino acid sequence selected from SEQ ID NOs:51-53; (4) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:65; (5) an LC-FR3 comprising the amino acid sequence selected from SEQ ID NOs:55-58; (6) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:66; and (7) an LC-FR4 comprising the amino acid sequence of SEQ ID NO:60. In some embodiments, the antibody comprises: (a) heavy chain variable region comprising: (1) an HC-FR1 comprising the amino acid sequence of SEQ ID NO:26; (2) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:61; (3) an HC-FR2 comprising the amino acid sequence of SEQ ID NO:34; (4) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:62; (5) an HC-FR3 comprising the amino acid sequence of SEQ ID NO:38; (6) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:63; and (7) an HC-FR4 comprising the amino acid sequence of SEQ ID NOs:45; and/or (b) a light chain variable region comprising: (1) an LC-FR1 comprising the amino acid sequence of SEQ ID NO:48; (2) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:64; (3) an LC-FR2 comprising the amino acid sequence of SEQ ID NO:51; (4) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:65; (5) an LC-FR3 comprising the amino acid sequence of SEQ ID NO:55; (6) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:66; and (7) an LC-FR4 comprising the amino acid sequence of SEQ ID NO:60. In some embodiments, the antibody comprises: (a) heavy chain variable region comprising: (1) an HC-FR1 comprising the amino acid sequence of SEQ ID NO:26; (2) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:61; (3) an HC-FR2 comprising the amino acid sequence of SEQ ID NO:34; (4) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:62; (5) an HC-FR3 comprising the amino acid sequence of SEQ ID NO:38; (6) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:63; and (7) an HC-FR4 comprising the amino acid sequence of SEQ ID NOs:45; and/or (b) a light chain variable region comprising: (1) an LC-FR1 comprising the amino acid sequence of SEQ ID NO:48; (2) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:64; (3) an LC-FR2 comprising the amino acid sequence of SEQ ID NO:51; (4) an HVR-L2 comprising the amino

acid sequence of SEQ ID NO:65; (5) an LC-FR3 comprising the amino acid sequence of SEQ ID NO:58; (6) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:66; and (7) an LC-FR4 comprising the amino acid sequence of SEQ ID NO:60. In some embodiments, the antibody comprises a heavy chain variable region comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:88, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:91, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:94; and/or a light chain variable region comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:97, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:100, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:103. In some embodiments, the antibody comprises a heavy chain variable region comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:89, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:92, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:95; and/or a light chain variable region comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:98, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:101, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:104. In some embodiments, the antibody comprises a heavy chain variable region comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:90, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:93, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:96; and/or a light chain variable region comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:99, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:102, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:105. In some embodiments, the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:106; and/or a light chain variable region comprising the amino acid sequence of SEQ ID NO:109. In some embodiments, the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:107; and/or a light chain variable region comprising the amino acid sequence of SEQ ID NO:110. In some embodiments, the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:108; and/or a light chain variable region comprising the amino acid sequence of SEQ ID NO:111.

[0023] 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. These and other features of the invention will become apparent to one of skill in the art. These and other embodiments of the invention are further described by the detailed description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] **FIG. 1** is a graph showing inhibition of neutrophil influx into the bronchoalveolar space in Siglec-8 transgenic mice with bleomycin-induced lung fibrosis due to treatment with anti-Siglec-8 antibodies. (1) Isotype indicates murine IgG1 isotype control antibody; (2) m2E2 Depleting indicates a murine monoclonal anti-Siglec-8 antibody with a murine IgG2a isotype that kills eosinophils and mast cells by ADCC activity; (3) m2E2 Inhibitory indicates a murine monoclonal anti-Siglec-8 antibody with a murine IgG1 isotype that kills eosinophils and inhibits mast cell activity; and (4) Naive indicates Siglec-8 transgenic mice that were not administered an antibody or bleomycin. p-values were determined by comparing the mouse IgG1 isotype control antibody study group to the m2E2 Depleting antibody study group, the m2E2 Inhibitory antibody study group or the naive study group. % of neutrophils indicates the percentage of neutrophils relative to the total number of leukocytes in the sample.

[0025] **FIG. 2A and 2B** is a series of graphs showing prevention of collagen accumulation in Siglec-8 transgenic mice with bleomycin-induced lung fibrosis due to treatment with anti-Siglec-8 antibodies. **FIG. 2A)** Experiment 1 and **FIG. 2B)** Experiment 2 are duplicate experiments except Experiment 2 includes a group of mice treated with m2E2 Depleting antibody. BAL indicates bronchoalveolar lavage; (1) Isotype indicates murine IgG1 isotype control antibody; (2) m2E2 Depleting indicates a murine monoclonal anti-Siglec-8 antibody with a murine IgG2a isotype that kills eosinophils and mast cells by ADCC activity; (3) m2E2 Inhibitory indicates a

murine monoclonal anti-Siglec-8 antibody with a murine IgG1 isotype that kills eosinophils and inhibits mast cell activity; and (4) Naive indicates Siglec-8 transgenic mice that were not administered an antibody or bleomycin. p-values were determined by comparing the mouse IgG1 isotype control antibody study group to the m2E2 Depleting antibody study group, the m2E2 Inhibitory antibody study group or the naive study group.

[0026] FIG. 3 is a graph showing that anti-Siglec-8 antibodies protect Siglec-8 transgenic mice from bleomycin-induced weight loss. (1) Isotype indicates murine IgG1 isotype control antibody; (2) m2E2 Depleting indicates a murine monoclonal anti-Siglec-8 antibody with a murine IgG2a isotype that kills eosinophils and mast cells by ADCC activity; (3) m2E2 Inhibitory indicates a murine monoclonal anti-Siglec-8 antibody with a murine IgG1 isotype that kills eosinophils and inhibits mast cell activity; and (4) Naive indicates Siglec-8 transgenic mice that were not administered an antibody or bleomycin. p-values were determined by comparing the mouse IgG1 isotype control antibody study group to the m2E2 Depleting antibody study group.

[0027] FIG. 4 is a graph showing inhibition of elevated cytokines in the bronchoalveolar lavage fluid after bleomycin-induced lung fibrosis in Siglec-8 transgenic mice due to treatment with anti-Siglec-8 antibodies. (1) Isotype indicates murine IgG1 isotype control antibody; (2) m2E2 Depleting indicates a murine monoclonal anti-Siglec-8 antibody with a murine IgG2a isotype that kills eosinophils and mast cells by ADCC activity; (3) m2E2 Inhibitory indicates a murine monoclonal anti-Siglec-8 antibody with a murine IgG1 isotype that kills eosinophils and inhibits mast cell activity; and (4) Naive indicates Siglec-8 transgenic mice that were not administered an antibody or bleomycin. p-values were determined by comparing the mouse IgG1 isotype control antibody study group to the m2E2 Depleting antibody study group, the m2E2 Inhibitory antibody study group or the naive study group. G-CSF indicates granulocyte colony-stimulating factor; IL-6 indicates interleukin-6; IP-10 indicates interferon gamma-induced protein 10; KC indicates keratinocyte-derived cytokine; RANTES indicates regulated on activation normal T cell expressed and secreted protein; and active TGF- β 2 indicates transforming growth factor- β 2 in active form.

[0028] FIG. 5 is a graph showing reduction of an Ashcroft score from lungs of Siglec-8 transgenic mice with bleomycin-induced lung fibrosis due to treatment with anti-Siglec-8 antibodies. (1) Isotype indicates murine IgG1 isotype control antibody; (2) m2E2 Depleting indicates a murine monoclonal anti-Siglec-8 antibody with a murine IgG2a isotype that kills eosinophils and mast cells by ADCC activity; (3) m2E2 Inhibitory indicates a murine monoclonal anti-Siglec-8 antibody with a murine IgG1 isotype that kills eosinophils and inhibits mast cell activity; and (4) Naive indicates Siglec-8 transgenic mice that were not administered

an antibody or bleomycin. p-values were determined by comparing the mouse IgG1 isotype control antibody study group to the m2E2 Depleting antibody study group, the m2E2 Inhibitory antibody study group or the naive study group.

[0029] FIG. 6 is a graph showing inhibition of neutrophil influx into the bronchoalveolar space in Siglec-8 transgenic mice with bleomycin-induced lung fibrosis due to therapeutic treatment with anti-Siglec-8 antibodies. (1) Isotype indicates murine IgG1 isotype control antibody; (3) m2E2 Inhibitory indicates a murine monoclonal anti-Siglec-8 antibody with a murine IgG1 isotype that kills eosinophils and inhibits mast cell activity; and (4) Naive indicates Siglec-8 transgenic mice that were not administered an antibody or bleomycin. p-values were determined by comparing the mouse IgG1 isotype control antibody study group to the m2E2 Inhibitory antibody study group or the naive study group. % of neutrophils indicates the percentage of neutrophils relative to the total number of leukocytes in the sample.

[0030] FIG. 7 is a graph showing prevention and inhibition of collagen deposition in humanized mice with foreign body-induced fibrosis in peritoneum due to treatment with anti-Siglec-8 antibodies. (1) hIgG4 isotype control indicates human IgG4 isotype control antibody; (2) c2E2 IgG4 antibody at day -1 indicates a chimeric monoclonal anti-Siglec-8 antibody with a human IgG4 isotype and murine 2E2 variable domains that was dosed one day prior to implantation of beads; (3) c2E2 IgG4 antibody at day 7 indicates a chimeric monoclonal anti-Siglec-8 antibody with a human IgG4 isotype and murine 2E2 variable domains that was dosed seven days after the implantation of beads. p-values were determined by comparing the human IgG4 isotype control antibody study group to the c2E2 IgG4 antibody study groups.

[0031] FIG. 8 is a graph showing prevention and inhibition of myofibroblasts accumulation in humanized mice with foreign body-induced fibrosis in peritoneum due to treatment with anti-Siglec-8 antibodies. (1) hIgG4 isotype control indicates human IgG4 isotype control antibody; (2) c2E2 IgG4 antibody at day -1 indicates a chimeric monoclonal anti-Siglec-8 antibody with a human IgG4 isotype and murine 2E2 variable domains that was dosed one day prior to implantation of beads; (3) c2E2 IgG4 antibody at day 7 indicates a chimeric monoclonal anti-Siglec-8 antibody with a human IgG4 isotype and murine 2E2 variable domains that was dosed seven days after the implantation of beads. α -smooth muscle actin is a marker for myofibroblasts. % Fibrotic Beads indicates the number of recovered polystyrene beads associated with myofibroblasts.

[0032] FIG. 9 is a graph showing decreased lung weights in Siglec-8 transgenic mice with bleomycin-induced cutaneous fibrosis that were treated with anti-Siglec-8 antibodies. (1) Isotype indicates murine IgG1 isotype control antibody; (2) m2E2 inhibitory at day 7 indicates a murine monoclonal anti-Siglec-8 antibody with a murine IgG1 isotype that kills eosinophils and inhibits

mast cell activity. Mice were dosed starting 7 days after initial bleomycin administration; (3) m2E2 inhibitory at day -1 indicates a murine monoclonal anti-Siglec-8 antibody with a murine IgG1 isotype that kills eosinophils and inhibits mast cell activity. Mice were dosed starting 1 day before initial bleomycin administration; and (4) Naive indicates Siglec-8 transgenic mice that were not administered an antibody or bleomycin. p-values were determined by comparing the mouse IgG1 isotype control antibody study group to the m2E2 inhibitory antibody study group treated at day 7, the m2E2 inhibitory antibody study group treated at day -1 or the naive study group.

[0033] **FIG. 10A and 10B** is a series of graphs showing prevention of hydroxyproline accumulation in the skin and lungs of Siglec-8 transgenic mice with bleomycin-induced cutaneous fibrosis due to treatment an anti-Siglec-8 antibody. **FIG. 10A**) is a graph showing decreased hydroxyproline accumulation in the lung, whereas **FIG. 10B**) is showing decreased hydroxyproline in the skin of Siglec-8 transgenic mice with bleomycin-induced cutaneous fibrosis. (1) Isotype indicates murine IgG1 isotype control antibody; (2) m2E2 inhibitory at day 7 indicates a murine monoclonal anti-Siglec-8 antibody with a murine IgG1 isotype that kills eosinophils and inhibits mast cell activity. Mice were dosed starting 7 days after initial bleomycin administration; (3) m2E2 inhibitory at day -1 indicates a murine monoclonal anti-Siglec-8 antibody with a murine IgG1 isotype that kills eosinophils and inhibits mast cell activity. Mice were dosed starting 1 day before initial bleomycin administration; and (4) Naive indicates Siglec-8 transgenic mice that were not administered an antibody or bleomycin. p-values were determined by comparing the mouse IgG1 isotype control antibody study group to the m2E2 inhibitory antibody study group treated at day 7, the m2E2 inhibitory antibody study group treated at day -1 or the naive study group.

[0034] **FIG. 11A and 11B** is a series of graphs showing decreased expression of pro-fibrotic mediators in the skin lesion due to treatment with an anti-Siglec-8 antibody in Siglec-8 transgenic mice with bleomycin-induced cutaneous fibrosis. **FIG. 11A** is a graph showing decreased TGF β RNA expression and **FIG. 11B** is a graph showing decreased IL-13 RNA expression in the skin following m2E2 treatment in bleomycin-induced cutaneous fibrosis. IL-13 indicates Interleukin-13; TGF β indicates transforming growth factor beta; (1) Isotype indicates murine IgG1 isotype control antibody; (2) m2E2 inhibitory at day 7 indicates a murine monoclonal anti-Siglec-8 antibody with a murine IgG1 isotype that kills eosinophils and inhibits mast cell activity. Mice were dosed starting 7 days after initial bleomycin administration; (3) m2E2 inhibitory at day -1 indicates a murine monoclonal anti-Siglec-8 antibody with a murine IgG1 isotype that kills eosinophils and inhibits mast cell activity. Mice were dosed starting 1 day before initial bleomycin administration; and (4) Naive indicates Siglec-8 transgenic mice

that were not administered an antibody or bleomycin. p-values were determined by comparing the mouse IgG1 isotype control antibody study group to the m2E2 inhibitory antibody study group treated at day 7, the m2E2 inhibitory antibody study group treated at day -1 or the naive study group.

[0035] FIG. 12 is a graph showing a decreased trend in the visual dermal score in Siglec-8 transgenic mice with bleomycin-induced cutaneous fibrosis that were treated with anti-Siglec-8 antibodies. (1) Isotype indicates murine IgG1 isotype control antibody; (2) m2E2 inhibitory at day 7 indicates a murine monoclonal anti-Siglec-8 antibody with a murine IgG1 isotype that kills eosinophils and inhibits mast cell activity. Mice were dosed starting 7 days after initial bleomycin administration; (3) m2E2 inhibitory at day -1 indicates a murine monoclonal anti-Siglec-8 antibody with a murine IgG1 isotype that kills eosinophils and inhibits mast cell activity. Mice were dosed starting 1 day before initial bleomycin administration; and (4) Naive indicates Siglec-8 transgenic mice that were not administered an antibody or bleomycin. p-values were determined by comparing the mouse IgG1 isotype control antibody study group to the m2E2 inhibitory antibody study group treated at day 7, the m2E2 inhibitory antibody study group treated at day -1 or the naive study group.

DETAILED DESCRIPTION

I. Definitions.

[0036] Before describing the invention in detail, it is to be understood that this invention is not limited to particular compositions or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting. As used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a molecule" optionally includes a combination of two or more such molecules, and the like.

[0037] The term "about" as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se.

[0038] It is understood that aspects and embodiments of the invention described herein include "comprising," "consisting," and "consisting essentially of" aspects and embodiments.

[0038a] In the claims which follow and in the description of the invention, except where the context requires otherwise due to express language or necessary implication, the word "comprise" or variations such as "comprises" or "comprising" is used in an inclusive sense, i.e.

to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the invention.

[0039] The term “antibody” includes polyclonal antibodies, monoclonal antibodies (including full length antibodies which have an immunoglobulin Fc region), antibody compositions with polyepitopic specificity, multispecific antibodies (*e.g.*, bispecific antibodies, diabodies, and

single-chain molecules, as well as antibody fragments (*e.g.*, Fab, F(ab')₂, and Fv). The term “immunoglobulin” (Ig) is used interchangeably with “antibody” herein.

[0040] The basic 4-chain antibody unit is a heterotetrameric glycoprotein composed of two identical light (L) chains and two identical heavy (H) chains. An IgM antibody consists of 5 of the basic heterotetramer units along with an additional polypeptide called a J chain, and contains 10 antigen binding sites, while IgA antibodies comprise from 2-5 of the basic 4-chain units which can polymerize to form polyvalent assemblages in combination with the J chain. In the case of IgGs, the 4-chain unit is generally about 150,000 daltons. Each L chain is linked to an H chain by one covalent disulfide bond, while the two H chains are linked to each other by one or more disulfide bonds depending on the H chain isotype. Each H and L chain also has regularly spaced intrachain disulfide bridges. Each H chain has at the N-terminus, a variable domain (V_H) followed by three constant domains (C_H) for each of the α and γ chains and four C_H domains for μ and ϵ isotypes. Each L chain has at the N-terminus, a variable domain (V_L) followed by a constant domain at its other end. The V_L is aligned with the V_H and the C_L is aligned with the first constant domain of the heavy chain (C_{H1}). Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains. The pairing of a V_H and V_L together forms a single antigen-binding site. For the structure and properties of the different classes of antibodies, see *e.g.*, *Basic and Clinical Immunology*, 8th Edition, Daniel P. Sties, Abba I. Terr and Tristram G. Parslow (eds), Appleton & Lange, Norwalk, CT, 1994, page 71 and Chapter 6.

[0041] The L chain from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains (CH), immunoglobulins can be assigned to different classes or isotypes. There are five classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM, having heavy chains designated α , δ , ϵ , γ and μ , respectively. The γ and α classes are further divided into subclasses on the basis of relatively minor differences in the CH sequence and function, *e.g.*, humans express the following subclasses: IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. IgG1 antibodies can exist in multiple polymorphic variants termed allotypes (reviewed in Jefferis and Lefranc 2009. *mAbs* Vol 1 Issue 4 1-7) any of which are suitable for use in the invention. Common allotypic variants in human populations are those designated by the letters a, f, n, z.

[0042] An “isolated” antibody is one that has been identified, separated and/or recovered from a component of its production environment (*e.g.*, naturally or recombinantly). In some embodiments, the isolated polypeptide is free of association with all other components from its production environment. Contaminant components of its production environment, such as that

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

[0043] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations and/or post-translation modifications (*e.g.*, isomerizations, amidations) that may be present in minor amounts. In some embodiments, monoclonal antibodies have a C-terminal cleavage at the heavy chain and/or light chain. For example, 1, 2, 3, 4, or 5 amino acid residues are cleaved at the C-terminus of heavy chain and/or light chain. In some embodiments, the C-terminal cleavage removes a C-terminal lysine from the heavy chain. In some embodiments, monoclonal antibodies have an N-terminal cleavage at the heavy chain and/or light chain. For example, 1, 2, 3, 4, or 5 amino acid residues are cleaved at the N-terminus of heavy chain and/or light chain. In some embodiments, monoclonal antibodies are highly specific, being directed against a single antigenic site. In some embodiments, monoclonal antibodies are highly specific, being directed against multiple antigenic sites (such as a bispecific antibody or a multispecific antibody). The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including, for example, the hybridoma method, recombinant DNA methods, phage-display technologies, and technologies for producing human or human-like antibodies in animals that have parts or all of the human immunoglobulin loci or genes encoding human immunoglobulin sequences.

[0044] The term "naked antibody" refers to an antibody that is not conjugated to a cytotoxic moiety or radiolabel.

[0045] The terms “full-length antibody,” “intact antibody” or “whole antibody” are used interchangeably to refer to an antibody in its substantially intact form, as opposed to an antibody fragment. Specifically whole antibodies include those with heavy and light chains including an Fc region. The constant domains may be native sequence constant domains (*e.g.*, human native sequence constant domains) or amino acid sequence variants thereof. In some cases, the intact antibody may have one or more effector functions.

[0046] An “antibody fragment” comprises a portion of an intact antibody, the antigen binding and/or the variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', F(ab')₂ and Fv fragments; diabodies; linear antibodies (see U.S. Pat. No. 5,641,870, Example 2; Zapata *et al.*, *Protein Eng.* 8(10): 1057-1062 [1995]); single-chain antibody molecules and multispecific antibodies formed from antibody fragments.

[0047] Papain digestion of antibodies produced two identical antigen-binding fragments, called “Fab” fragments, and a residual “Fc” fragment, a designation reflecting the ability to crystallize readily. The Fab fragment consists of an entire L chain along with the variable region domain of the H chain (V_H), and the first constant domain of one heavy chain (C_H1). Each Fab fragment is monovalent with respect to antigen binding, *i.e.*, it has a single antigen-binding site. Pepsin treatment of an antibody yields a single large F(ab')₂ fragment which roughly corresponds to two disulfide linked Fab fragments having different antigen-binding activity and is still capable of cross-linking antigen. Fab' fragments differ from Fab fragments by having a few additional residues at the carboxy terminus of the C_H1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. F(ab')₂ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0048] The Fc fragment comprises the carboxy-terminal portions of both H chains held together by disulfides. The effector functions of antibodies are determined by sequences in the Fc region, the region which is also recognized by Fc receptors (FcR) found on certain types of cells.

[0049] “Fv” is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This fragment consists of a dimer of one heavy- and one light-chain variable region domain in tight, non-covalent association. From the folding of these two domains emanate six hypervariable loops (3 loops each from the H and L chain) that contribute the amino acid residues for antigen binding and confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three HVRs specific

for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0050] “Single-chain Fv” also abbreviated as “sFv” or “scFv” are antibody fragments that comprise the V_H and V_L antibody domains connected into a single polypeptide chain. In some embodiments, the sFv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the sFv to form the desired structure for antigen binding. For a review of the sFv, see Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

[0051] “Functional fragments” of the antibodies of the invention comprise a portion of an intact antibody, generally including the antigen binding or variable region of the intact antibody or the Fv region of an antibody which retains or has modified FcR binding capability. Examples of antibody fragments include linear antibody, single-chain antibody molecules and multispecific antibodies formed from antibody fragments.

[0052] The monoclonal antibodies herein specifically include “chimeric” antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is (are) identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Pat. No. 4,816,567; Morrison *et al.*, *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). Chimeric antibodies of interest herein include PRIMATIZED[®] antibodies wherein the antigen-binding region of the antibody is derived from an antibody produced by, *e.g.*, immunizing macaque monkeys with an antigen of interest. As used herein, “humanized antibody” is used as a subset of “chimeric antibodies.”

[0053] “Humanized” forms of non-human (*e.g.*, murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. In one embodiment, a humanized antibody is a human immunoglobulin (recipient antibody) in which residues from an HVR of the recipient are replaced by residues from an HVR of a non-human species (donor antibody) such as mouse, rat, rabbit or non-human primate having the desired specificity, affinity, and/or capacity. In some instances, FR residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications may be made to further refine antibody performance, such as binding affinity. In general, a humanized antibody will comprise substantially all of at least one, and typically two,

variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin sequence, and all or substantially all of the FR regions are those of a human immunoglobulin sequence, although the FR regions may include one or more individual FR residue substitutions that improve antibody performance, such as binding affinity, isomerization, immunogenicity, *etc.* In some embodiments, the number of these amino acid substitutions in the FR are no more than 6 in the H chain, and in the L chain, no more than 3. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see, *e.g.*, Jones *et al.*, *Nature* 321:522-525 (1986); Riechmann *et al.*, *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992). See also, for example, Vaswani and Hamilton, *Ann. Allergy, Asthma & Immunol.* 1:105-115 (1998); Harris, *Biochem. Soc. Transactions* 23:1035-1038 (1995); Hurle and Gross, *Curr. Op. Biotech.* 5:428-433 (1994); and U.S. Pat. Nos. 6,982,321 and 7,087,409. In some embodiments, humanized antibodies are directed against a single antigenic site. In some embodiments, humanized antibodies are directed against multiple antigenic sites. An alternative humanization method is described in U.S. Pat. No. 7,981,843 and U.S. Patent Application Publication No. 2006/0134098.

[0054] The “variable region” or “variable domain” of an antibody refers to the amino-terminal domains of the heavy or light chain of the antibody. The variable domains of the heavy chain and light chain may be referred to as “VH” and “VL”, respectively. These domains are generally the most variable parts of the antibody (relative to other antibodies of the same class) and contain the antigen binding sites.

[0055] The term “hypervariable region,” “HVR,” or “HV,” when used herein refers to the regions of an antibody-variable domain that are hypervariable in sequence and/or form structurally defined loops. Generally, antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). In native antibodies, H3 and L3 display the most diversity of the six HVRs, and H3 in particular is believed to play a unique role in conferring fine specificity to antibodies. See, *e.g.*, Xu *et al.* *Immunity* 13:37-45 (2000); Johnson and Wu in *Methods in Molecular Biology* 248:1-25 (Lo, ed., Human Press, Totowa, NJ, 2003)). Indeed, naturally occurring camelid antibodies consisting of a heavy chain only are functional and stable in the absence of light chain. See, *e.g.*, Hamers-Casterman *et al.*, *Nature* 363:446-448 (1993) and Sheriff *et al.*, *Nature Struct. Biol.* 3:733-736 (1996).

[0056] A number of HVR delineations are in use and are encompassed herein. The HVRs that are Kabat complementarity-determining regions (CDRs) are based on sequence variability and are the most commonly used (Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institute of Health, Bethesda, MD (1991)). Chothia HVRs

refer instead to the location of the structural loops (Chothia and Lesk *J. Mol. Biol.* 196:901-917 (1987)). The “contact” HVRs are based on an analysis of the available complex crystal structures. The residues from each of these HVRs are noted below.

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

[0057] Unless otherwise indicated, the variable-domain residues (HVR residues and framework region residues) are numbered according to Kabat *et al.*, *supra*.

[0058] “Framework” or “FR” residues are those variable-domain residues other than the HVR residues as herein defined.

[0059] The expression “variable-domain residue-numbering as in Kabat” or “amino-acid-position numbering as in Kabat,” and variations thereof, refers to the numbering system used for heavy-chain variable domains or light-chain variable domains of the compilation of antibodies in Kabat *et al.*, *supra*. Using this numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or HVR of the variable domain. For example, a heavy-chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues (*e.g.* residues 82a, 82b, and 82c, *etc.* according to Kabat) after heavy-chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a “standard” Kabat numbered sequence.

[0060] An “acceptor human framework” for the purposes herein is a framework comprising the amino acid sequence of a VL or VH framework derived from a human immunoglobulin framework or a human consensus framework. An acceptor human framework “derived from” a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain pre-existing amino acid sequence changes. In some embodiments, the number of pre-existing amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less.

[0061] “Percent (%) amino acid sequence identity” with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are

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

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

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

[0062] An antibody that “binds to”, “specifically binds to” or is “specific for” a particular polypeptide or an epitope on a particular polypeptide is one that binds to that particular polypeptide or epitope on a particular polypeptide without substantially binding to any other polypeptide or polypeptide epitope. In some embodiments, binding of an anti-Siglec-8 antibody described herein (*e.g.*, an antibody that binds to human Siglec-8) to an unrelated non-Siglec-8 polypeptide is less than about 10% of the antibody binding to Siglec-8 as measured by methods known in the art (*e.g.*, enzyme-linked immunosorbent assay (ELISA)). In some embodiments, an antibody that binds to a Siglec-8 (*e.g.*, an antibody that binds to human Siglec-8) has a dissociation constant (K_d) of $\leq 1 \mu\text{M}$, $\leq 100 \text{ nM}$, $\leq 10 \text{ nM}$, $\leq 2 \text{ nM}$, $\leq 1 \text{ nM}$, $\leq 0.7 \text{ nM}$, $\leq 0.6 \text{ nM}$, $\leq 0.5 \text{ nM}$, $\leq 0.1 \text{ nM}$, $\leq 0.01 \text{ nM}$, or $\leq 0.001 \text{ nM}$ (*e.g.* 10^{-8} M or less, *e.g.* from 10^{-8} M to 10^{-13} M , *e.g.*, from 10^{-9} M to 10^{-13} M).

[0063] The term “anti-Siglec-8 antibody” or “an antibody that binds to human Siglec-8” refers to an antibody that binds to a polypeptide or an epitope of human Siglec-8 without substantially binding to any other polypeptide or epitope of an unrelated non-Siglec-8 polypeptide.

[0064] The term “Siglec-8” as used herein refers to a human Siglec-8 protein. The term also includes naturally occurring variants of Siglec-8, including splice variants or allelic variants. The amino acid sequence of an exemplary human Siglec-8 is shown in SEQ ID NO:72. The

amino acid sequence of another exemplary human Siglec-8 is shown in SEQ ID NO:73. In some embodiments, a human Siglec-8 protein comprises the human Siglec-8 extracellular domain fused to an immunoglobulin Fc region. The amino acid sequence of an exemplary human Siglec-8 extracellular domain fused to an immunoglobulin Fc region is shown in SEQ ID NO:74. The amino acid sequence underlined in SEQ ID NO:74 indicates the Fc region of the Siglec-8 Fc fusion protein amino acid sequence.

Human Siglec-8 Amino Acid Sequence

GYLLQVQELVTVQEGLCVHVPCSFSSYPQDGWTDSDPVHGYWFRAGDRPYQDAPVATN
 NPDREVQAETQGRFQLLGDIWSNDCSLSDARKRDKGSYFFRLERGSMSKWSYKSQLN
 YKTKQLSVFVTALTHRPDILILGTLESQHSRLTCSVPWACKQGTPPMISWIGASVSSPG
 PTTARSSVLTLTPKPQDHGTSITCQVTLPGTGVTTTSTVRLDVSYPWNLTMTVFQGD
 TASTALGNGSSLSVLEGQSLRLVCAVNSNPPARLSWTRGSLTLCPSRSSNPGLLELPRVH
 VRDEGEFTCRAQNAQGSQHISLSLSLQNEGTTGSRPVSQVTLAAVGGAGATALAFLSFC
 IIFIIVRSCRKKSARPAAGVGDTGMEDAKAIRGSASQGPLETSWKDGNPLKKPPPAVAPS
 SGEEGELHYATLSFHKVKPQDPQGQEATDSEYSEIKIHKRETAETQAACLRNHNPSSEV
 RG (SEQ ID NO:72)

Human Siglec-8 Amino Acid Sequence

GYLLQVQELVTVQEGLCVHVPCSFSSYPQDGWTDSDPVHGYWFRAGDRPYQDAPVATN
 NPDREVQAETQGRFQLLGDIWSNDCSLSDARKRDKGSYFFRLERGSMSKWSYKSQLN
 YKTKQLSVFVTALTHRPDILILGTLESQHSRLTCSVPWACKQGTPPMISWIGASVSSPG
 PTTARSSVLTLTPKPQDHGTSITCQVTLPGTGVTTTSTVRLDVSYPWNLTMTVFQGD
 TASTALGNGSSLSVLEGQSLRLVCAVNSNPPARLSWTRGSLTLCPSRSSNPGLLELPRVH
 VRDEGEFTCRAQNAQGSQHISLSLSLQNEGTTGSRPVSQVTLAAVGGAGATALAFLSFC
 IIFIIVRSCRKKSARPAAGVGDTGMEDAKAIRGSASQGPLETSWKDGNPLKKPPPAVAPS
 SGEEGELHYATLSFHKVKPQDPQGQEATDSEYSEIKIHKRETAETQAACLRNHNPSSEV
 RG (SEQ ID NO:73)

Siglec-8 Fc Fusion Protein Amino Acid Sequence

GYLLQVQELVTVQEGLCVHVPCSFSSYPQDGWTDSDPVHGYWFRAGDRPYQDAPVATN
 NPDREVQAETQGRFQLLGDIWSNDCSLSDARKRDKGSYFFRLERGSMSKWSYKSQLN
 YKTKQLSVFVTALTHRPDILILGTLESQHSRLTCSVPWACKQGTPPMISWIGASVSSPG
 PTTARSSVLTLTPKPQDHGTSITCQVTLPGTGVTTTSTVRLDVSYPWNLTMTVFQGD
 TASTALGNGSSLSVLEGQSLRLVCAVNSNPPARLSWTRGSLTLCPSRSSNPGLLELPRVH

VRDEGEFTCRAQNAQGSQHISLSLSLQNEGTGTSRPVSQVTLAAVGGIEGRSDKTHTCPP
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE
PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSF
FLYSKLTVDKSRWQOGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:74)

[0065] Antibodies that “induce apoptosis” or are “apoptotic” are those that induce programmed cell death as determined by standard apoptosis assays, such as binding of annexin V, fragmentation of DNA, cell shrinkage, dilation of endoplasmic reticulum, cell fragmentation, and/or formation of membrane vesicles (called apoptotic bodies). For example, the apoptotic activity of the anti-Siglec-8 antibodies (*e.g.*, an antibody that binds to human Siglec-8) of the present invention can be showed by staining cells with annexin V.

[0066] Antibody “effector functions” refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody, and vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.*, B cell receptors); and B cell activation.

[0067] “Antibody-dependent cell-mediated cytotoxicity” or “ADCC” refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs) present on certain cytotoxic cells (*e.g.*, natural killer (NK) cells, neutrophils and macrophages) enable these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell with cytotoxins. The antibodies “arm” the cytotoxic cells and are required for killing of the target cell by this mechanism. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. Fc expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9: 457-92 (1991). In some embodiments, an anti-Siglec-8 antibody (*e.g.*, an antibody that binds to human Siglec-8) described herein enhances ADCC. To assess ADCC activity of a molecule of interest, an *in vitro* ADCC assay, such as that described in U.S. Pat. No. 5,500,362 or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and natural killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, *e.g.*, in an animal model such as that disclosed in Clynes *et al.*, *PNAS USA* 95:652-656 (1998). Other Fc variants that alter ADCC activity and other antibody properties include those disclosed by Ghetie *et al.*, *Nat Biotech.* 15:637-40, 1997; Duncan *et al.*, *Nature* 332:563-564, 1988; Lund *et al.*, *J. Immunol* 147:2657-

2662, 1991; Lund et al, Mol Immunol 29:53-59, 1992; Alegre et al, Transplantation 57:1537-1543, 1994; Hutchins et al., Proc Natl. Acad Sci USA 92:11980-11984, 1995; Jefferis et al, Immunol Lett. 44:111-117, 1995; Lund et al., FASEB J9:115-119, 1995; Jefferis et al, Immunol Lett 54:101-104, 1996; Lund et al, J Immunol 157:4963-4969, 1996; Armour et al., Eur J Immunol 29:2613-2624, 1999; Idusogie et al, J Immunol 164:4178-4184, 2000; Reddy et al, J Immunol 164:1925-1933, 2000; Xu et al., Cell Immunol 200:16-26, 2000; Idusogie et al, J Immunol 166:2571-2575, 2001; Shields et al., J Biol Chem 276:6591-6604, 2001; Jefferis et al, Immunol Lett 82:57-65, 2002; Presta et al., Biochem Soc Trans 30:487-490, 2002; Lazar et al., Proc. Natl. Acad. Sci. USA 103:4005-4010, 2006; U.S. Pat. Nos. 5,624,821; 5,885,573; 5,677,425; 6,165,745; 6,277,375; 5,869,046; 6,121,022; 5,624,821; 5,648,260; 6,194,551; 6,737,056; 6,821,505; 6,277,375; 7,335,742; and 7,317,091.

[0068] The term “Fc region” herein is used to define a C-terminal region of an immunoglobulin heavy chain, including native-sequence Fc regions and variant Fc regions. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy-chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. Suitable native-sequence Fc regions for use in the antibodies of the invention include human IgG1, IgG2, IgG3 and IgG4. A single amino acid substitution (S228P according to Kabat numbering; designated IgG4Pro) may be introduced to abolish the heterogeneity observed in recombinant IgG4 antibody. *See* Angal, S. et al. (1993) Mol Immunol 30, 105–108.

[0069] “Non-fucosylated” or “fucose-deficient” antibody refers to a glycosylation antibody variant comprising an Fc region wherein a carbohydrate structure attached to the Fc region has reduced fucose or lacks fucose. In some embodiments, an antibody with reduced fucose or lacking fucose has improved ADCC function. Non-fucosylated or fucose-deficient antibodies have reduced fucose relative to the amount of fucose on the same antibody produced in a cell line. In some embodiments, a non-fucosylated or fucose-deficient antibody composition contemplated herein is a composition wherein less than about 50% of the N-linked glycans attached to the Fc region of the antibodies in the composition comprise fucose.

[0070] The terms “fucosylation” or “fucosylated” refers to the presence of fucose residues within the oligosaccharides attached to the peptide backbone of an antibody. Specifically, a fucosylated antibody comprises α (1,6)-linked fucose at the innermost N-acetylglucosamine (GlcNAc) residue in one or both of the N-linked oligosaccharides attached to the antibody Fc region, *e.g.* at position Asn 297 of the human IgG1 Fc domain (EU numbering of Fc region residues). Asn297 may also be located about + 3 amino acids upstream or downstream of

position 297, i.e. between positions 294 and 300, due to minor sequence variations in immunoglobulins.

[0071] The "degree of fucosylation" is the percentage of fucosylated oligosaccharides relative to all oligosaccharides identified by methods known in the art *e.g.*, in an N-glycosidase F treated antibody composition assessed by matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI TOF MS). In a composition of a "fully fucosylated antibody" essentially all oligosaccharides comprise fucose residues, *i.e.* are fucosylated. In some embodiments, a composition of a fully fucosylated antibody has a degree of fucosylation of at least about 90%. Accordingly, an individual antibody in such a composition typically comprises fucose residues in each of the two N-linked oligosaccharides in the Fc region. Conversely, in a composition of a "fully non-fucosylated" antibody essentially none of the oligosaccharides are fucosylated, and an individual antibody in such a composition does not contain fucose residues in either of the two N-linked oligosaccharides in the Fc region. In some embodiments, a composition of a fully non-fucosylated antibody has a degree of fucosylation of less than about 10%. In a composition of a "partially fucosylated antibody" only part of the oligosaccharides comprise fucose. An individual antibody in such a composition can comprise fucose residues in none, one or both of the N-linked oligosaccharides in the Fc region, provided that the composition does not comprise essentially all individual antibodies that lack fucose residues in the N-linked oligosaccharides in the Fc region, nor essentially all individual antibodies that contain fucose residues in both of the N-linked oligosaccharides in the Fc region. In one embodiment, a composition of a partially fucosylated antibody has a degree of fucosylation of about 10% to about 80% (*e.g.*, about 50% to about 80%, about 60% to about 80%, or about 70% to about 80%).

[0072] "Binding affinity" as used herein refers to the strength of the non-covalent interactions between a single binding site of a molecule (*e.g.*, an antibody) and its binding partner (*e.g.*, an antigen). In some embodiments, the binding affinity of an antibody for a Siglec-8 (which may be a dimer, such as the Siglec-8-Fc fusion protein described herein) can generally be represented by a dissociation constant (*K_d*). Affinity can be measured by common methods known in the art, including those described herein.

[0073] "Binding avidity" as used herein refers to the binding strength of multiple binding sites of a molecule (*e.g.*, an antibody) and its binding partner (*e.g.*, an antigen).

[0074] An "isolated" nucleic acid molecule encoding the antibodies herein is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the environment in which it was produced. In some embodiments, the isolated nucleic acid is free of association with all components associated with the production environment. The isolated nucleic acid molecules encoding the polypeptides and

antibodies herein is in a form other than in the form or setting in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from nucleic acid encoding the polypeptides and antibodies herein existing naturally in cells.

[0075] The term “pharmaceutical formulation” refers to a preparation that is in such form as to permit the biological activity of the active ingredient to be effective, and that contains no additional components that are unacceptably toxic to an individual to which the formulation would be administered. Such formulations are sterile.

[0076] “Carriers” as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers that are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™.

[0077] As used herein, the term “treatment” or “treating” refers to clinical intervention designed to alter the natural course of the individual or cell being treated during the course of clinical pathology. Desirable effects of treatment include decreasing the rate of disease progression, ameliorating or palliating the disease state, and remission or improved prognosis. An individual is successfully “treated”, for example, if one or more symptoms associated with a disorder (*e.g.*, a fibrotic disease) are mitigated or eliminated. For example, an individual is successfully “treated” if treatment results in increasing the quality of life of those suffering from a disease, decreasing the dose of other medications required for treating the disease, reducing the frequency of recurrence of the disease, lessening severity of the disease, delaying the development or progression of the disease, and/or prolonging survival of individuals.

[0078] As used herein, “in conjunction with” refers to administration of one treatment modality in addition to another treatment modality. As such, “in conjunction with” refers to administration of one treatment modality before, during or after administration of the other treatment modality to the individual.

[0079] As used herein, the term “prevention” or “preventing” includes providing prophylaxis with respect to occurrence or recurrence of a disease in an individual. An individual may be predisposed to, susceptible to a disorder, or at risk of developing a disorder, but has not yet been

diagnosed with the disorder. In some embodiments, anti-Siglec-8 antibodies (*e.g.*, an antibody that binds to human Siglec-8) described herein are used to delay development of a disorder (*e.g.*, a fibrotic disease).

[0080] As used herein, an individual “at risk” of developing a disorder (*e.g.*, a fibrotic disease) may or may not have detectable disease or symptoms of disease, and may or may not have displayed detectable disease or symptoms of disease prior to the treatment methods described herein. “At risk” denotes that an individual has one or more risk factors, which are measurable parameters that correlate with development of the disease (*e.g.*, a fibrotic disease), as known in the art. An individual having one or more of these risk factors has a higher probability of developing the disorder than an individual without one or more of these risk factors.

[0081] An “effective amount” refers to at least an amount effective, at dosages and for periods of time necessary, to achieve the desired or indicated effect, including a therapeutic or prophylactic result. An effective amount can be provided in one or more administrations. A “therapeutically effective amount” is at least the minimum concentration required to effect a measurable improvement of a particular disorder. A therapeutically effective amount herein may vary according to factors such as the disease state, age, sex, and weight of the patient, and the ability of the antibody to elicit a desired response in the individual. A therapeutically effective amount may also be one in which any toxic or detrimental effects of the antibody are outweighed by the therapeutically beneficial effects. A “prophylactically effective amount” refers to an amount effective, at the dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically but not necessarily, since a prophylactic dose is used in individuals prior to or at the earlier stage of disease, the prophylactically effective amount can be less than the therapeutically effective amount.

[0082] “Chronic” administration refers to administration of the medicament(s) in a continuous as opposed to acute mode, so as to maintain the initial therapeutic effect (activity) for an extended period of time. “Intermittent” administration is treatment that is not consecutively done without interruption, but rather is cyclic in nature.

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

II. Compositions and Methods

A. Methods of the Invention

[0084] Provided herein are methods for treating or preventing a fibrotic disease (*e.g.*, idiopathic pulmonary fibrosis) in an individual comprising administering to the individual an effective amount of an antibody described herein that binds to human Siglec-8 (*e.g.*, an anti-Siglec-8 antibody), or compositions thereof. Also provided herein are methods for treating or preventing a fibrotic disease (*e.g.*, idiopathic pulmonary fibrosis) in an individual comprising administering to the individual an effective amount of an agonist described herein that binds to human Siglec-8 (*e.g.*, a 6'-sulfo-sLe^X-containing agonist or agonist antibodies), or compositions thereof. In some embodiments, the individual (*e.g.*, a human) has been diagnosed with a fibrotic disease (*e.g.*, idiopathic pulmonary fibrosis) or is at risk of developing the fibrotic disease. Non-limiting examples of fibrotic diseases that are treatable with the antibodies and agonists, and compositions thereof, of the present invention include pulmonary fibrosis (*e.g.*, idiopathic pulmonary fibrosis), hepatic fibrosis (*e.g.*, cirrhosis), renal fibrosis (*e.g.*, renal interstitial fibrosis), cardiac fibrosis (*e.g.*, endomyocardial fibrosis and atrial fibrosis), spleen fibrosis, ocular fibrosis, mechanical-induced fibrosis (*e.g.*, ventilator-induced pulmonary fibrosis), implant-induced fibrosis, radiation-induced fibrosis, drug-induced fibrosis (*e.g.*, bleomycin-induced pulmonary fibrosis), viral-induced fibrosis, cystic fibrosis, cancer-associated fibrosis, atherosclerosis, bone marrow fibrosis (*e.g.*, myelofibrosis), scleroderma (*e.g.*, systemic sclerosis), mediastinal fibrosis and retroperitoneal cavity fibrosis. In some embodiments, the fibrotic disease is a mast cell-mediated disorder.

[0085] Also provided herein are methods for treating or preventing a pre-fibrotic disease (*e.g.*, chronic hypersensitivity pneumonitis) in an individual comprising administering to the individual an effective amount of an antibody described herein that binds to human Siglec-8 (*e.g.*, an anti-Siglec-8 antibody), or compositions thereof. Also provided herein are methods for treating or preventing a pre-fibrotic disease (*e.g.*, chronic hypersensitivity pneumonitis) in an individual comprising administering to the individual an effective amount of an agonist described herein that binds to human Siglec-8 (*e.g.*, a 6'-sulfo-sLe^X-containing agonist or agonist antibodies), or compositions thereof. In some embodiments, the individual (*e.g.*, a human) has been diagnosed with a pre-fibrotic disease (*e.g.*, chronic hypersensitivity pneumonitis) or is at risk of developing a fibrotic disease (*e.g.*, idiopathic pulmonary fibrosis). Non-limiting examples of pre-fibrotic diseases that are treatable with the antibodies and agonists, and compositions thereof, of the present invention include bleomycin-induced pneumonitis, chronic hypersensitivity pneumonitis, polycythemia vera, essential thrombocythemia, age-related macular degeneration, diabetic retinopathy, neovascular

glaucoma, retinopathy of prematurity, and proliferative vitreoretinopathy. In some embodiments, the pre-fibrotic disease is a mast cell-mediated disorder.

[0086] Fibrotic disease is characterized by excessive connective tissue accumulation and tissue contraction that can lead to progressive deterioration in the normal structure and function of organs and tissues. Fibrotic disease generally arises due to a normal wound-healing process that has gone awry. After tissue injury, the normal wound-healing process involves epithelial and/or endothelial cells which release inflammatory mediators that initiate an antifibrinolytic-coagulation cascade, which triggers blood clot formation. This is followed by an inflammatory and proliferative phase, wherein leukocytes are recruited and then activated and induced to proliferate by chemokines and growth factors. The activated leukocytes secrete profibrotic cytokines such as IL-13 and TGF- β . Stimulated epithelial cells, endothelial cells, and myofibroblasts also produce matrix metalloproteinases (MMPs), and additional cytokines and chemokines that recruit and activate neutrophils, macrophages, T cells, B cells, and eosinophils, which are important components of reparative tissue. Shortly after the initial inflammatory phase, myofibroblasts produce extracellular matrix (ECM) components, and endothelial cells form new blood vessels. In the subsequent remodeling phase, the activated myofibroblasts stimulate wound contraction. Collagen fibers also become more organized, blood vessels are restored to normal, scar tissue is eliminated, and epithelial and/or endothelial cells divide and migrate over the basal layers to regenerate the epithelium or endothelium, respectively, restoring the damaged tissue to its normal appearance. In the case of chronic wounds, this normal wound-healing process is disrupted. Persistent inflammation, tissue necrosis, and infection lead to chronic myofibroblast activation and excessive accumulation of ECM components, which promotes the formation of a fibrotic scar. *See* Wynn T.A., *J Clin Invest.*, 2007, 114(3):524-529. There are many fibrotic diseases of the organs and tissues that are caused by a variety of injuries. Injuries that lead to fibrosis include, but are not limited to, infection by an infectious agent (*e.g.*, bacteria, virus, fungi and multicellular parasites), mechanical stress (*e.g.*, respiratory ventilator), exposure to environmental agents (*e.g.*, dust, mold, asbestos, etc.), radiation exposure, drug exposure (*e.g.*, chemotherapeutic drugs), and pre-fibrotic diseases that are characterized by chronic injury that leads to abnormal wound healing of tissues and/or organs. Although there are various types of fibrotic diseases, they all contain a common hallmark of disease that is well known to one of skill in the art, accumulation of excess ECM components. *See* Wynn et al., *Nature Medicine*, 2012, 18:1028-1040.

[0087] In some embodiments, provided herein is a method for treating or preventing fibrotic disease of an organ in an individual comprising administering to the individual an effective amount of an antibody (*e.g.*, an anti-Siglec-8 antibody) or an agonist (*e.g.*, a 6'-sulfo-sLe^X-

containing agonist) that binds to human Siglec-8. In some embodiments, the fibrotic disease of an organ is selected from the group consisting of: pulmonary fibrosis, hepatic fibrosis, renal fibrosis, cardiac fibrosis, spleen fibrosis, and ocular fibrosis. In some embodiments, the pulmonary fibrosis is, but is not limited to, idiopathic pulmonary fibrosis (IPF), idiopathic nonspecific interstitial pneumonitis (NSIP), cryptogenic organizing pneumonia (COP), Hamman-Rich syndrome (also known as acute interstitial pneumonia), lymphocytic interstitial pneumonitis (LIP), respiratory bronchiolitis interstitial lung disease, desquamative interstitial pneumonitis or idiopathic lymphoid interstitial pneumonia, or idiopathic pleuroparenchymal fibroelastosis. In some embodiments, the pulmonary fibrosis is idiopathic pulmonary fibrosis. In some embodiments, the pulmonary fibrosis is associated with chronic obstructive pulmonary disease. In some embodiments, the hepatic fibrosis is, but is not limited to, cirrhosis, alcohol-induced hepatic fibrosis, hepatitis C virus (HCV)-induced hepatic fibrosis, hepatitis B virus (HBV)-induced fibrosis, or nonalcoholic steatohepatitis. In some embodiments, the renal fibrosis is, but is not limited to, renal interstitial fibrosis or glomerulosclerosis. In some embodiments, the cardiac fibrosis is, but is not limited to, endomyocardial fibrosis or atrial fibrosis.

[0088] In some embodiments, provided herein is a method for treating or preventing fibrotic disease induced by an environmental agent in an individual comprising administering to the individual an effective amount of an antibody (*e.g.*, an anti-Siglec-8 antibody) or an agonist (*e.g.*, a 6'-sulfo-sLe^X-containing agonist) that binds to human Siglec-8. In some embodiments, the fibrotic disease induced by an environmental agent is selected from the group consisting of: mechanical-induced fibrosis, implant-induced fibrosis, radiation-induced fibrosis, drug-induced fibrosis, and viral-induced fibrosis. In some embodiments, the mechanical-induced fibrosis is, but is not limited to, ventilator-induced pulmonary fibrosis. In some embodiments, the drug-induced fibrosis is, but is not limited to, bleomycin-induced pulmonary fibrosis, methotrexate-induced fibrosis, and cyclophosphamide-induced fibrosis, amiodarone-induced fibrosis, propranolol-induced fibrosis, nitrofurantoin-induced fibrosis, and sulfasalazine-induced fibrosis. In some embodiments, the viral-induced fibrosis is, but is not limited to, HCV-induced fibrosis, HBV-induced fibrosis, TT virus-induced fibrosis, adenovirus-induced fibrosis, human cytomegalovirus-induced fibrosis, and Epstein-Barr virus-induced fibrosis.

[0089] In some embodiments, provided herein is a method for treating or preventing fibrotic disease in an individual comprising administering to the individual an effective amount of an antibody (*e.g.*, an anti-Siglec-8 antibody) or an agonist (*e.g.*, a 6'-sulfo-sLe^X-containing agonist) that binds to human Siglec-8. In some embodiments herein, the fibrotic disease is selected from the group consisting of: cystic fibrosis, cancer-associated fibrosis, atherosclerosis, bone marrow

fibrosis, scleroderma, mediastinal fibrosis and retroperitoneal cavity fibrosis. In some embodiments, the fibrotic disease is associated with a cancer (*i.e.*, a cancer-associated fibrosis). In some embodiments, the cancer is, but is not limited to, sarcomas such as fibrosarcoma, malignant fibrous histiocytoma, liposarcoma, rhabdomyosarcoma, leiomyosarcoma, angiosarcoma, Kaposi's sarcoma, lymphangiosarcoma, synovial sarcoma, chondrosarcoma, and osteosarcoma, brain tumor, head and neck carcinoma, breast carcinoma, lung carcinoma, esophageal carcinoma, stomach carcinoma, duodenal carcinoma, appendiceal carcinoma, colon carcinoma, rectal carcinoma, hepatic carcinoma, pancreatic carcinoma, gallbladder carcinoma, bile duct carcinoma, anal carcinoma, kidney carcinoma, ureteral carcinoma, bladder carcinoma, prostate carcinoma, penile carcinoma, testicular carcinoma, uterine carcinoma, ovarian carcinoma, vulvar carcinoma, vaginal carcinoma, and skin carcinoma and, furthermore, leukemia, or malignant lymphoma. In some embodiments, the cancer-associated fibrosis is present at, but not limited to, the brain, head and neck, breast, limbs, lung, heart, thymus, esophagus, stomach, small intestine (duodenum, jejunum, ileum), large intestine (colon, cecum, appendix, rectum), liver, pancreas, gallbladder, anus, kidney, ureter, bladder, prostate, penis, testis, uterus, ovary, vulva, vagina, skin, striated muscle, smooth muscle, synovial membrane, cartilage, bone, thyroid, adrenal gland, peritoneum, mesentery, bone marrow, blood, vascular system, lymphatic system such as lymph nodes, or lymphatic fluid. See U.S. Patent No. 8,686,052. In some embodiments, the fibrotic disease is a bone marrow fibrosis. In a further embodiment, the bone marrow fibrosis is, but is not limited to, myelofibrosis. In some embodiments, the fibrotic disease is scleroderma. In a further embodiment, the scleroderma is localized scleroderma or systemic scleroderma. In a further embodiment, the scleroderma is, but is not limited to, systemic morphea, linear scleroderma, systemic sclerosis, limited scleroderma or diffuse scleroderma. In some embodiments, the fibrotic disease is Dupuytren's contracture.

[0090] Types of fibrotic disease and methods of diagnosing fibrotic disease are well known to one of skill in the art. For example, symptoms of idiopathic pulmonary fibrosis (IPF) include, but are not limited to, shortness of breath, dry cough, weight loss, fatigue, malaise, muscle pain, joint pain, and finger and toe clubbing. IPF disease progression can be monitored with techniques such as high-resolution CT (HRCT) scanning. In a diagnosis by HRCT, the presence of the following characteristics is noted: (1) presence of reticular abnormality and/or traction bronchiectasis with basal and peripheral predominance; (2) presence of honeycombing with basal and peripheral predominance; and (3) absence of atypical features such as micronodules, peribronchovascular nodules, consolidation, isolated (non-honeycomb) cysts, ground glass attenuation (or, if present, is less extensive than reticular opacity), and mediastinal adenopathy (or, if present, is not extensive enough to be visible on chest x-ray). A diagnosis of definite IPF

is made if characteristics (1), (2), and (3) are met. A diagnosis of probable IPF is made if characteristics (1) and (3) are met. Pulmonary function can also be monitored in IPF patients to determine progression of disease. Pulmonary function values are well known in the art. The following is an example of pulmonary function values that may be used. The values include, but are not limited to, vital capacity (VC), residual volume (V), forced expiratory volume (FEV_1), forced vital capacity (FVC), forced expiratory flow (FEF), peak expiratory flow rate (PEFR), inspiratory reserve volume (IRV), functional residual capacity (FRC), inspirator capacity (IC), total lung capacity (TLC), expiratory reserve volume (ERV), tidal volume (TV) and maximum voluntary ventilation (MVV). FEV_1 measures the volume of air exhaled over a pre-determined period of time by a forced expiration immediately after a full inspiration. FVC measures the total volume of air exhaled immediately after a full inspiration. FEF measures the volume of air exhaled during a FVC divided by the time in seconds. PEFR measures the maximum flow rate during a forced exhale starting from full inspiration. V is the volume of air remaining in the lungs after a full expiration. Lung volumes are usually reduced as IPF progresses. Lung volumes can be measured by determining functional residual capacity (FRC), total lung capacity (TLC), and residual value (V). Patients with IPF also develop more rapid shallow breaths as the diseases progresses, and therefore the work of breathing is increased. Expiratory flow rates, forced expiratory volume (FEV_1), and forced vital capacity (FVC) are often decreased because of the reduction in lung volume, but the FEV_1 -to-FVC ratio is maintained or increased in IPF. Gas exchange at rest and during exercise as well as pulmonary hemodynamics can also be pulmonary functions that are tested to diagnose and monitor IPF. See *American Journal of Respiratory and Critical Care Medicine*, 2000, 161(2):646-664; Travis et al., *Am J Respir Crit Care Med*, 2013, 188(6):733-748; and U.S. Patent Number 8,247,379.

[0091] As another example of fibrotic disease, pulmonary fibrosis can be associated with chronic obstructive pulmonary disease (COPD). COPD is characterized by the presence of chronic bronchitis and/or emphysema. Lung tissue remodeling in COPD involves processes such as epithelial disruption, smooth muscle hypertrophy, smooth muscle hyperplasia, airway wall fibrosis, and alveolar destruction. See Salazar et al., *Lung*, 2011, 189(2):101-109. In addition to some individuals with COPD having fibrosis due to lung tissue remodeling, a subpopulation of individuals with COPD can also have pulmonary fibrosis. For example, idiopathic pulmonary fibrosis (IPF) is now recognized to co-exist with COPD with a recent study finding the prevalence of IPF in people with COPD is as high as 6%. See Divo et al., *Am J Respir Crit Care Med.*, 2012, 186(2):155-161. Variants of pulmonary fibrosis associated with emphysema have also been described, also known as combined pulmonary fibrosis and emphysema (CPFE), which is characterized by exertional dyspnea, upper-lobe emphysema and

lower-lobe fibrosis, preserved lung volume, diminished capacity of gas exchange and poor survival. See Cottin et al., *Eur. Respir. J.*, 2005, 26(4):586-593.

[0092] As another representative example of fibrotic disease, hepatic fibrosis can be diagnosed by assessing liver enzyme levels that have been traditionally used to assess liver disease, such as serum alanine aminotransferase (ALT) and aspartate amino transferase (AST). An AST/ALT (AAR) ratio of >1 is predictive of cirrhosis. See Bataller et al., *J Clin Invest.*, 2005, 115(2):209-218.

[0093] In another example, in cardiac fibrosis, fibrotic tissues accumulate in the heart and blood vessels as a result of hypertension, hypertensive heart disease, atherosclerosis, and myocardial infarction. These cardiovascular diseases all exhibit an accumulation of extra-cellular matrix or fibrotic deposition which results in stiffening of the vasculature and stiffening of the cardiac tissue itself. Typically the symptoms of cardiac fibrosis relate to the specific chambers and valves where the disease is most extensive. Symptoms of cardiac fibrosis include, but are not limited to, lower extremity swelling, increasing abdominal girth, dyspnea, fatigue, nausea, chest pain, palpitations and fainting. See U.S. Patent No. 8,461,303.

[0094] In another example of fibrotic disease, individuals may require mechanical ventilation to improve oxygenation and facilitate organ repair when recovering from illnesses such as sepsis, acute lung injury and acute respiratory distress syndrome. Evidence from experimental and clinical studies suggests that mechanical ventilation can cause lung fibrosis. There is a need to prevent or treat pulmonary fibrosis associated with mechanical ventilation. Mechanical, drug (*e.g.*, bleomycin), and radiation-induced fibrosis exhibit similar cellular mechanisms, namely activated myofibroblasts which produce collagen. This indicates that a therapeutic agent that can effectively treat drug-induced fibrosis can be effective for treating mechanical-induced fibrosis and radiation-induced fibrosis. See Cabrera-Benitez et al., *Anesthesiology*, 2014, 121:189-198; Villar et al., *Critical Care*, 2015, 19:138; and Schaefer et al., *Eur Respir Rev.*, 2011, 20(120):85-97.

[0095] In another example of fibrotic disease, implant-induced fibrosis occurs when an implant is placed in the body and encounters a foreign body response from the surrounding host tissue. The response is characterized by the infiltration of inflammatory cells to the area to destroy or remove the implant, followed by the repair and regeneration of injured tissue. If the implant cannot be removed, the inflammatory response persists until the implant becomes encapsulated in a layer of fibrotic connective tissue that shields it from the immune system. Fibrosis due to the implant or fibrous encapsulation of the implant can compromise the efficiency of the implant and can lead to implant failure. See Rolfe B. et al., *Regenerative Medicine and Tissue Engineering-Cells and Biomaterials, InTech*, 2011, pages 551-568. In

some embodiments, implant-induced fibrosis occurs in an individual after the individual has been implanted with an implant during a medical procedure. In some embodiments, implant-induced fibrosis occurs in an individual after the individual has been implanted with an implant during a cosmetic procedure. An implant contemplated herein includes, but is not limited to, an implant used for cosmetic surgery (*e.g.*, breast augmentation, breast reconstruction, etc.), joint replacement surgery (*e.g.*, hip joint replacement, knee joint replacement, shoulder joint replacement, ankle joint replacement, etc.), hernia repair surgery, graft surgery (*e.g.*, artificial vascular graft surgery, skin graft surgery, etc.), stent placement, organ transplant surgery, neurosurgery, heart surgery, or any other medical surgery. An implant contemplated herein also includes, but is not limited to, an orthopedic implant, a dental implant, a breast implant, a chin implant, a cheek implant, a buttock implant, nose implant, penile implant, a pacemaker, a suture, a graft (*e.g.*, a vascular graft, skin graft, a bone graft, etc.), a heart valve, intraocular lens, a controlled drug delivery device, a biosensor, a stent (*e.g.*, gastrointestinal stent, tracheal stent, bronchial stent, urinary stent, ear stent, nose stent, etc.), a surgical mesh or film, a biomaterial, a medical device, a catheter, glaucoma drainage device, a pressure monitor device, contraceptive implant, an artificial joint, an organ transplant (*e.g.*, kidney transplant, lung transplant, heart transplant, etc.) or any other medical implant or cosmetic implant. In some embodiments, the graft is an autograft, an isograft, an allograft or a xenograft. In some embodiments, the antibody (*e.g.*, an anti-Siglec-8 antibody) or an agonist (*e.g.*, a 6'-sulfo-sLe^X-containing agonist) that binds to human Siglec-8 contemplated herein can be administered to an individual before and/or after implantation of an implant contemplated herein in the individual. In some embodiments, the antibody (*e.g.*, an anti-Siglec-8 antibody) or an agonist (*e.g.*, a 6'-sulfo-sLe^X-containing agonist) that binds to human Siglec-8 contemplated herein can be administered to an individual during implantation of an implant contemplated herein in the individual. In some embodiments, the antibody (*e.g.*, an anti-Siglec-8 antibody) or an agonist (*e.g.*, a 6'-sulfo-sLe^X-containing agonist) that binds to human Siglec-8 contemplated herein can be administered to an individual at about the same time the individual is implanted with an implant contemplated herein. In some embodiments, an implant contemplated herein can be coated with the antibody (*e.g.*, an anti-Siglec-8 antibody) or an agonist (*e.g.*, a 6'-sulfo-sLe^X-containing agonist) that binds to human Siglec-8. In some embodiments, an implant contemplated herein can be embedded with the antibody (*e.g.*, an anti-Siglec-8 antibody) or an agonist (*e.g.*, a 6'-sulfo-sLe^X-containing agonist) that binds to human Siglec-8.

[0096] In another example of fibrotic disease, scleroderma is an autoimmune, rheumatic, and chronic disease that affects the body by hardening of connective tissue, such as in the skin, fingers or toes, digestive system, heart, lungs and kidneys. There are two major types of

scleroderma, localized scleroderma and systemic scleroderma. Skin lesions appear in both types of scleroderma and appear in different shapes and colors. Skin lesions are considered to be a part of the skin that has an abnormal growth or appearance compared to the skin around it. Morphea, a form of localized scleroderma, is characterized by waxy patches on the skin of various sizes and color. Linear scleroderma, also a form of localized scleroderma, starts as a streak or line of hardened waxy skin, usually on an arm, leg or head and neck area. In systemic sclerosis, hardening of connective tissue can occur in the internal systems of the body and result in major organ dysfunction. Limited scleroderma or diffuse scleroderma are two types of systemic sclerosis and are based on the extent of skin tightening. *See LeRoy et al., J Rheumatol.*, 1988, 15:202-205. In limited scleroderma, skin tightening is confined to the fingers, hands, and forearms distal to the elbows, with or without tightening of skin of the feet and of the legs distal to the knees. In diffuse disease, the skin of the proximal extremities and trunk is also involved. Symptoms of scleroderma can include, but are not limited to, joint pain, skin rashes, swollen blood vessels in the skin, skin ulcers, weight loss, tightened and hardened fingers, acid reflux, chronic cough, difficulty swallowing, dry mouth, fatigue, headache, joint stiffness, sensitivity to cold, or shortness of breath. In some of the embodiments herein, the fibrotic disease is scleroderma. In a further embodiment, the scleroderma is localized scleroderma or systemic scleroderma. In another further embodiment, the scleroderma is, but is not limited to, systemic morphea, linear scleroderma, systemic sclerosis, limited scleroderma or diffuse scleroderma.

[0097] In some embodiments, provided herein is a method for treating or preventing pre-fibrotic disease in an individual comprising administering to the individual an effective amount of an antibody (*e.g.*, an anti-Siglec-8 antibody) or an agonist (*e.g.*, a 6'-sulfo-sLe^X-containing agonist) that binds to human Siglec-8. In some embodiments, the pre-fibrotic disease is bleomycin-induced pneumonitis. In some embodiments, the pre-fibrotic disease is chronic hypersensitivity pneumonitis. In some embodiments, the pre-fibrotic disease is polycythemia vera. In some embodiments, the pre-fibrotic disease is essential thrombocythemia. In some embodiments, the pre-fibrotic disease is a pre-fibrotic disease of the eye. In some embodiments, the pre-fibrotic disease is selected from the group consisting of: age-related macular degeneration, diabetic retinopathy, neovascular glaucoma, retinopathy of prematurity, and proliferative vitreoretinopathy.

[0098] Types of pre-fibrotic disease and methods of diagnosing pre-fibrotic disease are well known to one of skill in the art. Pre-fibrotic disease as contemplated herein includes a disease that results in an individual to be more susceptible to or be at risk of developing a fibrotic disease, such as a fibrotic disease contemplated herein. For example, chronic hypersensitivity

pneumonitis is a pre-fibrotic disease in which lungs become inflamed from breathing in foreign substances such as mold, dust, and chemicals. Symptoms of chronic hypersensitivity pneumonitis include, but are not limited to, cough, shortness of breath, fatigue, weight loss, and finger or toe clubbing. Chronic hypersensitivity pneumonitis can cause long-term lung damage such as pulmonary fibrosis.

[0099] In another example of pre-fibrotic disease, polycythemia vera is a pre-fibrotic disease of the bone marrow that leads to an abnormal increase in the number of blood cells. Symptoms of polycythemia vera include, but are not limited to, trouble breathing when lying down, dizziness, excess bleeding, full feeling in the left upper abdomen due to enlarged spleen, headache, itchiness, red skin coloring, shortness of breath, bluish skin color, fatigue, red skin spots, vision problems, and phlebitis. A complication of polycythemia vera is the development of the fibrotic disease, myelofibrosis, a progressive bone marrow disorder that results in bone marrow scarring, severe anemia, and enlargement of liver and spleen.

[0100] In another representative example of pre-fibrotic disease, essential thrombocythemia is a pre-fibrotic disease in which the body produces too many blood platelets. Symptoms of essential thrombocythemia include, but are not limited to, headache, dizziness, chest pain, weakness, fainting, temporary vision changes, numbness or tingling of the hands and feet, pain in hands and feet, mildly enlarged spleen, nosebleeds, bruising, bleeding from mouth and gums, and bloody stool. A complication of essential thrombocythemia is the development of the fibrotic disease, myelofibrosis.

[0101] The retina of the eye consists of multiple layers of neurons, blood vessels, ECM, and various resident and transient cells such as glial cells and monocytes. The vascular supply of the retina consists of the retinal blood vessels and the choriocapillaris. The photoreceptors are in the outermost portion of the neurosensory retina and rest on a monolayer of cells, the retinal pigmented epithelium (RPE). The RPE rests on a collagenous basement membrane (Bruch membrane), and directly beneath this structure flows the choriocapillaris, providing blood supply for the outer third of the retina. Diseases that lead to vision loss can be as a result of abnormalities in the retinal or choroidal vasculature. These diseases, characterized by macula edema, retinal and vitreous hemorrhage, and fibrovascular scarring, include age-related macular degeneration, diabetic retinopathy, neovascular glaucoma, retinopathy of prematurity, and neovascular glaucoma. The final common pathophysiological denominator in all of these diseases is the retinal response to injury, with chronic wound healing leading to fibrosis. *See* Friedlander, M., *J Clin Invest.*, 2007, 117(3):576-586.

[0102] Methodologies and assays known in the art and described herein can be used for assessment of any fibrotic disease (*e.g.*, idiopathic pulmonary fibrosis) or any pre-fibrotic

disease described herein (*e.g.*, chronic hypersensitivity pneumonitis) or symptom of a fibrotic disease or a pre-fibrotic disease described herein.

[0103] In some embodiments of the methods provided herein, the method further comprises a step of diagnosing an individual (*e.g.*, a patient) with a fibrotic disease (*e.g.*, idiopathic pulmonary fibrosis), selecting an individual (*e.g.*, a patient) with a fibrotic disease (*e.g.*, idiopathic pulmonary fibrosis) for treatment, and/or determining if an individual (*e.g.*, a patient) has a fibrotic disease (*e.g.*, idiopathic pulmonary fibrosis). In some embodiments, the method further comprises a step of diagnosing an individual with a fibrotic disease, selecting an individual with a fibrotic disease for treatment, and/or determining if an individual has a fibrotic disease before treating or preventing a fibrotic disease (*e.g.*, idiopathic pulmonary fibrosis) in the individual, wherein the method comprises administering an effective amount of an antibody (*e.g.*, an anti-Siglec-8 antibody) or an agonist (*e.g.*, a 6'-sulfo-sLe^X-containing agonist) that binds to human Siglec-8. In some embodiments, the method further comprises a step of diagnosing an individual with a fibrotic disease, selecting an individual with a fibrotic disease for treatment, and/or determining if an individual has a fibrotic disease after treating or preventing a fibrotic disease (*e.g.*, idiopathic pulmonary fibrosis) in the individual, wherein the method comprises administering an effective amount of an antibody (*e.g.*, an anti-Siglec-8 antibody) or an agonist (*e.g.*, a 6'-sulfo-sLe^X-containing agonist) that binds to human Siglec-8.

[0104] In some embodiments of the methods provided herein, the method further comprises a step of diagnosing an individual (*e.g.*, a patient) with a pre-fibrotic disease (*e.g.*, chronic hypersensitivity pneumonitis), selecting an individual (*e.g.*, a patient) with a pre-fibrotic disease (*e.g.*, chronic hypersensitivity pneumonitis) for treatment, and/or determining if an individual (*e.g.*, a patient) has a pre-fibrotic disease (*e.g.*, chronic hypersensitivity pneumonitis). In some embodiments, the method further comprises a step of diagnosing an individual with a pre-fibrotic disease, selecting an individual with a pre-fibrotic disease for treatment, and/or determining if an individual has a pre-fibrotic disease before treating or preventing a pre-fibrotic disease (*e.g.*, chronic hypersensitivity pneumonitis) in the individual, wherein the method comprises administering an effective amount of an antibody (*e.g.*, an anti-Siglec-8 antibody) or an agonist (*e.g.*, a 6'-sulfo-sLe^X-containing agonist) that binds to human Siglec-8. In some embodiments, the method further comprises a step of diagnosing an individual with a pre-fibrotic disease, selecting an individual with a pre-fibrotic disease for treatment, and/or determining if an individual has a pre-fibrotic disease after treating or preventing a pre-fibrotic disease (*e.g.*, chronic hypersensitivity pneumonitis) in the individual, wherein the method comprises administering an effective amount of an antibody (*e.g.*, an anti-Siglec-8 antibody) or an agonist (*e.g.*, a 6'-sulfo-sLe^X-containing agonist) that binds to human Siglec-8.

[0105] In some embodiments, provided herein is a method for treating or preventing a fibrotic disease (*e.g.*, idiopathic pulmonary fibrosis) in an individual comprising administering to the individual an effective amount of an antibody (*e.g.*, an anti-Siglec-8 antibody) or an agonist (*e.g.*, a 6'-sulfo-sLe^X-containing agonist) that binds to human Siglec-8. In some embodiments, the fibrotic disease is selected from the group consisting of: pulmonary fibrosis, hepatic fibrosis, renal fibrosis, cardiac fibrosis, spleen fibrosis, and ocular fibrosis. In some embodiments, the pulmonary fibrosis is idiopathic pulmonary fibrosis. In some embodiments, the pulmonary fibrosis is associated with chronic obstructive pulmonary disease. In some embodiments, the fibrotic disease is selected from the group consisting of: mechanical-induced fibrosis, implant-induced fibrosis, radiation-induced fibrosis, drug-induced fibrosis, and viral-induced fibrosis. In some embodiments, the mechanical-induced fibrosis is ventilator-induced pulmonary fibrosis. In some embodiments, the drug-induced fibrosis is bleomycin-induced pulmonary fibrosis. In some embodiments, the fibrotic disease is selected from the group consisting of: cystic fibrosis, cancer-associated fibrosis, atherosclerosis, bone marrow fibrosis, scleroderma, mediastinal fibrosis and retroperitoneal cavity fibrosis. In some embodiments, the bone marrow fibrosis is myelofibrosis. In some embodiments, the scleroderma is systemic fibrosis. In some embodiments, the scleroderma is systemic sclerosis. In some of the embodiments herein, one or more symptom in an individual with fibrotic disease (*e.g.*, idiopathic pulmonary fibrosis) is reduced or improved (*e.g.*, a reference value) relative to baseline after administration of the antibody (*e.g.*, an anti-Siglec-8 antibody) or the agonist (*e.g.*, a 6'-sulfo-sLe^X-containing agonist) that binds to human Siglec-8. In a further embodiment, the one or more symptom can be a symptom associated with a fibrotic disease disclosed herein, such as, but not limited to, shortness of breath, dry cough, weight loss, fatigue, malaise, finger or toe clubbing, muscle pain, or joint pain. In some embodiments, one or more pulmonary function in the individual with pulmonary fibrosis (*e.g.*, idiopathic pulmonary fibrosis) is increased by at least 5% (*e.g.*, a reference value) relative to baseline after administration of the antibody (*e.g.*, an anti-Siglec-8 antibody) or the agonist (*e.g.*, a 6'-sulfo-sLe^X-containing agonist) that binds to human Siglec-8. In a further embodiment, the one or more pulmonary function is selected from the group consisting of: vital capacity (VC), residual volume (V), forced expiratory volume (FEV₁), forced vital capacity (FVC), forced expiratory flow (FEF), peak expiratory flow rate (PEFR), inspiratory reserve volume (IRV), functional residual capacity (FRC), inspirator capacity (IC), total lung capacity (TLC), expiratory reserve volume (ERV), tidal volume (TV) and maximum voluntary ventilation (MVV). In some embodiments, one or more pathologic parameter in the individual with fibrotic disease (*e.g.*, idiopathic pulmonary fibrosis) is reduced by at least 5% (*e.g.*, a reference value) relative to baseline after administration of the antibody (*e.g.*, an anti-

Siglec-8 antibody) or the agonist (*e.g.*, a 6'-sulfo-sLe^X-containing agonist) that binds to human Siglec-8. In a further embodiment, the one or more pathologic parameter is selected from the group consisting of: neutrophil influx, number of mast cells, cytokine release, collagen accumulation, fibroblast or myofibroblast infiltration and fibroblastic foci formation. In some embodiments herein, the individual is a human. In some of the embodiments herein, the antibody is in a pharmaceutical composition comprising the antibody and a pharmaceutically acceptable carrier. In some of the embodiments herein, the agonist is in a pharmaceutical composition comprising the agonist and a pharmaceutically acceptable carrier.

[0106] In some embodiments, provided herein is a method for treating or preventing a pre-fibrotic disease (*e.g.*, chronic hypersensitivity pneumonitis) in an individual comprising administering to the individual an effective amount of an antibody (*e.g.*, an anti-Siglec-8 antibody) or an agonist (*e.g.*, a 6'-sulfo-sLe^X-containing agonist) that binds to human Siglec-8. In some embodiments, the pre-fibrotic disease is selected from the group consisting of: bleomycin-induced pneumonitis, chronic hypersensitivity pneumonitis, polycythemia vera, essential thrombocythemia, age-related macular degeneration, diabetic retinopathy, neovascular glaucoma, retinopathy of prematurity, and proliferative vitreoretinopathy. In some embodiments, the pre-fibrotic disease is bleomycin-induced pneumonitis. In some embodiments, the pre-fibrotic disease is chronic hypersensitivity pneumonitis. In some of the embodiments herein, one or more symptom in an individual with pre-fibrotic disease (*e.g.*, chronic hypersensitivity pneumonitis) is reduced or improved (*e.g.*, a reference value) relative to baseline after administration of the antibody (*e.g.*, an anti-Siglec-8 antibody) or the agonist (*e.g.*, a 6'-sulfo-sLe^X-containing agonist) that binds to human Siglec-8. In a further embodiment, the one or more symptom can be a symptom associated with a pre-fibrotic disease disclosed herein, such as, but not limited to, shortness of breath, dry cough, weight loss, fatigue, malaise, finger or toe clubbing, muscle pain, or joint pain. In some embodiments, one or more pathologic parameter in the individual with pre-fibrotic disease (*e.g.*, chronic hypersensitivity pneumonitis) is reduced by at least 5% (*e.g.*, a reference value) relative to baseline after administration of the antibody (*e.g.*, an anti-Siglec-8 antibody) or the agonist (*e.g.*, a 6'-sulfo-sLe^X-containing agonist) that binds to human Siglec-8. In a further embodiment, the one or more pathologic parameter is selected from the group consisting of: neutrophil influx, number of mast cells, cytokine release, collagen accumulation, fibroblast or myofibroblast infiltration and fibroblastic foci formation. In some embodiments herein, the individual is a human. In some of the embodiments herein, the antibody is in a pharmaceutical composition comprising the antibody and a pharmaceutically acceptable carrier. In some of the embodiments herein, the agonist is in a pharmaceutical composition comprising the agonist and a pharmaceutically acceptable carrier.

[0107] The terms “baseline” or “baseline value” used interchangeably herein can refer to a measurement or characterization of a symptom (e.g., cytokine release, collagen accumulation, neutrophil influx, etc.) before the administration of the therapy (e.g., an anti-Siglec-8 antibody) or at the beginning of administration of the therapy. The baseline value can be compared to a reference value in order to determine the reduction or improvement of a symptom of a fibrotic disease or a pre-fibrotic disease contemplated herein. The terms “reference” or “reference value” used interchangeably herein can refer to a measurement or characterization of a symptom after administration of the therapy (e.g., an anti-Siglec-8 antibody). The reference value can be measured one or more times during a dosage regimen or treatment cycle or at the completion of the dosage regimen or treatment cycle. A “reference value” can be an absolute value; a relative value; a value that has an upper and/or lower limit; a range of values; an average value; a median value; a mean value; or a value as compared to a baseline value. Similarly, a “baseline value” can be an absolute value; a relative value; a value that has an upper and/or lower limit; a range of values; an average value; a median value; a mean value; or a value as compared to a reference value. The reference value and/or baseline value can be obtained from one individual, from two different individuals or from a group of individuals (e.g., a group of two, three, four, five or more individuals). For example, an individual with a fibrotic disease (e.g., idiopathic pulmonary fibrosis) can have a reduced level of collagen accumulation after administration of the antibody that binds to human Siglec-8 (e.g., a reference value) as compared to the level of collagen accumulation before or at the beginning of administration of the antibody that binds to human Siglec-8 in the individual (e.g., a baseline value). In another example, an individual with a fibrotic disease (e.g., idiopathic pulmonary fibrosis) can have a reduced level of collagen accumulation after administration of the antibody that binds to human Siglec-8 (e.g., a reference value) as compared to the level of collagen accumulation before or at the beginning of administration of the antibody that binds to human Siglec-8 in a different individual (e.g., a baseline value). In yet another example, an individual with a fibrotic disease (e.g., idiopathic pulmonary fibrosis) can have a reduced level of collagen accumulation after administration of the antibody that binds to human Siglec-8 (e.g., a reference value) as compared to the level of collagen accumulation before or at the beginning of administration of the antibody that binds to human Siglec-8 in a group of individuals (e.g., a baseline value). In another example, a group of individuals with a fibrotic disease (e.g., idiopathic pulmonary fibrosis) can have a reduced level of collagen accumulation after administration of the antibody that binds to human Siglec-8 (e.g., a reference value) as compared to the level of collagen accumulation before or at the beginning of administration of the antibody that binds to human Siglec-8 in a group of individuals (e.g., a baseline value). In any of the embodiments herein, the baseline value can be obtained from one

individual, from two different individuals or from a group of individuals (e.g., a group of two, three, four, five or more individuals) that are not treated with an antibody that binds to human Siglec-8.

[0108] Mast cells play a role in both allergic immune responses (e.g., an IgE mediated cell signaling pathway) and non-allergic cellular functions (e.g., a non-IgE mediated cell signaling pathway). Non-IgE mediated mast cell functions include the regulation of epithelial function (e.g., secretion and epithelial permeability), smooth-muscle function (e.g., peristalsis and bronchioconstriction), endothelial functions (e.g., blood flow, coagulation and vascular permeability), neuronal functions and other tissue functions (e.g., wound healing and fibrosis). These functions are mediated by a variety of cell signaling agents (i.e., an agent of the non-IgE mediated cell signaling pathway) such as growth factors, tissue factors, infectious agents, neuropeptides, and protein antigens. See Bischoff et al., Nature Reviews Immunology, 2007, 7:93-104. A method of treatment contemplated herein is a method for reducing mast cell activation by an agent of a non-IgE mediated cell signaling pathway in an individual with an antibody or agonist described herein that binds to human Siglec-8, or compositions thereof. In some of the embodiments herein, the agent of the non-IgE mediated cell signaling pathway is selected from the group consisting of: thymic stromal lymphopoietin (TSLP), Stem cell factor (SCF), Toll-like Receptor 3 (TLR3), Interleukin 33 (IL-33), and complement proteins. In some embodiments, mast cell activation is reduced in the individual relative to baseline after administration of the antibody or the agonist that binds to human Siglec-8. In some embodiments, the individual has a fibrotic disease described herein. In some embodiments, the fibrotic disease is selected from the group consisting of: pulmonary fibrosis, hepatic fibrosis, renal fibrosis, cardiac fibrosis, spleen fibrosis, and ocular fibrosis. In a further embodiment, the pulmonary fibrosis is idiopathic pulmonary fibrosis. In some embodiments, the pulmonary fibrosis is associated with chronic obstructive pulmonary disease. In some embodiments herein, the fibrotic disease is selected from the group consisting of: mechanical-induced fibrosis, implant-induced fibrosis, radiation-induced fibrosis, drug-induced fibrosis and viral-induced fibrosis. In a further embodiment, the mechanical induced fibrosis is ventilator-induced pulmonary fibrosis. In yet another further embodiment, the drug-induced fibrosis is bleomycin-induced pulmonary fibrosis. In some embodiments, the fibrotic disease is selected from the group consisting of: cystic fibrosis, cancer-associated fibrosis, atherosclerosis, bone marrow fibrosis, scleroderma, mediastinal fibrosis and retroperitoneal cavity fibrosis. In some embodiments, the scleroderma is systemic sclerosis. In some embodiments, the individual has a pre-fibrotic disease described herein. In some embodiments, the pre-fibrotic disease is selected from the group consisting of: bleomycin-induced pneumonitis, chronic hypersensitivity

pneumonitis, polycythemia vera, essential thrombocythemia, age-related macular degeneration, diabetic retinopathy, neovascular glaucoma, retinopathy of prematurity, and proliferative vitreoretinopathy.

[0109] In some embodiments, an individual described herein is administered an effective amount of an antibody or agonist that binds to human Siglec-8, or compositions thereof, for depletion or reduction of eosinophils (e.g., eosinophils expressing Siglec-8). In some embodiments, the anti-Siglec-8 antibody depletes or reduces at least about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90% or about 100% of the eosinophils (e.g., eosinophils expressing Siglec-8) in a sample obtained from the subject as compared to a baseline level before treatment. In some embodiments, the anti-Siglec-8 antibody depletes or reduces at least about 20% of the eosinophils (e.g., eosinophils expressing Siglec-8) in a sample obtained from the subject as compared to a baseline level before treatment. In some embodiments, the depletion or reduction of eosinophils is measured by comparing the eosinophil population number in a sample (e.g., a tissue sample or a biological fluid sample) from an individual after treatment with the antibody or agonist to the eosinophil population number in a sample from an individual before treatment with the antibody or agonist. In some embodiments, the depletion or reduction of eosinophils is measured by comparing the eosinophil population number in a sample (e.g., a tissue sample or a biological fluid sample) from an individual after treatment with the antibody or agonist to the eosinophil population number in a sample from another individual without the antibody treatment or the agonist treatment or average eosinophil population number in samples from individuals without the antibody treatment or the agonist treatment. In some embodiments, the sample is a tissue sample (e.g., a skin sample, a lung sample, a bone marrow sample, etc.). In some embodiments herein, the antibody depletes eosinophils in a tissue sample (e.g., a lung sample). In some embodiments, the sample is a biological fluid sample (e.g., a blood sample, a bronchoalveolar lavage sample, etc.). In some embodiments herein, the antibody depletes eosinophils in a biological fluid sample (e.g., a bronchoalveolar lavage sample). In some embodiments of the methods herein, the effective amount of an antibody or agonist that binds to human Siglec-8, or compositions thereof, induces apoptosis of activated eosinophils. Eosinophils can be activated or sensitized by cytokines or hormones such as, but not limited to, IL-5, GM-CSF, IL-33, IFN- γ , TNF- α , and leptin. In some embodiments of the methods herein, the effective amount of an antibody or agonist described herein that binds to human Siglec-8, or compositions thereof, induces apoptosis of resting eosinophils. In some embodiments, the effective amount of an antibody or agonist described herein that binds to human Siglec-8, or compositions thereof, has antibody-dependent cell-mediated cytotoxicity (ADCC) activity against eosinophils. In some embodiments, the effective

amount of an antibody or agonist described herein that binds to human Siglec-8, or compositions thereof, prevents or reduces eosinophil production of inflammatory mediators. Exemplary inflammatory mediators include, but are not limited to, reactive oxygen species, granule proteins (e.g., eosinophil cationic protein, major basic protein, eosinophil-derived neurotoxin, eosinophil peroxidase, etc.), lipid mediators (e.g., PAF, PGE1, PGE2, etc.), enzymes (e.g., elastase), growth factors (e.g., VEGF, PDGF, TGF- α , TGF- β , etc.), chemokines (e.g., RANTES, MCP-1, MCP-3, MCP4, eotaxin, etc.) and cytokines (e.g., IL-3, IL-5, IL-10, IL-13, IL-15, IL-33, TNF- α , etc.).

[0110] In some embodiments, an individual described herein is administered an effective amount of an antibody or agonist that binds to human Siglec-8, or compositions thereof, for depletion or reduction of mast cells. In some embodiments, the depletion or reduction of mast cells is measured by comparing the mast cell population number in a sample (e.g., a tissue sample or a biological fluid sample) from an individual after treatment with the antibody or agonist to the mast cell population number in a sample from an individual before treatment with the antibody or agonist. In some embodiments, the depletion or reduction of mast cells is measured by comparing the mast cell population number in a sample (e.g., a tissue sample or a biological fluid sample) from an individual after treatment with the antibody or agonist to the mast cell population number in a sample from another individual without the antibody treatment or agonist treatment or average mast cell population number in samples from individuals without the antibody treatment or agonist treatment. In some embodiments, the sample is a tissue sample (e.g., a skin sample, a lung sample, a bone marrow sample, etc.). In some embodiments, the sample is a biological fluid sample (e.g., a blood sample, a bronchoalveolar lavage sample, etc.). In some embodiments, the effective amount of an antibody or agonist described herein that binds to human Siglec-8, or compositions thereof, has antibody-dependent cell-mediated cytotoxicity (ADCC) activity against mast cells. In some embodiments, depletion or reduction of mast cells is the reduction or prevention of preformed or newly formed inflammatory mediators produced from mast cells. Exemplary inflammatory mediators include, but are not limited to, histamine, N-methyl histamine, enzymes (e.g., tryptase, chymase, cathepsin G, carboxypeptidase, etc.), lipid mediators (e.g., prostaglandin D2, prostaglandin E2, leukotriene B4, leukotriene C4, platelet-activating factor, 11-beta-prostaglandin F2, etc.), chemokines (e.g., CCL2, CCL3, CCL4, CCL11 (i.e., eotaxin), CXCL1, CXCL2, CXCL3, CXCL10, etc.), and cytokines (e.g., IL-3, IL-4, IL-5, IL-15, IL-33, GM-CSF, TNF, etc.).

[0111] In some embodiments, an individual described herein is administered an effective amount of an antibody or agonist that binds to human Siglec-8, or compositions thereof, for depleting mast cells expressing Siglec-8, wherein the anti-Siglec-8 antibody kills mast cells

expressing Siglec-8 by ADCC activity. In some embodiments, the anti-Siglec-8 antibody depletes at least about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90% or about 100% of the mast cells expressing Siglec-8 in a sample obtained from the subject as compared to a baseline level before treatment. In some embodiments, the anti-Siglec-8 antibody depletes at least about 20% of the mast cells expressing Siglec-8 in a sample obtained from the subject as compared to a baseline level before treatment. In some embodiments, the depletion or killing of mast cells is measured by comparing the mast cell population number in a sample (e.g., a tissue sample or a biological fluid sample) from a subject after treatment with the antibody to the mast cell population number in a sample from a subject before treatment with the antibody. In some embodiments, the depletion or killing of mast cells is measured by comparing the mast cell population number in a sample (e.g., a tissue sample or a biological fluid sample) from a subject after treatment with the antibody to the mast cell population number in a sample from another subject without the antibody treatment or average mast cell population number in samples from subjects without the antibody treatment. In some embodiments, the sample is a tissue sample (e.g., a skin sample, a lung sample, a bone marrow sample, etc.). In some embodiments, the sample is a biological fluid sample (e.g., a blood sample, a bronchoalveolar lavage sample, etc.). In some embodiments, the anti-Siglec-8 antibody has been engineered to improve ADCC activity. In some embodiments, the anti-Siglec-8 antibody comprises at least one amino acid substitution in the Fc region that improves ADCC activity. In some embodiments, at least one or two of the heavy chains of the antibody is non-fucosylated. In some embodiments, depletion or killing of mast cells is the reduction or prevention of preformed or newly formed inflammatory mediators produced from mast cells. Exemplary inflammatory mediators include, but are not limited to, histamine, N-methyl histamine, enzymes (e.g., tryptase, chymase, cathepsin G, carboxypeptidase, etc.), lipid mediators (e.g., prostaglandin D2, prostaglandin E2, leukotriene B4, leukotriene C4, platelet-activating factor, 11-beta-prostaglandin F2, etc.), chemokines (e.g., CCL2, CCL3, CCL4, CCL11 (i.e., eotaxin), CXCL1, CXCL2, CXCL3, CXCL10, etc.), and cytokines (e.g., IL-3, IL-4, IL-5, IL-13, IL-15, IL-33, GM-CSF, TNF, etc.).

[0112] In some embodiments, an individual described herein is administered an effective amount of an antibody or agonist that binds to human Siglec-8, or compositions thereof, for the inhibition of mast cell-mediated activity. In some embodiments, the inhibition of mast cell-mediated activity is measured by comparing the mast cell-mediated activity in a sample (e.g., a tissue sample or a biological fluid sample) from an individual after treatment with the antibody or agonist to the mast cell-mediated activity in a sample from an individual before treatment with the antibody or agonist. In some embodiments, the inhibition of mast cell-mediated activity

is measured by comparing the mast cell-mediated activity in a sample (e.g., a tissue sample or a biological fluid sample) from an individual after treatment with the antibody or agonist to the mast cell-mediated activity in a sample from another individual without the antibody treatment or agonist treatment or average mast cell-mediated activity in samples from individuals without the antibody treatment or agonist treatment. In some embodiments, the sample is a tissue sample (e.g., a skin sample, a lung sample, a bone marrow sample, etc.). In some embodiments, the sample is a biological fluid sample (e.g., a blood sample, a bronchoalveolar lavage sample, etc.). In some embodiments, inhibition of mast cell-mediated activity is the inhibition of mast cell degranulation. In some embodiments, inhibition of mast cell-mediated activity is the inhibition of neutrophil influx to sites of fibrotic disease. In some embodiments, inhibition of mast cell-mediated activity is the inhibition of collagen accumulation at sites of fibrotic disease. In some embodiments, inhibition of mast cell-mediated activity is the inhibition of cytokine release at sites of fibrotic disease. In some embodiments, inhibition of mast cell-mediated activity is the reduction in the number of mast cells in the individual. In some embodiments, inhibition of mast cell-mediated activity is the inhibition of release of preformed or newly formed inflammatory mediators from mast cells. Exemplary inflammatory mediators include, but are not limited to, histamine, N-methyl histamine, enzymes (e.g., tryptase, chymase, cathepsin G, carboxypeptidase, etc.), lipid mediators (e.g., prostaglandin D2, prostaglandin E2, leukotriene B4, leukotriene C4, platelet-activating factor, 11-beta-prostaglandin F2, etc.), chemokines (e.g., CCL2, CCL3, CCL4, CCL11 (i.e., eotaxin), CXCL1, CXCL2, CXCL3, CXCL10, etc.), and cytokines (e.g., IL-3, IL-4, IL-5, IL-13, IL-15, IL-33, GM-CSF, TNF, etc.).

[0113] For the prevention or treatment of disease, the appropriate dosage of an active agent, will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the agent is administered for preventive or therapeutic purposes, previous therapy, the individual's clinical history and response to the agent, and the discretion of the attending physician. The agent is suitably administered to the individual at one time or over a series of treatments. In some embodiments of the methods described herein, an interval between administrations of an anti-Siglec-8 antibody (e.g., an antibody that binds to human Siglec-8) or agonist described herein is about one month or longer. In some embodiments, the interval between administrations is about two months, about three months, about four months, about five months, about six months or longer. As used herein, an interval between administrations refers to the time period between one administration of the antibody or agonist and the next administration of the antibody or agonist. As used herein, an interval of about one month includes four weeks. Accordingly, in some embodiments, the interval between administrations is about four weeks, about five weeks, about six weeks, about seven weeks, about eight weeks,

about nine weeks, about ten weeks, about eleven weeks, about twelve weeks, about sixteen weeks, about twenty weeks, about twenty four weeks, or longer. In some embodiments, the interval between administrations is about one day, about two days, about three days, about four days, about five days, about five days, about seven days or longer. In some embodiments, the treatment includes multiple administrations of the antibody or agonist, wherein the interval between administrations may vary. For example, the interval between the first administration and the second administration is about one month, and the intervals between the subsequent administrations are about three months. In some embodiments, the interval between the first administration and the second administration is about one month, the interval between the second administration and the third administration is about two months, and the intervals between the subsequent administrations are about three months. In some embodiments, an anti-Siglec-8 antibody described herein (e.g., an antibody that binds to human Siglec-8) or agonist described herein is administered at a flat dose. In some embodiments, an anti-Siglec-8 antibody described herein (e.g., an antibody that binds to human Siglec-8) or agonist described herein is administered to an individual at a dosage from about 0.1 mg to about 1800 mg per dose. In some embodiments, the anti-Siglec-8 antibody (e.g., an antibody that binds to human Siglec-8) or agonist is administered to an individual at a dosage of about any of 0.1 mg, 0.5 mg, 1 mg, 5 mg, 10 mg, 20 mg, 30 mg, 40 mg, 50 mg, 60 mg, 70 mg, 80 mg, 90 mg, 100 mg, 150 mg, 200 mg, 250 mg, 300 mg, 350 mg, 400 mg, 450 mg, 500 mg, 550 mg, 600 mg, 650 mg, 700 mg, 750 mg, 800 mg, 850 mg, 900 mg, 950 mg, 1000 mg, 1100 mg, 1200 mg, 1300 mg, 1400 mg, 1500 mg, 1600 mg, 1700 mg, and 1800 mg per dose. In some embodiments, an anti-Siglec-8 antibody described herein (e.g., an antibody that binds to human Siglec-8) or agonist described herein is administered to an individual at a dosage from about 150 mg to about 450 mg per dose. In some embodiments, the anti-Siglec-8 antibody (e.g., an antibody that binds to human Siglec-8) or agonist is administered to an individual at a dosage of about any of 150 mg, 200 mg, 250 mg, 300 mg, 350 mg, 400 mg, and 450 mg per dose. In some embodiments, an anti-Siglec-8 antibody described herein (e.g., an antibody that binds to human Siglec-8) or agonist described herein is administered to an individual at a dosage from about 0.1 mg/kg to about 20 mg/kg per dose. In some embodiments, an anti-Siglec-8 antibody described herein (e.g., an antibody that binds to human Siglec-8) or agonist described herein is administered to an individual at a dosage from about 0.01 mg/kg to about 10 mg/kg per dose. In some embodiments, an anti-Siglec-8 antibody described herein (e.g., an antibody that binds to human Siglec-8) or agonist described herein is administered to a subject at a dosage from about 0.1 mg/kg to about 10 mg/kg or about 1.0 mg/kg to about 10 mg/kg. In some embodiments, an anti-Siglec-8 antibody described herein is administered to a subject at a dosage of about any of 0.1 mg/kg, 0.5 mg/kg, 1.0 mg/kg, 1.5

mg/kg, 2.0 mg/kg, 2.5 mg/kg, 3.0 mg/kg, 3.5 mg/kg, 4.0 mg/kg, 4.5 mg/kg, 5.0 mg/kg, 5.5 mg/kg, 6.0 mg/kg, 6.5 mg/kg, 7.0 mg/kg, 7.5 mg/kg, 8.0 mg/kg, 8.5 mg/kg, 9.0 mg/kg, 9.5 mg/kg, or 10.0 mg/kg. Any of the dosing frequency described above may be used. Any dosing frequency described above may be used in the methods or uses of the compositions described herein. Efficacy of treatment with an antibody described herein (e.g., an antibody that binds to human Siglec-8) or agonist described herein can be assessed using any of the methodologies or assays described herein at intervals ranging between every week and every three months. In some embodiments of the methods described herein, efficacy of treatment (e.g., reduction or improvement of one or more symptom) is assessed about every one month, about every two months, about every three months, about every four months, about every five months, about every six months or longer after administration of an antibody or agonist that binds to human Siglec-8. In some embodiments of the methods described herein, efficacy of treatment (e.g., reduction or improvement of one or more symptom) is assessed about every one week, about every two weeks, about every three weeks, about every four weeks, about every five weeks, about every six weeks, about every seven weeks, about every eight weeks, about every nine weeks, about every ten weeks, about every eleven weeks, about every twelve weeks, about every sixteen weeks, about every twenty weeks, about every twenty four weeks, or longer. In some embodiments of the methods described herein, efficacy of treatment (e.g., reduction or improvement of one or more symptom) is assessed about every day, about every two days, about every three days, about every four days, about every five days, about every six days, about every seven days or longer.

Agonists of Siglec-8

[0114] In one aspect, the present invention provides for agonists for use in any of the methods herein. In some embodiments, an agonist is an agent that binds to Siglec-8 expressed on eosinophils and induces apoptosis of eosinophils *in vitro* or *in vivo*. In some embodiments, an agonist is an agent that binds to Siglec-8 expressed on mast cells and inhibits activation of mast cells *in vitro* or *in vivo*. In some embodiments, an agonist is an agent that binds to Siglec-8 expressed on mast cells and depletes or reduces the number of mast cells *in vitro* or *in vivo*. In some embodiments, the agonist is an agonist antibody. In some embodiments, the agonist antibody (e.g., antibody 2E2 provide herein) crosslinks Siglec-8 expressed by eosinophils and induces activation of one or more caspases (e.g., caspase-8, caspase-3, and caspase-9) in eosinophils and/or loss of mitochondrial membrane potential. See Nutku *et al.*, *Biochem Biophys Res Commun.*, 336:918-924, 2005. Siglec-8 binds to the glycan 6'-sulfo-sialyl Lewis X (also referred to herein as 6'-sulfo-sLe^X) and engagement to this glycan induces apoptosis of Siglec-8 expressing cells (e.g., eosinophils). See Hudson *et al.*, *J Pharmacol Exp Ther.*,

330(2):608-12, 2009. In some embodiments herein, an agonist of Siglec-8 is a molecule having a 6'-sulfo-sLe^X attached or linked to a molecule (*e.g.*, a polymer, an oligosaccharide, a polypeptide, a glycoprotein, etc.). In some embodiments herein, the agonist is a 6'-sulfo-sLe^X-containing agonist molecule (*e.g.*, a 6'-sulfo-sLe^X-containing ligand, a 6'-sulfo-sLe^X-containing oligosaccharide, a 6'-sulfo-sLe^X-containing polypeptide, and a 6'-sulfo-sLe^X-containing glycoprotein).

[0115] Agonists may be identified from a variety of sources, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. Such agonists may be natural or modified substrates, ligands, receptors, oligonucleotides, polypeptides, or antibodies that contain the glycan 6'-sulfo-sLeX and bind to Siglec-8, or may be structural or functional mimetics thereof. Structural or functional mimetics of such natural or modified substrates, ligands, receptors, oligonucleotides, or antibodies that contain the glycan 6'-sulfo-sLeX are referred to herein as a "6'-sulfo-sLeX-containing glycomimetic." See Coligan et al., *Current Protocols in Immunology* 1(2):Chapter 5, 1991. For example, a 6'-sulfo-sLeX-containing glycomimetic may be a synthetic polymer-based ligand decorated with 6'-sulfo-sLeX that structurally or functionally mimics the activity of the natural ligand of Siglec-8. See Hudson et al., *J Pharmacol Exp Ther.*, 330(2):608-12, 2009 for examples of glycomimetics contemplated herein. Other examples of potential agonists include antibodies or, in some cases, oligonucleotides or polypeptides which are closely related to the natural ligand of Siglec-8, or small molecules which bind to Siglec-8. Synthetic compounds that mimic the conformation and desirable features of a particular polysaccharide ligand (*e.g.*, a 6'-sulfo-sLeX-containing ligand) that binds to Siglec-8, and preferably avoid at least some undesirable features (such as low binding affinity, short half-life in vivo, and the like) of the original polysaccharide ligand of interest (*e.g.*, a 6'-sulfo-sLeX-containing ligand), are referred to herein as "mimetics". See U.S. Pat. No. 8,178,512 for examples of mimetics contemplated herein.

[0116] In some aspects, an agonist that binds to human Siglec-8 (*e.g.*, 6'-sulfo-sLeX-containing agonist or an antibody) described herein induces apoptosis of eosinophils. Apoptosis of eosinophils can be assessed by methods well known in the art. See Hudson *et al.*, *J Pharmacol Exp Ther.*, 330(2):608-12, 2009 and Nutku *et al.*, *Biochem Biophys Res Commun.*, 336:918-924, 2005. For example, human eosinophils are isolated from peripheral blood, purified, and cultured for 24 or 72 hours in IL-5 followed by incubation with the agonist that binds to human Siglec-8 for an additional 24 hours. Cell survival is then assessed by flow cytometric analysis after labeling with annexin-V and propidium iodide. Agonist activity may also be assessed using by detecting activation of caspases (*e.g.*, caspase-8, caspase-3, and

caspase-9) in eosinophils and/or loss of mitochondrial membrane potential in eosinophils. These assays are described in Nutku *et al.*, *Biochem Biophys Res Commun.*, 336:918-924, 2005.

Antibodies

[0117] In one aspect, the invention provides isolated antibodies that bind to a human Siglec-8 (e.g., an agonist antibody that binds to human Siglec-8). In some embodiments, an anti-Siglec-8 antibody described herein has one or more of the following characteristics: (1) binds a human Siglec-8; (2) binds to an extracellular domain of a human Siglec-8; (3) binds a human Siglec-8 with a higher affinity than mouse antibody 2E2 and/or mouse antibody 2C4; (4) binds a human Siglec-8 with a higher avidity than mouse antibody 2E2 and/or mouse antibody 2C4; (5) has a T_m of about 70°C-72°C or higher in a thermal shift assay; (6) has a reduced degree of fucosylation or is non-fucosylated; (7) binds a human Siglec-8 expressed on eosinophils and induces apoptosis of eosinophils; (8) binds a human Siglec-8 expressed on mast cells and depletes or reduces the number of mast cells; (9) binds a human Siglec-8 expressed on mast cells and inhibits FcεRI-dependent activities of mast cells (e.g., histamine release, PGD₂ release, Ca²⁺ flux, and/or β-hexosaminidase release, etc.); (10) has been engineered to improve ADCC activity; and (11) binds a human Siglec-8 expressed on mast cells and inhibits the activation of mast cells by an agent of a non-IgE mediated cell signaling pathway (e.g., complement proteins).

[0118] In one aspect, the invention provides antibodies that bind to a human Siglec-8. In some embodiments, the human Siglec-8 comprises an amino acid sequence of SEQ ID NO:72. In some embodiments, the human Siglec-8 comprises an amino acid sequence of SEQ ID NO:73. In some embodiments, an antibody described herein binds to a human Siglec-8 expressed on eosinophils and induces apoptosis of eosinophils. In some embodiments, an antibody described herein binds to a human Siglec-8 expressed on mast cells and depletes or reduces the number of mast cells. In some embodiments, an antibody described herein binds to a human Siglec-8 expressed on mast cells and inhibits mast cell-mediated activity. In some embodiments, an antibody described herein binds to a human Siglec-8 expressed on mast cells and reduces mast cell activation by an agent of a non-IgE mediated immune response pathway. In a further embodiment, the agent of the non-IgE mediated cell signaling pathway is selected from the group consisting of: thymic stromal lymphopoietin (TSLP), Stem cell factor (SCF), Toll-like Receptor 3 (TLR3), Interleukin 33 (IL-33), and complement proteins.

[0119] In one aspect, an anti-Siglec-8 antibody described herein is a monoclonal antibody. In one aspect, an anti-Siglec-8 antibody described herein is an antibody fragment (including antigen-binding fragment), e.g., a Fab, Fab'-SH, Fv, scFv, or (Fab')₂ fragment. In one aspect, an anti-Siglec-8 antibody described herein is a chimeric, humanized, or human antibody. In one aspect, any of the anti-Siglec-8 antibodies described herein are purified.

[0120] In one aspect, anti-Siglec-8 antibodies that compete with murine 2E2 antibody and murine 2C4 antibody binding to Siglec-8 are provided. Anti-Siglec-8 antibodies that bind to the same epitope as murine 2E2 antibody and murine 2C4 antibody are also provided. Murine antibodies to Siglec-8, 2E2 and 2C4 antibody are described in U.S. Pat. No. 8,207,305; U.S. Pat. No. 8,197,811, U.S. Pat. No. 7,871,612, and U.S. Pat. No. 7,557,191.

[0121] In one aspect, anti-Siglec-8 antibodies that compete with any anti-Siglec-8 antibody described herein (e.g., HEKA, HEKF, 1C3, 1H10, 4F11) for binding to Siglec-8 are provided. Anti-Siglec-8 antibodies that bind to the same epitope as any anti-Siglec-8 antibody described herein (e.g., HEKA, HEKF, 1C3, 1H10, 4F11) are also provided.

[0122] In one aspect of the invention, polynucleotides encoding anti-Siglec-8 antibodies are provided. In certain embodiments, vectors comprising polynucleotides encoding anti-Siglec-8 antibodies are provided. In certain embodiments, host cells comprising such vectors are provided. In another aspect of the invention, compositions comprising anti-Siglec-8 antibodies or polynucleotides encoding anti-Siglec-8 antibodies are provided. In certain embodiments, a composition of the invention is a pharmaceutical formulation for the treatment of a fibrotic disease (e.g., idiopathic pulmonary fibrosis) or pre-fibrotic disease (e.g., chronic hypersensitivity pneumonitis), such as those enumerated herein. In certain embodiments, a composition of the invention is a pharmaceutical formulation for the prevention of a fibrotic disease (e.g., idiopathic pulmonary fibrosis) or pre-fibrotic disease (e.g., chronic hypersensitivity pneumonitis), such as those enumerated herein.

[0123] In one aspect, provided herein is an anti-Siglec-8 antibody comprising 1, 2, 3, 4, 5, or 6 of the HVR sequences of the murine antibody 2C4. In one aspect, provided herein is an anti-Siglec-8 antibody comprising 1, 2, 3, 4, 5, or 6 of the HVR sequences of the murine antibody 2E2. In some embodiments, the HVR is a Kabat CDR or a Chothia CDR.

[0124] In one aspect, provided herein is an anti-Siglec-8 antibody comprising 1, 2, 3, 4, 5, or 6 of the HVR sequences of the murine antibody 1C3. In one aspect, provided herein is an anti-Siglec-8 antibody comprising 1, 2, 3, 4, 5, or 6 of the HVR sequences of the murine antibody 4F11. In one aspect, provided herein is an anti-Siglec-8 antibody comprising 1, 2, 3, 4, 5, or 6 of the HVR sequences of the murine antibody 1H10. In some embodiments, the HVR is a Kabat CDR or a Chothia CDR.

[0125] In one aspect, provided herein is an isolated anti-Siglec-8 antibody that binds to human Siglec-8 and non-human primate Siglec-8. In some embodiments, the antibody that binds to a human Siglec-8 and a non-human primate Siglec-8, binds to an epitope in Domain 1 of human Siglec-8. In some embodiments, the antibody that binds to a human Siglec-8 and a non-human primate Siglec-8, binds to an epitope in Domain 3 of human Siglec-8. In some embodiments,

the antibody that binds to a human Siglec-8 and a non-human primate Siglec-8 is a humanized antibody, a chimeric antibody, or a human antibody. In some embodiments, the antibody that binds to a human Siglec-8 and a non-human primate Siglec-8 is a murine antibody. In some embodiments, the antibody that binds to a human Siglec-8 and a non-human primate Siglec-8 is a human IgG1 antibody.

[0126] In one aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:61, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:62, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:63; and/or wherein the light chain variable region comprises (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:64, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:65, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:66.

[0127] In one aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:61, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:62, and (iii) HVR-H3 comprising the amino acid sequence selected from SEQ ID NOs:67-70; and/or wherein the light chain variable region comprises (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:64, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:65, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:66.

[0128] In one aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:61, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:62, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:63; and/or wherein the light chain variable region comprises (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:64, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:65, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:71.

[0129] In another aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:61, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:62, and (iii) HVR-H3 comprising the amino acid sequence selected from SEQ ID NOs:67-70; and/or wherein the light chain variable region comprises (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:64, (ii) HVR-L2

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

[0130] In another aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:88, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:91, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:94; and/or a light chain variable region comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:97, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:100, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:103.

[0131] In another aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:89, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:92, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:95; and/or a light chain variable region comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:98, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:101, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:104.

[0132] In another aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:90, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:93, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:96; and/or a light chain variable region comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:99, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:102, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:105.

[0133] An anti-Siglec-8 antibody described herein may comprise any suitable framework variable domain sequence, provided that the antibody retains the ability to bind human Siglec-8. As used herein, heavy chain framework regions are designated "HC-FR1-FR4," and light chain framework regions are designated "LC-FR1-FR4." In some embodiments, the anti-Siglec-8 antibody comprises a heavy chain variable domain framework sequence of SEQ ID NO:26, 34, 38, and 45 (HC-FR1, HC-FR2, HC-FR3, and HC-FR4, respectively). In some embodiments, the anti-Siglec-8 antibody comprises a light chain variable domain framework sequence of SEQ ID NO:48, 51, 55, and 60 (LC-FR1, LC-FR2, LC-FR3, and LC-FR4, respectively). In some embodiments, the anti-Siglec-8 antibody comprises a light chain variable domain framework

sequence of SEQ ID NO:48, 51, 58, and 60 (LC-FR1, LC-FR2, LC-FR3, and LC-FR4, respectively).

[0134] In one embodiment, an anti-Siglec-8 antibody comprises a heavy chain variable domain comprising a framework sequence and hypervariable regions, wherein the framework sequence comprises the HC-FR1-HC-FR4 sequences SEQ ID NOs:26-29 (HC-FR1), SEQ ID NOs:31-36 (HC-FR2), SEQ ID NOs:38-43 (HC-FR3), and SEQ ID NOs:45 or 46 (HC-FR4), respectively; the HVR-H1 comprises the amino acid sequence of SEQ ID NO:61; the HVR-H2 comprises the amino acid sequence of SEQ ID NO:62; and the HVR-H3 comprises an amino acid sequence of SEQ ID NO:63. In one embodiment, an anti-Siglec-8 antibody comprises a heavy chain variable domain comprising a framework sequence and hypervariable regions, wherein the framework sequence comprises the HC-FR1-HC-FR4 sequences SEQ ID NOs:26-29 (HC-FR1), SEQ ID NOs:31-36 (HC-FR2), SEQ ID NOs:38-43 (HC-FR3), and SEQ ID NOs:45 or 46 (HC-FR4), respectively; the HVR-H1 comprises the amino acid sequence of SEQ ID NO:61; the HVR-H2 comprises the amino acid sequence of SEQ ID NO:62; and the HVR-H3 comprises an amino acid sequence selected from SEQ ID NOs:67-70. In one embodiment, an anti-Siglec-8 antibody comprises a light chain variable domain comprising a framework sequence and hypervariable regions, wherein the framework sequence comprises the LC-FR1-LC-FR4 sequences SEQ ID NOs:48 or 49 (LC-FR1), SEQ ID NOs:51-53 (LC-FR2), SEQ ID NOs:55-58 (LC-FR3), and SEQ ID NO:60 (LC-FR4), respectively; the HVR-L1 comprises the amino acid sequence of SEQ ID NO:64; the HVR-L2 comprises the amino acid sequence of SEQ ID NO:65; and the HVR-L3 comprises an amino acid sequence of SEQ ID NO:66. In one embodiment, an anti-Siglec-8 antibody comprises a light chain variable domain comprising a framework sequence and hypervariable regions, wherein the framework sequence comprises the LC-FR1-LC-FR4 sequences SEQ ID NOs:48 or 49 (LC-FR1), SEQ ID NOs:51-53 (LC-FR2), SEQ ID NOs:55-58 (LC-FR3), and SEQ ID NO:60 (LC-FR4), respectively; the HVR-L1 comprises the amino acid sequence of SEQ ID NO:64; the HVR-L2 comprises the amino acid sequence of SEQ ID NO:65; and the HVR-L3 comprises an amino acid sequence of SEQ ID NO:71. In one embodiment of these antibodies, the heavy chain variable domain comprises an amino acid sequence selected from SEQ ID NOs:2-10 and the light chain variable domain comprises an amino acid sequence selected from SEQ ID NOs:16-22. In one embodiment of these antibodies, the heavy chain variable domain comprises an amino acid sequence selected from SEQ ID NOs:2-10 and the light chain variable domain comprises an amino acid sequence selected from SEQ ID NOs:23 or 24. In one embodiment of these antibodies, the heavy chain variable domain comprises an amino acid sequence selected from SEQ ID NOs:11-14 and the light chain variable domain comprises an amino acid sequence selected from SEQ ID NOs:16-

22. In one embodiment of these antibodies, the heavy chain variable domain comprises an amino acid sequence selected from SEQ ID NOs:11-14 and the light chain variable domain comprises an amino acid sequence selected from SEQ ID NOs:23 or 24. In one embodiment of these antibodies, the heavy chain variable domain comprises an amino acid sequence of SEQ ID NO:6 and the light chain variable domain comprises an amino acid sequence of SEQ ID NO:16. In one embodiment of these antibodies, the heavy chain variable domain comprises an amino acid sequence of SEQ ID NO:6 and the light chain variable domain comprises an amino acid sequence of SEQ ID NO:21.

[0135] In some embodiments, the heavy chain HVR sequences comprise the following:

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

[0136] In some embodiments, the heavy chain HVR sequences comprise the following:

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

[0137] In some embodiments, the heavy chain FR sequences comprise the following:

- a) HC-FR1 (EVQLVESGGGLVQPGGSLRLSCAASGFSLT (SEQ ID NO:26); EVQLVESGGGLVQPGGSLRLSCAVSGFSLT (SEQ ID NO:27); QVQLQESGPGLVKPSSETLSLTCTVSGGSIS (SEQ ID NO:28); or QVQLQESGPGLVKPSSETLSLTCTVSGFSLT (SEQ ID NO:29));
- b) HC-FR2 (WVRQAPGKGLEWVS (SEQ ID NO:31); WVRQAPGKGLEWLG (SEQ ID NO:32); WVRQAPGKGLEWLS (SEQ ID NO:33); WVRQAPGKGLEWVG (SEQ ID NO:34); WVRQPPGKGLEWIG (SEQ ID NO:35); or WVRQPPGKGLEWLG (SEQ ID NO:36));
- c) HC-FR3 (RFTISKDNSKNTVYLQMNSLRAEDTAVYYCAR (SEQ ID NO:38); RLSISKDNSKNTVYLQMNSLRAEDTAVYYCAR (SEQ ID NO:39); RLTISKDNSKNTVYLQMNSLRAEDTAVYYCAR (SEQ ID NO:40); RFSISKDNSKNTVYLQMNSLRAEDTAVYYCAR (SEQ ID NO:41);

RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR (SEQ ID NO:42); or
 RLSISKDNSKNQVSLKLSSVTAADTAVYYCAR (SEQ ID NO:43)); and
 d) HC-FR4 (WGQGTITVTVSS (SEQ ID NO:45); or WGQGTLVTVSS (SEQ ID NO:46)).

[0138] In some embodiments, the light chain HVR sequences comprise the following:

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

[0139] In some embodiments, the light chain HVR sequences comprise the following:

- a) HVR-L1 (SASSSVSYMH (SEQ ID NO:97); RASQDITNYLN (SEQ ID NO:98); or SASSSVSYMY (SEQ ID NO:99));
- b) HVR-L2 (DTSKLAY (SEQ ID NO:100); FTSRLHS (SEQ ID NO:101); or DTSSLAS (SEQ ID NO:102)); and
- c) HVR-L3 (QQWSSNPPT (SEQ ID NO:103); QQGNTLPWT (SEQ ID NO:104); or QQWNSDPYT (SEQ ID NO:105)).

[0140] In some embodiments, the light chain FR sequences comprise the following:

- a) LC-FR1 (EIVLTQSPATLSLSPGERATLSC (SEQ ID NO:48); or EIILTQSPATLSLSPGERATLSC (SEQ ID NO:49));
- b) LC-FR2 (WFQQKPGQAPRLLIY (SEQ ID NO:51); WFQQKPGQAPRLWIY (SEQ ID NO:52); or WYQQKPGQAPRLLIY (SEQ ID NO: 53));
- c) LC-FR3 (GIPARFSGSGSGTDFTLTSSLEPEDFAVYYC (SEQ ID NO:55); GVPARFSGSGSGTDYTLTSSLEPEDFAVYYC (SEQ ID NO:56); GVPARFSGSGSGTDFTLTSSLEPEDFAVYYC (SEQ ID NO:57); or GIPARFSGSGSGTDYTLTSSLEPEDFAVYYC (SEQ ID NO:58)); and
- d) LC-FR4 (FGPGTKLDIK (SEQ ID NO:60)).

[0141] In some embodiments, provided herein is an anti-Siglec-8 antibody (*e.g.*, a humanized anti-Siglec-8) antibody that binds to human Siglec-8, wherein the antibody comprises a heavy chain variable region and a light chain variable region, wherein the antibody comprises:

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

- (7) an HC-FR4 comprising the amino acid sequence selected from SEQ ID NOs:45-46, and/or
- (b) a light chain variable domain comprising:
 - (1) an LC-FR1 comprising the amino acid sequence selected from SEQ ID NOs:48-49;
 - (2) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:64;
 - (3) an LC-FR2 comprising the amino acid sequence selected from SEQ ID NOs:51-53;
 - (4) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:65;
 - (5) an LC-FR3 comprising the amino acid sequence selected from SEQ ID NOs:55-58;
 - (6) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:66; and
 - (7) an LC-FR4 comprising the amino acid sequence of SEQ ID NO:60.

[0142] In one aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable domain selected from SEQ ID NOs:2-10 and/or comprising a light chain variable domain selected from SEQ ID NOs:16-22. In one aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable domain selected from SEQ ID NOs:2-10 and/or comprising a light chain variable domain selected from SEQ ID NO:23 or 24. In one aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable domain selected from SEQ ID NOs:11-14 and/or comprising a light chain variable domain selected from SEQ ID NOs:16-22. In one aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable domain selected from SEQ ID NOs:11-14 and/or comprising a light chain variable domain selected from SEQ ID NO:23 or 24. In one aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable domain of SEQ ID NO:6 and/or comprising a light chain variable domain selected from SEQ ID NO:16 or 21.

[0143] In one aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable domain selected from SEQ ID NOs:106-108 and/or comprising a light chain variable domain selected from SEQ ID NOs:109-111. In one aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable domain of SEQ ID NO:106 and/or comprising a light chain variable domain of SEQ ID NO:109. In one aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable domain of SEQ ID NO:107 and/or comprising a light chain variable domain of SEQ ID NO:110. In one aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable domain of SEQ ID NO:108 and/or comprising a light chain variable domain of SEQ ID NO:111.

[0144] In some embodiments, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence selected from SEQ ID NOs:2-14. In some embodiments, provided herein is an anti-Siglec-8 antibody

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

[0145] In some embodiments, provided herein is an anti-Siglec-8 antibody comprising a light chain variable domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence selected from SEQ ID NOs:16-24. In some embodiments, provided herein is an anti-Siglec-8 antibody comprising a light chain variable domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence selected from SEQ ID NOs:109-111. In some embodiments, an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity contains substitutions, insertions, or deletions relative to the reference sequence, but an antibody comprising that amino acid sequence retains the ability to bind to human Siglec-8. In some embodiments, the substitutions, insertions, or deletions (*e.g.*, 1, 2, 3, 4, or 5 amino acids) occur in regions outside the HVRs (*i.e.*, in the FRs). In some embodiments, an anti-Siglec-8 antibody comprises a light chain variable domain comprising an amino acid sequence of SEQ ID NO:16 or 21. In some embodiments, an anti-Siglec-8 antibody comprises a heavy chain variable domain comprising an amino acid sequence selected from SEQ ID NOs:109-111.

[0146] In one aspect, the invention provides an anti-Siglec-8 antibody comprising (a) one, two, or three VH HVRs selected from those shown in Table 2 and/or (b) one, two, or three VL HVRs selected from those shown in Table 2.

[0147] In one aspect, the invention provides an anti-Siglec-8 antibody comprising (a) one, two, or three VH HVRs selected from those shown in Table 5 and/or (b) one, two, or three VL HVRs selected from those shown in Table 5.

[0148] In one aspect, the invention provides an anti-Siglec-8 antibody comprising (a) one, two, three or four VH FRs selected from those shown in Table 3 and/or (b) one, two, three or four VL FRs selected from those shown in Table 3.

[0149] In some embodiments, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable domain and/or a light chain variable domain of an antibody shown in Table 4, for example, HAKA antibody, HAKB antibody, HAKC antibody, etc.

Table 2. Amino acid sequences of HVRs of antibodies

Antibody Chain	HVR1	HVR2	HVR3
<i>2E2 antibody</i>			
Heavy chain	IYGAH SEQ ID NO:61	VIWAGGSTNYNSALMS SEQ ID NO:62	DGSSPYYYSM EY SEQ ID NO:63
Light chain	SATSSVSYMH SEQ ID NO:64	STSNLAS SEQ ID NO:65	QQRSSYPFT SEQ ID NO:66
<i>Humanized Heavy Chain Variants 2E2 RHA, 2E2 RHB, 2E2 RHC, 2E2 RHD, 2E2 RHE, 2E2 RHF, 2E2 RHG, 2E2 RHA2, and 2E2 RHB2</i>			
Heavy chain	IYGAH SEQ ID NO:61	VIWAGGSTNYNSALMS SEQ ID NO:62	DGSSPYYYSM EY SEQ ID NO:63
<i>Humanized Light Chain Variants 2E2 RKA, 2E2 RKB, 2E2 RKC, 2E2 RKD, 2E2 RKE, 2E2 RKF, and 2E2 RKG</i>			
Light chain	SATSSVSYMH SEQ ID NO:64	STSNLAS SEQ ID NO:65	QQRSSYPFT SEQ ID NO:66
<i>Humanized Heavy Chain Variants 2E2 RHE S-G, 2E2 RHE E-D, 2E2 RHE Y-V, and 2E2 RHE triple</i>			
2E2 RHE S-G	IYGAH SEQ ID NO:61	VIWAGGSTNYNSALMS SEQ ID NO:62	DGSSPYYYGMEY SEQ ID NO:67
2E2 RHE E-D	IYGAH SEQ ID NO:61	VIWAGGSTNYNSALMS SEQ ID NO:62	DGSSPYYYSM DY SEQ ID NO:68
2E2 RHE Y-V	IYGAH SEQ ID NO:61	VIWAGGSTNYNSALMS SEQ ID NO:62	DGSSPYYYSM EV SEQ ID NO:69
2E2 RHE triple	IYGAH SEQ ID NO:61	VIWAGGSTNYNSALMS SEQ ID NO:62	DGSSPYYYGM DV SEQ ID NO:70
<i>Humanized Light Chain Variants 2E2 RKA F-Y and 2E2 RKF F-Y</i>			
2E2 RKA F-Y	SATSSVSYMH SEQ ID NO:64	STSNLAS SEQ ID NO:65	QQRSSYPYT SEQ ID NO:71
2E2 RKF F-Y	SATSSVSYMH SEQ ID NO:64	STSNLAS SEQ ID NO:65	QQRSSYPYT SEQ ID NO:71

Table 3. Amino acid sequences of FRs of antibodies

Heavy Chain	FR1	FR2	FR3	FR4
2E2	QVQLKESGPG LVA PSQSL SITCTVSGFS LT (SEQ ID NO:25)	WVRQPPGKGLEW LG (SEQ ID NO:30)	RLSISKDNSK SQVF LKINSLQTDD TAL YYCAR (SEQ ID NO:37)	WGQGTSVTVSS (SEQ ID NO:44)

2E2 RHA	EVQLVESGGGLVQ PGGSLRLSCAASGF SLT (SEQ ID NO:26)	WVRQAPGKGLEW VS (SEQ ID NO:31)	RFTISKDNSKNTVY LQMNSLRAEDTAV YYCAR (SEQ ID NO:38)	WGQGTTVTVSS (SEQ ID NO:45)
2E2 RHB	EVQLVESGGGLVQ PGGSLRLSCAVSGF SLT (SEQ ID NO:27)	WVRQAPGKGLEW LG (SEQ ID NO:32)	RLSISKDNSKNTVY LQMNSLRAEDTAV YYCAR (SEQ ID NO:39)	WGQGTTVTVSS (SEQ ID NO:45)
2E2 RHC	EVQLVESGGGLVQ PGGSLRLSCAVSGF SLT (SEQ ID NO:27)	WVRQAPGKGLEW VS (SEQ ID NO:31)	RFTISKDNSKNTVY LQMNSLRAEDTAV YYCAR (SEQ ID NO:38)	WGQGTTVTVSS (SEQ ID NO:45)
2E2 RHD	EVQLVESGGGLVQ PGGSLRLSCAASGF SLT (SEQ ID NO:26)	WVRQAPGKGLEW LS (SEQ ID NO:33)	RFTISKDNSKNTVY LQMNSLRAEDTAV YYCAR (SEQ ID NO:38)	WGQGTTVTVSS (SEQ ID NO:45)
2E2 RHE	EVQLVESGGGLVQ PGGSLRLSCAASGF SLT (SEQ ID NO:26)	WVRQAPGKGLEW VG (SEQ ID NO:34)	RFTISKDNSKNTVY LQMNSLRAEDTAV YYCAR (SEQ ID NO:38)	WGQGTTVTVSS (SEQ ID NO:45)
2E2 RHF	EVQLVESGGGLVQ PGGSLRLSCAASGF SLT (SEQ ID NO:26)	WVRQAPGKGLEW VS (SEQ ID NO:31)	RLTISKDNSKNTV YLQMNSLRAEDTA VYYCAR (SEQ ID NO:40)	WGQGTTVTVSS (SEQ ID NO:45)
2E2 RHG	EVQLVESGGGLVQ PGGSLRLSCAASGF SLT (SEQ ID NO:26)	WVRQAPGKGLEW VS (SEQ ID NO:31)	RFSISKDNSKNTVY LQMNSLRAEDTAV YYCAR (SEQ ID NO:41)	WGQGTTVTVSS (SEQ ID NO:45)
2E2 RHA2	QVQLQESGPGLVK PSETLSLTCTVSGG SIS (SEQ ID NO:28)	WIRQPPGKGLEWI G (SEQ ID NO:35)	RVTISVDTSKNQFS LKLSSVTAADTAV YYCAR (SEQ ID NO:42)	WGQGTTLVTVSS (SEQ ID NO:46)
2E2 RHB2	QVQLQESGPGLVK PSETLSLTCTVSGF SLT (SEQ ID NO:29)	WVRQPPGKGLEW LG (SEQ ID NO:36)	RLSISKDNSKNQVS LKLSSVTAADTAV YYCAR (SEQ ID NO:43)	WGQGTTLVTVSS (SEQ ID NO:46)
2E2 RHE S-G	EVQLVESGGGLVQ PGGSLRLSCAASGF SLT (SEQ ID NO:26)	WVRQAPGKGLEW VG (SEQ ID NO:34)	RFTISKDNSKNTVY LQMNSLRAEDTAV YYCAR (SEQ ID NO:38)	WGQGTTVTVSS (SEQ ID NO:45)
2E2 RHE E-D	EVQLVESGGGLVQ	WVRQAPGKGLEW	RFTISKDNSKNTVY	WGQGTTVTVSS

	PGGSLRLSCAASGF SLT (SEQ ID NO:26)	VG (SEQ ID NO:34)	LQMNSLRAEDTAV YYCAR (SEQ ID NO:38)	(SEQ ID NO:45)
2E2 RHE Y-V	EVQLVESGGGLVQ PGGSLRLSCAASGF SLT (SEQ ID NO:26)	WVRQAPGKGLEW VG (SEQ ID NO:34)	RFTISKDNSKNTVY LQMNSLRAEDTAV YYCAR (SEQ ID NO:38)	WGQGTTVTVSS (SEQ ID NO:45)
2E2 RHE triple	EVQLVESGGGLVQ PGGSLRLSCAASGF SLT (SEQ ID NO:26)	WVRQAPGKGLEW VG (SEQ ID NO:34)	RFTISKDNSKNTVY LQMNSLRAEDTAV YYCAR (SEQ ID NO:38)	WGQGTTVTVSS (SEQ ID NO:45)
Light Chain	FR1	FR2	FR3	FR4
2E2	QHILTSQSPAIMSASP GEKVSITC (SEQ ID NO:47)	WFQQKPGTSPKLW IY (SEQ ID NO:50)	GVPVRFSGSGSGTS YSLTISRMEAEDA ATYYC (SEQ ID NO:54)	FGSGTKLEIK (SEQ ID NO:59)
RKA	EIVLTQSPATLSLSP GERATLSC (SEQ ID NO:48)	WFQQKPGQAPRL IY (SEQ ID NO:51)	GIPARFSGSGSGTD FTLTISLEPEDFAV YYC (SEQ ID NO:55)	FGPGTKLDIK (SEQ ID NO:60)
RKB	EHILTQSPATLSLSP GERATLSC (SEQ ID NO:49)	WFQQKPGQAPRL WIY (SEQ ID NO:52)	GVPARFSGSGSGT DYTLTISLEPEDF AVYYC (SEQ ID NO:56)	FGPGTKLDIK (SEQ ID NO:60)
RKC	EHILTQSPATLSLSP GERATLSC (SEQ ID NO:49)	WFQQKPGQAPRL IY (SEQ ID NO:51)	GIPARFSGSGSGTD FTLTISLEPEDFAV YYC (SEQ ID NO:55)	FGPGTKLDIK (SEQ ID NO:60)
RKD	EIVLTQSPATLSLSP GERATLSC (SEQ ID NO:48)	WFQQKPGQAPRL WIY (SEQ ID NO:52)	GIPARFSGSGSGTD FTLTISLEPEDFAV YYC (SEQ ID NO:55)	FGPGTKLDIK (SEQ ID NO:60)
RKE	EIVLTQSPATLSLSP GERATLSC (SEQ ID NO:48)	WFQQKPGQAPRL IY (SEQ ID NO:51)	GVPARFSGSGSGT DFTLTISLEPEDFA VYYC (SEQ ID NO:57)	FGPGTKLDIK (SEQ ID NO:60)
RKF	EIVLTQSPATLSLSP GERATLSC (SEQ ID NO:48)	WFQQKPGQAPRL IY (SEQ ID NO:51)	GIPARFSGSGSGTD YTLTISLEPEDFA VYYC (SEQ ID NO:58)	FGPGTKLDIK (SEQ ID NO:60)
RKG	EIVLTQSPATLSLSP	WYQQKPGQAPRL	GIPARFSGSGSGTD	FGPGTKLDIK

	GERATLSC (SEQ ID NO:48)	LIY (SEQ ID NO:53)	FTLTISLEPEDFAV YYC (SEQ ID NO:55)	(SEQ ID NO:60)
RKA F-Y	EIVLTQSPATLSLSP GERATLSC (SEQ ID NO:48)	WFQKPGQAPRLI Y (SEQ ID NO:51)	GIPARFSGSGSGTD FTLTISLEPEDFAV YYC (SEQ ID NO:55)	FGPGTKLDIK (SEQ ID NO:60)
RKF F-Y	EIVLTQSPATLSLSP GERATLSC (SEQ ID NO:48)	WFQKPGQAPRLI Y (SEQ ID NO:51)	GIPARFSGSGSGTD YTLTISLEPEDFA VYYC (SEQ ID NO:58)	FGPGTKLDIK (SEQ ID NO:60)

Table 4. Amino acid sequences of variable regions of antibodies

Antibody Name	Variable Heavy Chain	Variable Light Chain
ch2C4	ch2C4 VH	ch2C4 VK
ch2E2	ch2E2 VH (SEQ ID NO:1)	ch2E2 VK (SEQ ID NO:15)
cVHKA	ch2E2 VH (SEQ ID NO:1)	2E2 RKA (SEQ ID NO:16)
cVHKB	ch2E2 VH (SEQ ID NO:1)	2E2 RKB (SEQ ID NO:17)
HAcVK	2E2 RHA (SEQ ID NO:2)	ch2E2 VK (SEQ ID NO:15)
HBcVK	2E2 RHB (SEQ ID NO:3)	ch2E2 VK (SEQ ID NO:15)
HAKA	2E2 RHA (SEQ ID NO:2)	2E2 RKA (SEQ ID NO:16)
HAKB	2E2 RHA (SEQ ID NO:2)	2E2 RKB (SEQ ID NO:17)
HAKC	2E2 RHA (SEQ ID NO:2)	2E2 RKC (SEQ ID NO:18)
HAKD	2E2 RHA (SEQ ID NO:2)	2E2 RKD (SEQ ID NO:19)
HAKE	2E2 RHA (SEQ ID NO:2)	2E2 RKE (SEQ ID NO:20)
HAKF	2E2 RHA (SEQ ID NO:2)	2E2 RKF (SEQ ID NO:21)
HAKG	2E2 RHA (SEQ ID NO:2)	2E2 RKG (SEQ ID NO:22)
HBKA	2E2 RHB (SEQ ID NO:3)	2E2 RKA (SEQ ID NO:16)
HBKB	2E2 RHB (SEQ ID NO:3)	2E2 RKB (SEQ ID NO:17)
HBKC	2E2 RHB (SEQ ID NO:3)	2E2 RKC (SEQ ID NO:18)
HBKD	2E2 RHB (SEQ ID NO:3)	2E2 RKD (SEQ ID NO:19)
HBKE	2E2 RHB (SEQ ID NO:3)	2E2 RKE (SEQ ID NO:20)

HBKF	2E2 RHB (SEQ ID NO:3)	2E2 RKF (SEQ ID NO:21)
HBKG	2E2 RHB (SEQ ID NO:3)	2E2 RKG (SEQ ID NO:22)
HCKA	2E2 RHC (SEQ ID NO:4)	2E2 RKA (SEQ ID NO:16)
HCKB	2E2 RHC (SEQ ID NO:4)	2E2 RKB (SEQ ID NO:17)
HCKC	2E2 RHC (SEQ ID NO:4)	2E2 RKC (SEQ ID NO:18)
HCKD	2E2 RHC (SEQ ID NO:4)	2E2 RKD (SEQ ID NO:19)
HCKE	2E2 RHC (SEQ ID NO:4)	2E2 RKE (SEQ ID NO:20)
HCKF	2E2 RHC (SEQ ID NO:4)	2E2 RKF (SEQ ID NO:21)
HCKG	2E2 RHC (SEQ ID NO:4)	2E2 RKG (SEQ ID NO:22)
HDKA	2E2 RHD (SEQ ID NO:5)	2E2 RKA (SEQ ID NO:16)
HDKB	2E2 RHD (SEQ ID NO:5)	2E2 RKB (SEQ ID NO:17)
HDKC	2E2 RHD (SEQ ID NO:5)	2E2 RKC (SEQ ID NO:18)
HDKD	2E2 RHD (SEQ ID NO:5)	2E2 RKD (SEQ ID NO:19)
HDKE	2E2 RHD (SEQ ID NO:5)	2E2 RKE (SEQ ID NO:20)
HDKF	2E2 RHD (SEQ ID NO:5)	2E2 RKF (SEQ ID NO:21)
HDKG	2E2 RHD (SEQ ID NO:5)	2E2 RKG (SEQ ID NO:22)
HEKA	2E2 RHE (SEQ ID NO:6)	2E2 RKA (SEQ ID NO:16)
HEKB	2E2 RHE (SEQ ID NO:6)	2E2 RKB (SEQ ID NO:17)
HEKC	2E2 RHE (SEQ ID NO:6)	2E2 RKC (SEQ ID NO:18)
HEKD	2E2 RHE (SEQ ID NO:6)	2E2 RKD (SEQ ID NO:19)
HEKE	2E2 RHE (SEQ ID NO:6)	2E2 RKE (SEQ ID NO:20)
HEKF	2E2 RHE (SEQ ID NO:6)	2E2 RKF (SEQ ID NO:21)
HEKG	2E2 RHE (SEQ ID NO:6)	2E2 RKG (SEQ ID NO:22)
HFKA	2E2 RHF (SEQ ID NO:7)	2E2 RKA (SEQ ID NO:16)
HFKB	2E2 RHF (SEQ ID NO:7)	2E2 RKB (SEQ ID NO:17)
HFKC	2E2 RHF (SEQ ID NO:7)	2E2 RKC (SEQ ID NO:18)
HFKD	2E2 RHF (SEQ ID NO:7)	2E2 RKD (SEQ ID NO:19)
HFKE	2E2 RHF (SEQ ID NO:7)	2E2 RKE (SEQ ID NO:20)
HFKF	2E2 RHF (SEQ ID NO:7)	2E2 RKF (SEQ ID NO:21)
HFKG	2E2 RHF (SEQ ID NO:7)	2E2 RKG (SEQ ID NO:22)

HGKA	2E2 RHG (SEQ ID NO:8)	2E2 RKA (SEQ ID NO:16)
HGKB	2E2 RHG (SEQ ID NO:8)	2E2 RKB (SEQ ID NO:17)
HGKC	2E2 RHG (SEQ ID NO:8)	2E2 RKC (SEQ ID NO:18)
HGKD	2E2 RHG (SEQ ID NO:8)	2E2 RKD (SEQ ID NO:19)
HGKE	2E2 RHG (SEQ ID NO:8)	2E2 RKE (SEQ ID NO:20)
HGKF	2E2 RHG (SEQ ID NO:8)	2E2 RKF (SEQ ID NO:21)
HGHG	2E2 RHG (SEQ ID NO:8)	2E2 RKG (SEQ ID NO:22)
HA2KA	2E2 RHA2 (SEQ ID NO:9)	2E2 RKA (SEQ ID NO:16)
HA2KB	2E2 RHA2 (SEQ ID NO:9)	2E2 RKB (SEQ ID NO:17)
HB2KA	2E2 RHB2 (SEQ ID NO:10)	2E2 RKA (SEQ ID NO:16)
HB2KB	2E2 RHB2 (SEQ ID NO:10)	2E2 RKB (SEQ ID NO:17)
HA2KF	2E2 RHA2 (SEQ ID NO:9)	2E2 RKF (SEQ ID NO:21)
HB2KF	2E2 RHB2 (SEQ ID NO:10)	2E2 RKF (SEQ ID NO:21)
HA2KC	2E2 RHA2 (SEQ ID NO:9)	2E2 RKC (SEQ ID NO:18)
HA2KD	2E2 RHA2 (SEQ ID NO:9)	2E2 RKD (SEQ ID NO:19)
HA2KE	2E2 RHA2 (SEQ ID NO:9)	2E2 RKE (SEQ ID NO:20)
HA2KF	2E2 RHA2 (SEQ ID NO:9)	2E2 RKF (SEQ ID NO:21)
HA2KG	2E2 RHA2 (SEQ ID NO:9)	2E2 RKG (SEQ ID NO:22)
HB2KC	2E2 RHB2 (SEQ ID NO:10)	2E2 RKC (SEQ ID NO:18)
HB2KD	2E2 RHB2 (SEQ ID NO:10)	2E2 RKD (SEQ ID NO:19)
HB2KE	2E2 RHB2 (SEQ ID NO:10)	2E2 RKE (SEQ ID NO:20)
HA2KFmut	2E2 RHA2 (SEQ ID NO:9)	2E2 RKF F-Y mut (SEQ ID NO:24)
HB2KFmut	2E2 RHB2 (SEQ ID NO:10)	2E2 RKF F-Y mut (SEQ ID NO:24)
HEKAmut	2E2 RHE (SEQ ID NO:6)	2E2 RKA F-Y mut (SEQ ID NO:23)
HEKFmut	2E2 RHE (SEQ ID NO:6)	2E2 RKF F-Y mut (SEQ ID NO:24)
HAKFmut	2E2 RHA (SEQ ID NO:2)	2E2 RKF F-Y mut (SEQ ID NO:24)
HBKFmut	2E2 RHB (SEQ ID NO:3)	2E2 RKF F-Y mut (SEQ ID NO:24)
HCKFmut	2E2 RHC (SEQ ID NO:4)	2E2 RKF F-Y mut (SEQ ID NO:24)
HDKFmut	2E2 RHD (SEQ ID NO:5)	2E2 RKF F-Y mut (SEQ ID NO:24)
HPKFmut	2E2 RHF (SEQ ID NO:7)	2E2 RKF F-Y mut (SEQ ID NO:24)

HGKFmut	2E2 RHG (SEQ ID NO:8)	2E2 RKF F-Y mut (SEQ ID NO:24)
RHE Y-VKA	2E2 RHE Y-V (SEQ ID NO:13)	2E2 RKA (SEQ ID NO:16)
RHE Y-VKB	2E2 RHE Y-V (SEQ ID NO:13)	2E2 RKB (SEQ ID NO:17)
RHE Y-VKC	2E2 RHE Y-V (SEQ ID NO:13)	2E2 RKC (SEQ ID NO:18)
RHE Y-VKD	2E2 RHE Y-V (SEQ ID NO:13)	2E2 RKD (SEQ ID NO:19)
RHE Y-VKE	2E2 RHE Y-V (SEQ ID NO:13)	2E2 RKE (SEQ ID NO:20)
RHE Y-VKF	2E2 RHE Y-V (SEQ ID NO:13)	2E2 RKF (SEQ ID NO:21)
RHE Y-VKG	2E2 RHE Y-V (SEQ ID NO:13)	2E2 RKG (SEQ ID NO:22)
RHE E-DKA	2E2 RHE E-D (SEQ ID NO:12)	2E2 RKA (SEQ ID NO:16)
RHE E-DKB	2E2 RHE E-D (SEQ ID NO:12)	2E2 RKB (SEQ ID NO:17)
RHE E-DKC	2E2 RHE E-D (SEQ ID NO:12)	2E2 RKC (SEQ ID NO:18)
RHE E-DKD	2E2 RHE E-D (SEQ ID NO:12)	2E2 RKD (SEQ ID NO:19)
RHE E-DKE	2E2 RHE E-D (SEQ ID NO:12)	2E2 RKE (SEQ ID NO:20)
RHE E-DKF	2E2 RHE E-D (SEQ ID NO:12)	2E2 RKF (SEQ ID NO:21)
RHE E-DKG	2E2 RHE E-D (SEQ ID NO:12)	2E2 RKG (SEQ ID NO:22)
RHE E-DKFmut	2E2 RHE E-D (SEQ ID NO:12)	2E2 RKF F-Y mut (SEQ ID NO:24)
RHE S-GKA	2E2 RHE S-G (SEQ ID NO:11)	2E2 RKA (SEQ ID NO:16)
RHE S-GKB	2E2 RHE S-G (SEQ ID NO:11)	2E2 RKB (SEQ ID NO:17)
RHE S-GKC	2E2 RHE S-G (SEQ ID NO:11)	2E2 RKC (SEQ ID NO:18)
RHE S-GKD	2E2 RHE S-G (SEQ ID NO:11)	2E2 RKD (SEQ ID NO:19)
RHE S-GKE	2E2 RHE S-G (SEQ ID NO:11)	2E2 RKE (SEQ ID NO:20)
RHE S-GKF	2E2 RHE S-G (SEQ ID NO:11)	2E2 RKF (SEQ ID NO:21)
RHE S-GKG	2E2 RHE S-G (SEQ ID NO:11)	2E2 RKG (SEQ ID NO:22)
RHE Triple-KA	2E2 RHE triple (SEQ ID NO:14)	2E2 RKA (SEQ ID NO:16)
RHE Triple-KB	2E2 RHE triple (SEQ ID NO:14)	2E2 RKB (SEQ ID NO:17)
RHE Triple-KC	2E2 RHE triple (SEQ ID NO:14)	2E2 RKC (SEQ ID NO:18)
RHE Triple-KD	2E2 RHE triple (SEQ ID NO:14)	2E2 RKD (SEQ ID NO:19)
RHE Triple-KE	2E2 RHE triple (SEQ ID NO:14)	2E2 RKE (SEQ ID NO:20)
RHE Triple-KF	2E2 RHE triple (SEQ ID NO:14)	2E2 RKF (SEQ ID NO:21)
RHE Triple-KG	2E2 RHE triple (SEQ ID NO:14)	2E2 RKG (SEQ ID NO:22)

RHE Triple-KFmut	2E2 RHE triple (SEQ ID NO:14)	2E2 RKF F-Y mut (SEQ ID NO:24)
RHE Y-VKFmut	2E2 RHE Y-V (SEQ ID NO:13)	2E2 RKF F-Y mut (SEQ ID NO:24)
RHE E-DKFmut	2E2 RHE E-D (SEQ ID NO:12)	2E2 RKF F-Y mut (SEQ ID NO:24)

Table 5. Amino acid sequences of HVRs from murine 1C3, 1H10, and 4F11 antibodies

Antibody	Chain	HVR1	HVR2	HVR3
1C3	Heavy	SYAMS	IISGGGSYTYYSDSVKG	HETAQAAWFAY
	Chain	SEQ ID NO:88	SEQ ID NO:91	SEQ ID NO:94
1H10	Heavy	DYYMY	RIAPEDGDTEYAPKFQG	EGNYYGSSILDY
	Chain	SEQ ID NO:89	SEQ ID NO:92	SEQ ID NO:95
4F11	Heavy	SSWMN	QIYPGDDYTNYNGKFKG	LGPYGPFPAD
	Chain	SEQ ID NO:90	SEQ ID NO:93	SEQ ID NO:96
1C3	Light Chain	SASSSVSYMH	DTSKLAY	QQWSSNPPT
		SEQ ID NO:97	SEQ ID NO:100	SEQ ID NO:103
1H10	Light Chain	RASQDITNYLN	FTSRLHS	QQGNTLPWT
		SEQ ID NO:98	SEQ ID NO:101	SEQ ID NO:104
4F11	Light Chain	SASSSVSYMY	DTSSLAS	QQWNSDPYT
		SEQ ID NO:99	SEQ ID NO:102	SEQ ID NO:105

[0150] There are five classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM, having heavy chains designated α , δ , ϵ , γ and μ , respectively. The γ and α classes are further divided into subclasses *e.g.*, humans express the following subclasses: IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. IgG1 antibodies can exist in multiple polymorphic variants termed allotypes (reviewed in Jefferis and Lefranc 2009. *mAbs* Vol 1 Issue 4 1-7) any of which are suitable for use in some of the embodiments herein. Common allotypic variants in human populations are those designated by the letters a,f,n,z or combinations thereof. In any of the embodiments herein, the antibody may comprise a heavy chain Fc region comprising a human IgG Fc region. In further embodiments, the human IgG Fc region comprises a human IgG1 or IgG4. In some embodiments, the human IgG4 comprises the amino acid substitution S228P, wherein the amino acid residues are numbered according to the EU index as in Kabat. In some embodiments, the human IgG1 comprises the amino acid sequence of SEQ ID NO:78. In some embodiments, the human IgG4 comprises the amino acid sequence of SEQ ID NO:79.

[0151] In some embodiments, provided herein is an anti-Siglec-8 antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:75; and/or a light chain comprising

the amino acid sequence selected from SEQ ID NOs:76 or 77. In some embodiments, the antibody may comprise a heavy chain comprising the amino acid sequence of SEQ ID NO:87; and/or a light chain comprising the amino acid sequence of SEQ ID NO:76. In some embodiments, the anti-Siglec-8 antibody depletes mast cells and inhibits mast cell activation. In some embodiments, the anti-Siglec-8 antibody depletes activated eosinophils and inhibits mast cell activation. In some embodiments, the anti-Siglec-8 antibody induces apoptosis of activated eosinophils. In some embodiments, the anti-Siglec-8 antibody induces apoptosis of resting eosinophils. In some embodiments herein, the antibody depletes eosinophils in a tissue (*e.g.*, nasal polyps). In some embodiments herein, the antibody depletes eosinophils in a biological fluid (*e.g.*, blood).

1. Antibody Affinity

[0152] In some aspects, an anti-Siglec-8 antibody described herein binds to human Siglec-8 with about the same or higher affinity and/or higher avidity as compared mouse antibody 2E2 and/or mouse antibody 2C4. In certain embodiments, an anti-Siglec-8 antibody provided herein has a dissociation constant (K_d) of $\leq 1\mu\text{M}$, $\leq 150\text{ nM}$, $\leq 100\text{ nM}$, $\leq 50\text{ nM}$, $\leq 10\text{ nM}$, $\leq 1\text{ nM}$, $\leq 0.1\text{ nM}$, $\leq 0.01\text{ nM}$, or $\leq 0.001\text{ nM}$ (*e.g.* 10^{-8} M or less, *e.g.* from 10^{-8} M to 10^{-13} M , *e.g.*, from 10^{-9} M to 10^{-13} M). In some embodiments, an anti-Siglec-8 antibody described herein binds to human Siglec-8 at about 1.5-fold, about 2-fold, about 3-fold, about 4-fold, about 5-fold, about 6-fold, about 7-fold, about 8-fold, about 9-fold or about 10-fold higher affinity than mouse antibody 2E2 and/or mouse antibody 2C4. In some embodiments herein, the anti-Siglec-8 antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:6; and/or a light chain variable region comprising the amino acid sequence selected from SEQ ID NOs:16 or 21.

[0153] In one embodiment, the binding affinity of the anti-Siglec-8 antibody can be determined by a surface plasmon resonance assay. For example, the K_d or K_d value can be measured by using a BIAcoreTM-2000 or a BIAcoreTM-3000 (BIAcore, Inc., Piscataway, N.J.) at 25° C with immobilized antigen CM5 chips at ~10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIAcore® Inc.) are activated with N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. Capture antibodies (*e.g.*, anti-human-Fc) are diluted with 10 mM sodium acetate, pH 4.8, before injection at a flow rate of 30 $\mu\text{l}/\text{minute}$ and further immobilized with an anti-Siglec-8 antibody. For kinetics measurements, two-fold serial dilutions of dimeric Siglec-8 are injected in PBS with 0.05% Tween 20 (PBST) at 25° C at a flow rate of approximately 25 $\mu\text{l}/\text{min}$. Association rates (k_{on}) and dissociation rates (k_{off}) are

calculated using a simple one-to-one Langmuir binding model (BIAcore® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (K_d) is calculated as the ratio k_{off}/k_{on} . See, *e.g.*, Chen, Y., et al., (1999) J. Mol. Biol. 293:865-881.

[0154] In another embodiment, biolayer interferometry may be used to determine the affinity of anti-Siglec-8 antibodies against Siglec-8. In an exemplary assay, Siglec-8-Fc tagged protein is immobilized onto anti-human capture sensors, and incubated with increasing concentrations of mouse, chimeric, or humanized anti-Siglec-8 Fab fragments to obtain affinity measurements using an instrument such as, for example, the Octet Red 384 System (ForteBio).

[0155] The binding affinity of the anti-Siglec-8 antibody can, for example, also be determined by the Scatchard analysis described in Munson et al., *Anal. Biochem.*, 107:220 (1980) using standard techniques well known in the relevant art. See also Scatchard, G., *Ann. N.Y. Acad. Sci.* 51:660 (1947).

1. Antibody Avidity

[0156] In one embodiment, the binding avidity of the anti-Siglec-8 antibody can be determined by a surface plasmon resonance assay. For example, the K_d or K_d value can be measured by using a BIAcore T100. Capture antibodies (*e.g.*, goat-anti-human-Fc and goat-anti-mouse-Fc) are immobilized on a CM5 chip. Flow-cells can be immobilized with anti-human or with anti-mouse antibodies. The assay is conducted at a certain temperature and flow rate, for example, at 25°C at a flow rate of 30 μ l/min. Dimeric Siglec-8 is diluted in assay buffer at various concentrations, for example, at a concentration ranging from 15nM to 1.88pM. Antibodies are captured and high performance injections are conducted, followed by dissociations. Flow cells are regenerated with a buffer, for example, 50mM glycine pH 1.5. Results are blanked with an empty reference cell and multiple assay buffer injections, and analyzed with 1:1 global fit parameters.

2. Competition Assays

[0157] Competition assays can be used to determine whether two antibodies bind the same epitope by recognizing identical or sterically overlapping epitopes or one antibody competitively inhibits binding of another antibody to the antigen. These assays are known in the art. Typically, antigen or antigen expressing cells is immobilized on a multi-well plate and the ability of unlabeled antibodies to block the binding of labeled antibodies is measured. Common labels for such competition assays are radioactive labels or enzyme labels. In some embodiments, an anti-Siglec-8 antibody described herein competes with a 2E2 antibody

described herein, for binding to the epitope present on the cell surface of a cell (*e.g.*, an eosinophil or a mast cell). In some embodiments, an anti-Siglec-8 antibody described herein competes with an antibody comprising a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:1, and a light chain variable region comprising the amino acid sequence of SEQ ID NO:15, for binding to the epitope present on the cell surface of a cell (*e.g.*, an eosinophil or a mast cell). In some embodiments, an anti-Siglec-8 antibody described herein competes with a 2C4 antibody described herein, for binding to the epitope present on the cell surface of a cell (*e.g.*, an eosinophil or a mast cell). In some embodiments, an anti-Siglec-8 antibody described herein competes with an antibody comprising a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:2 (as found in U.S. Pat. No. 8,207,305), and a light chain variable region comprising the amino acid sequence of SEQ ID NO:4 (as found in U.S. Pat. No. 8,207,305), for binding to the epitope present on the cell surface of a cell (*e.g.*, an eosinophil or a mast cell).

3. *Thermal Stability*

[0158] In some aspects, an anti-Siglec-8 described herein has a melting temperature (T_m) of at least about 70°C, at least about 71°C, or at least about 72°C in a thermal shift assay. In an exemplary thermal shift assay, samples comprising a humanized anti-Siglec-8 antibody are incubated with a fluorescent dye (Sypro Orange) for 71 cycles with 1°C increase per cycle in a qPCR thermal cycler to determine the T_m . In some embodiments herein, the anti-Siglec-8 antibody has a similar or higher T_m as compared to mouse 2E2 antibody and/or mouse 2C4 antibody. In some embodiments herein, the anti-Siglec-8 antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:6; and/or a light chain variable region comprising the amino acid sequence selected from SEQ ID NOs:16 or 21. In some embodiments, the anti-Siglec-8 antibody has the same or higher T_m as compared to a chimeric 2C4 antibody. In some embodiments, the anti-Siglec-8 antibody has the same or higher T_m as compared to an antibody having a heavy chain comprising the amino acid sequence of SEQ ID NO:84 and a light chain comprising the amino acid sequence of SEQ ID NO:85.

4. *Biological Activity Assays*

[0159] In some aspects, an anti-Siglec-8 antibody described herein induces apoptosis of eosinophils. In some other aspects, an anti-Siglec-8 antibody described herein depletes mast cells. Assays for assessing apoptosis of cells are well known in the art, for example staining with Annexin V and the TUNEL assay. In an exemplary cell apoptosis assay, fresh buffy coat

from a blood sample is resuspended in media and plated in a 96-well U-bottom plate. A series of serial 5-fold dilutions of anti-Siglec-8 antibody is added to each well and the plate is incubated at 37°C at 5% CO₂ for greater than four hours. The cells are fixed with paraformaldehyde diluted in PBS and stained with conjugated antibodies specific for eosinophils for detection using a microscope. The eosinophil population in the total peripheral blood leukocytes is evaluated when the buffy coat is incubated in the presence of the anti-Siglec-8 antibody as compared to when the buffy coat is not incubated in the presence of the anti-Siglec-8 antibody. In another exemplary assay, eosinophils purified from a blood sample (*e.g.*, Miltenyi Eosinophil Isolation Kit) are resuspended in media and cultured in the presence or absence of IL-5 overnight. The cultured eosinophils are subsequently harvested by centrifugation, resuspended in media, and plated in a 96-well U-bottom plate. A series of serial 5-fold dilutions of anti-Siglec-8 antibody is added to each well and the plate is incubated at 37°C at 5% CO₂ for greater than four hours. The cells are fixed and stained with Annexin-V using standard techniques well known in the art the number of eosinophils is detected using a microscope. The eosinophil population in the sample is evaluated when the purified cells are incubated in the presence of the anti-Siglec-8 antibody as compared to when the purified cells are not incubated in the presence of the anti-Siglec-8 antibody.

[0160] In some aspects, an anti-Siglec-8 antibody described herein induces ADCC activity. In some other aspects, an anti-Siglec-8 antibody described herein kills mast cells expressing Siglec-8 by ADCC activity. In some embodiments, a composition comprises non-fucosylated (*i.e.*, afucosylated) anti-Siglec-8 antibodies. In some embodiments, a composition comprising non-fucosylated anti-Siglec-8 antibodies described herein enhances ADCC activity as compared to a composition comprising partially fucosylated anti-Siglec-8 antibodies. Assays for assessing ADCC activity are well known in the art and described herein. In an exemplary assay, to measure ADCC activity, effector cells and target cells are used. Examples of effector cells include natural killer (NK) cells, large granular lymphocytes (LGL), lymphokine-activated killer (LAK) cells and PBMC comprising NK and LGL, or leukocytes having Fc receptors on the cell surfaces, such as neutrophils, eosinophils and macrophages. The target cell is any cell which expresses on the cell surface antigens that antibodies to be evaluated can recognize. An example of such a target cell is an eosinophil which expresses Siglec-8 on the cell surface. Another example of such a target cell is a mast cell which expresses Siglec-8 on the cell surface. Target cells are labeled with a reagent that enables detection of cytolysis. Examples of reagents for labeling include a radio-active substance such as sodium chromate (Na₂ ⁵¹CrO₄). See, *e.g.*, *Immunology*, 14, 181 (1968); *J. Immunol. Methods.*, 172, 227 (1994); and *J. Immunol. Methods.*, 184, 29 (1995).

[0161] In some aspects, an anti-Siglec-8 antibody described herein inhibits mast cell-mediated activities. Mast cell tryptase has been used as a biomarker for total mast cell number and activation. For example, total and active tryptase as well as histamine, N-methyl histamine, and 11-beta-prostaglandin F2 can be measured in blood or urine to assess the reduction in mast cells. See, *e.g.*, U.S. Patent Application Publication No. US 20110293631 for an exemplary mast cell activity assay. In an exemplary assay to assess ADCC and apoptotic activity of anti-Siglec-8 antibodies on mast cells, human mast cells are isolated from human tissues according to published protocols (Guhl et al., *Biosci. Biotechnol. Biochem.*, 2011, 75:382-384; Kulka et al., *In Current Protocols in Immunology*, 2001, (John Wiley & Sons, Inc.)) or differentiated from human hematopoietic stem cells, for example as described by Yokoi et al., *J Allergy Clin Immunol.*, 2008, 121:499-505. Purified mast cells are resuspended in Complete RPMI medium in a sterile 96-well U-bottom plate and incubated in the presence or absence of anti Siglec-8 antibodies for 30 minutes at concentrations ranging between 0.0001 ng/ml and 10 µg/ml. Samples are incubated for a further 4 to 16 hours with and without purified natural killer (NK) cells or fresh PBL to induce ADCC. Cell-killing by apoptosis or ADCC is analyzed by flow cytometry using fluorescent conjugated antibodies to detect mast cells (CD117 and FcεR1) and Annexin-V and 7AAD to discriminate live and dead or dying cells. Annexin-V and 7AAD staining are performed according to manufacturer's instructions.

[0162] In some aspects, an anti-Siglec-8 antibody described herein inhibits neutrophil influx to sites of fibrotic disease (*e.g.*, pulmonary fibrosis). Bleomycin is a glycopeptide antitumor antibiotic and antiviral drug. Bleomycin acts by induction of DNA strand breaks. The most serious complication of bleomycin is pulmonary fibrosis and impaired lung function. A well-known animal model of pulmonary fibrosis is generated by a single intratracheal or intranasal delivery of bleomycin to the lungs of a rodent (*e.g.*, a mouse or a rat). Administration of bleomycin generally results in dose-dependent damage to the lung, characterized by inflammatory cell infiltrates, collagen accumulation, and parenchymal consolidation. The lungs are generally studied 7 to 14 days following a single administration of bleomycin. See Mouratis et al., *Curr Opin Pulm Med.*, 2011, 17(5):355-61. In an exemplary assay, transgenic rodents expressing human Siglec-8 on eosinophils, mast cells, and basophils and with bleomycin-induced pulmonary fibrosis are treated with an anti-Siglec-8 antibody described herein at a dosage regimen of interest. Bronchoalveolar lavage (BAL) fluid is harvested from the treated rodents and processed for leukocyte evaluation. The BAL fluid is centrifuged and cell pellet is resuspended in an appropriate lysing buffer to lyse the red blood cells. Phosphate buffered saline (PBS) supplemented with Fetal Bovine Serum (FBS) is added to stop the lysis reaction

before centrifuging the sample again to obtain a leukocyte pellet. The number of leukocytes are counted using a hemocytometer and the trypan blue exclusion method as described herein.

[0163] In some aspects, an anti-Siglec-8 antibody described herein prevents collagen accumulation at sites of fibrotic disease (*e.g.*, pulmonary fibrosis). In an exemplary assay, transgenic rodents expressing human Siglec-8 on eosinophils, mast cells, and basophils and with bleomycin-induced pulmonary fibrosis are treated with an anti-Siglec-8 antibody described herein at a dosage regimen of interest. Bronchoalveolar lavage (BAL) fluid is harvested from the treated rodents and processed for collagen evaluation. The BAL fluid is centrifuged and the supernatant is analyzed for collagen using a collagen assay such as Sircol™ Collagen Assay (Biocolor Life Science Assays, United Kingdom).

Antibody Preparation

[0164] The antibody described herein (*e.g.*, an antibody that binds to human Siglec-8) is prepared using techniques available in the art for generating antibodies, exemplary methods of which are described in more detail in the following sections.

1. Antibody Fragments

[0165] The present invention encompasses antibody fragments. Antibody fragments may be generated by traditional means, such as enzymatic digestion, or by recombinant techniques. In certain circumstances there are advantages of using antibody fragments, rather than whole antibodies. For a review of certain antibody fragments, see Hudson et al. (2003) *Nat. Med.* 9:129-134.

[0166] Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, *e.g.*, Morimoto et al., *Journal of Biochemical and Biophysical Methods* 24:107-117 (1992); and Brennan et al., *Science*, 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. Fab, Fv and ScFv antibody fragments can all be expressed in and secreted from *E. coli*, thus allowing the facile production of large amounts of these fragments. Antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form F(ab')₂ fragments (Carter et al., *Bio/Technology* 10: 163-167 (1992)). According to another approach, F(ab')₂ fragments can be isolated directly from recombinant host cell culture. Fab and F(ab')₂ fragment with increased in vivo half-life comprising salvage receptor binding epitope residues are described in U.S. Pat. No. 5,869,046. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In certain embodiments, an antibody is a single chain Fv fragment (scFv). See WO 93/16185; U.S. Pat. Nos. 5,571,894; and

5,587,458. Fv and scFv are the only species with intact combining sites that are devoid of constant regions; thus, they may be suitable for reduced nonspecific binding during in vivo use. scFv fusion proteins may be constructed to yield fusion of an effector protein at either the amino or the carboxy terminus of an scFv. *See* Antibody Engineering, ed. Borrebaeck, supra. The antibody fragment may also be a “linear antibody”, *e.g.*, as described in U.S. Pat. No. 5,641,870, for example. Such linear antibodies may be monospecific or bispecific.

2. Humanized Antibodies

[0167] The invention encompasses humanized antibodies. Various methods for humanizing non-human antibodies are known in the art. For example, a humanized antibody can have one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as “import” residues, which are typically taken from an “import” variable domain. Humanization can be essentially performed following the method of Winter (Jones et al. (1986) *Nature* 321:522-525; Riechmann et al. (1988) *Nature* 332:323-327; Verhoeven et al. (1988) *Science* 239:1534-1536), by substituting hypervariable region sequences for the corresponding sequences of a human antibody. Accordingly, such “humanized” antibodies are chimeric antibodies (U.S. Pat. No. 4,816,567) wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some hypervariable region residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

[0168] The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies can be important to reduce antigenicity. According to the so-called “best-fit” method, the sequence of the variable domain of a rodent (*e.g.*, mouse) antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework for the humanized antibody (Sims et al. (1993) *J. Immunol.* 151:2296; Chothia et al. (1987) *J. Mol. Biol.* 196:901. Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (Carter et al. (1992) *Proc. Natl. Acad. Sci. USA*, 89:4285; Presta et al. (1993) *J. Immunol.*, 151:2623.

[0169] It is further generally desirable that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to one method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly

available and are familiar to those, skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, *i.e.*, the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the hypervariable region residues are directly and most substantially involved in influencing antigen binding.

3. Human Antibodies

[0170] Human anti-Siglec-8 antibodies of the invention can be constructed by combining Fv clone variable domain sequence(s) selected from human-derived phage display libraries with known human constant domain sequences(s). Alternatively, human monoclonal anti-Siglec-8 antibodies of the invention can be made by the hybridoma method. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described, for example, by Kozbor *J. Immunol.*, 133: 3001 (1984); Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., *J. Immunol.*, 147: 86 (1991).

[0171] It is possible to produce transgenic animals (*e.g.*, mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (JH) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, *e.g.*, Jakobovits et al., *Proc. Natl. Acad. Sci. USA*, 90: 2551 (1993); Jakobovits et al., *Nature*, 362: 255 (1993); Bruggermann et al., *Year in Immunol.*, 7: 33 (1993).

[0172] Gene shuffling can also be used to derive human antibodies from non-human (*e.g.*, rodent) antibodies, where the human antibody has similar affinities and specificities to the starting non-human antibody. According to this method, which is also called “epitope imprinting”, either the heavy or light chain variable region of a non-human antibody fragment obtained by phage display techniques as described herein is replaced with a repertoire of human V domain genes, creating a population of non-human chain/human chain scFv or Fab chimeras. Selection with antigen results in isolation of a non-human chain/human chain chimeric scFv or Fab wherein the human chain restores the antigen binding site destroyed upon removal of the

corresponding non-human chain in the primary phage display clone, *i.e.*, the epitope governs the choice of the human chain partner. When the process is repeated in order to replace the remaining non-human chain, a human antibody is obtained (see PCT WO 93/06213 published Apr. 1, 1993). Unlike traditional humanization of non-human antibodies by CDR grafting, this technique provides completely human antibodies, which have no FR or CDR residues of non-human origin.

4. Bispecific Antibodies

[0173] Bispecific antibodies are monoclonal antibodies that have binding specificities for at least two different antigens. In certain embodiments, bispecific antibodies are human or humanized antibodies. In certain embodiments, one of the binding specificities is for Siglec-8 and the other is for any other antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of Siglec-8. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express Siglec-8. Bispecific antibodies can be prepared as full length antibodies or antibody fragments (*e.g.* F(ab')₂ bispecific antibodies).

[0174] Methods for making bispecific antibodies are known in the art. See Milstein and Cuello, *Nature*, 305: 537 (1983), WO 93/08829 published May 13, 1993, and Traunecker et al., *EMBO J.*, 10: 3655 (1991). For further details of generating bispecific antibodies see, for example, Suresh et al., *Methods in Enzymology*, 121:210 (1986). Bispecific antibodies include cross-linked or “heteroconjugate” antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Heteroconjugate antibodies may be made using any convenient cross-linking method. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Pat. No. 4,676,980, along with a number of cross-linking techniques.

5. Single-Domain Antibodies

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

6. Antibody Variants

[0176] In some embodiments, amino acid sequence modification(s) of the antibodies described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of the antibody may be prepared by introducing appropriate changes into the nucleotide sequence encoding the

antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid alterations may be introduced in the subject antibody amino acid sequence at the time that sequence is made.

[0177] A useful method for identification of certain residues or regions of the antibody that are preferred locations for mutagenesis is called “alanine scanning mutagenesis” as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. Here, a residue or group of target residues are identified (*e.g.*, charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (*e.g.*, alanine or polyalanine) to affect the interaction of the amino acids with antigen. Those amino acid locations demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at, or for, the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation per se need not be predetermined. For example, to analyze the performance of a mutation at a given site, ala scanning or random mutagenesis is conducted at the target codon or region and the expressed immunoglobulins are screened for the desired activity.

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

[0179] In some embodiments, monoclonal antibodies have a C-terminal cleavage at the heavy chain and/or light chain. For example, 1, 2, 3, 4, or 5 amino acid residues are cleaved at the C-terminus of heavy chain and/or light chain. In some embodiments, the C-terminal cleavage removes a C-terminal lysine from the heavy chain. In some embodiments, monoclonal antibodies have an N-terminal cleavage at the heavy chain and/or light chain. For example, 1, 2, 3, 4, or 5 amino acid residues are cleaved at the N-terminus of heavy chain and/or light chain. In some embodiments, truncated forms of monoclonal antibodies can be made by recombinant techniques.

[0180] In certain embodiments, an antibody of the invention is altered to increase or decrease the extent to which the antibody is glycosylated. Glycosylation of polypeptides is typically either N-linked or O-linked. N-linked refers to the attachment of a carbohydrate moiety to the side

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

[0181] Addition or deletion of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that one or more of the above-described tripeptide sequences (for N-linked glycosylation sites) is created or removed. The alteration may also be made by the addition, deletion, or substitution of one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites).

[0182] Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. For example, antibodies with a mature carbohydrate structure that lacks fucose attached to an Fc region of the antibody are described in US Pat Appl No US 2003/0157108 (Presta, L.). *See also* US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Antibodies with a bisecting N-acetylglucosamine (GlcNAc) in the carbohydrate attached to an Fc region of the antibody are referenced in WO 2003/011878, Jean-Mairet et al. and U.S. Pat. No. 6,602,684, Umana et al. Antibodies with at least one galactose residue in the oligosaccharide attached to an Fc region of the antibody are reported in WO 1997/30087, Patel et al. *See, also*, WO 1998/58964 (Raju, S.) and WO 1999/22764 (Raju, S.) concerning antibodies with altered carbohydrate attached to the Fc region thereof. *See also* US 2005/0123546 (Umana et al.) on antigen-binding molecules with modified glycosylation.

[0183] In certain embodiments, a glycosylation variant comprises an Fc region, wherein a carbohydrate structure attached to the Fc region lacks fucose. Such variants have improved ADCC function. Optionally, the Fc region further comprises one or more amino acid substitutions therein which further improve ADCC, for example, substitutions at positions 298, 333, and/or 334 of the Fc region (Eu numbering of residues). Examples of publications related to “defucosylated” or “fucose-deficient” antibodies include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; Okazaki et al. *J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. *Arch. Biochem. Biophys.* 249:533-545 (1986); US Pat Appl No US 2003/0157108

A1, Presta, L; and WO 2004/056312 A1, Adams et al., especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, FUT8, knockout CHO cells (Yamane-Ohnuki et al. Biotech. Bioeng. 87: 614 (2004)), and cells overexpressing β 1,4-N-acetylglycosaminyltransferase III (GnT-III) and Golgi μ -mannosidase II (ManII).

[0184] Antibodies are contemplated herein that have reduced fucose relative to the amount of fucose on the same antibody produced in a wild-type CHO cell. For example, the antibody has a lower amount of fucose than it would otherwise have if produced by native CHO cells (*e.g.*, a CHO cell that produce a native glycosylation pattern, such as, a CHO cell containing a native FUT8 gene). In certain embodiments, an anti-Siglec-8 antibody provided herein is one wherein less than about 50%, 40%, 30%, 20%, 10%, 5% or 1% of the N-linked glycans thereon comprise fucose. In certain embodiments, an anti-Siglec-8 antibody provided herein is one wherein none of the N-linked glycans thereon comprise fucose, *i.e.*, wherein the antibody is completely without fucose, or has no fucose or is non-fucosylated or is afucosylated. The amount of fucose can be determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn297 (*e.g.*, complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (Eu numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, *i.e.*, between positions 294 and 300, due to minor sequence variations in antibodies. In some embodiments, at least one or two of the heavy chains of the antibody is non-fucosylated.

[0185] In one embodiment, the antibody is altered to improve its serum half-life. To increase the serum half-life of the antibody, one may incorporate a salvage receptor binding epitope into the antibody (especially an antibody fragment) as described in U.S. Pat. No. 5,739,277, for example. As used herein, the term “salvage receptor binding epitope” refers to an epitope of the Fc region of an IgG molecule (*e.g.*, IgG1, IgG2, IgG3, or IgG4) that is responsible for increasing the *in vivo* serum half-life of the IgG molecule (US 2003/0190311, U.S. Pat. No. 6,821,505; U.S. Pat. No. 6,165,745; U.S. Pat. No. 5,624,821; U.S. Pat. No. 5,648,260; U.S. Pat. No. 6,165,745; U.S. Pat. No. 5,834,597).

[0186] Another type of variant is an amino acid substitution variant. These variants have at least one amino acid residue in the antibody molecule replaced by a different residue. Sites of interest for substitutional mutagenesis include the hypervariable regions, but FR alterations are also contemplated. Conservative substitutions are shown in Table 1 under the heading of “preferred substitutions.” If such substitutions result in a desirable change in biological activity, then more substantial changes, denominated “exemplary substitutions” in Table 1, or as further

described below in reference to amino acid classes, may be introduced and the products screened.

Table 1.

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

[0187] Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or c) the bulk of the side chain. Amino acids may be grouped according to similarities in the properties of their side chains (in A. L. Lehninger, in Biochemistry, second ed., pp. 73-75, Worth Publishers, New York (1975)):

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

[0188] Alternatively, naturally occurring residues may be divided into groups based on common side-chain properties:

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

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

[0189] Non-conservative substitutions will entail exchanging a member of one of these classes for another class. Such substituted residues also may be introduced into the conservative substitution sites or, into the remaining (non-conserved) sites.

[0190] One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (*e.g.*, a humanized or human antibody). Generally, the resulting variant(s) selected for further development will have modified (*e.g.*, improved) biological properties relative to the parent antibody from which they are generated. A convenient way for generating such substitutional variants involves affinity maturation using phage display. Briefly, several hypervariable region sites (*e.g.*, 6-7 sites) are mutated to generate all possible amino acid substitutions at each site. The antibodies thus generated are displayed from filamentous phage particles as fusions to at least part of a phage coat protein (*e.g.*, the gene III product of M13) packaged within each particle. The phage-displayed variants are then screened for their biological activity (*e.g.*, binding affinity). In order to identify candidate hypervariable region sites for modification, scanning mutagenesis (*e.g.*, alanine scanning) can be performed to identify hypervariable region residues contributing significantly to antigen binding.

Alternatively, or additionally, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues are candidates for substitution according to techniques known in the art, including those elaborated herein. Once such variants are generated, the panel of variants is subjected to screening using techniques known in the art, including those described herein, and antibodies with superior properties in one or more relevant assays may be selected for further development.

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

[0192] It may be desirable to introduce one or more amino acid modifications in an Fc region of antibodies of the invention, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (*e.g.*, a human IgG1, IgG2, IgG3 or IgG4 Fc region)

comprising an amino acid modification (*e.g.*, a substitution) at one or more amino acid positions including that of a hinge cysteine. In some embodiments, the Fc region variant comprises a human IgG4 Fc region. In a further embodiment, the human IgG4 Fc region comprises the amino acid substitution S228P, wherein the amino acid residues are numbered according to the EU index as in Kabat.

[0193] In accordance with this description and the teachings of the art, it is contemplated that in some embodiments, an antibody of the invention may comprise one or more alterations as compared to the wild type counterpart antibody, *e.g.* in the Fc region. These antibodies would nonetheless retain substantially the same characteristics required for therapeutic utility as compared to their wild type counterpart. For example, it is thought that certain alterations can be made in the Fc region that would result in altered (*i.e.*, either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), *e.g.*, as described in WO99/51642. See also Duncan & Winter *Nature* 322:738-40 (1988); U.S. Pat. No. 5,648,260; U.S. Pat. No. 5,624,821; and WO94/29351 concerning other examples of Fc region variants. WO00/42072 (Presta) and WO 2004/056312 (Lowman) describe antibody variants with improved or diminished binding to FcRs. The content of these patent publications are specifically incorporated herein by reference. See, also, Shields et al. *J. Biol. Chem.* 9(2): 6591-6604 (2001). Antibodies with increased half-lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., *J. Immunol.* 117:587 (1976) and Kim et al., *J. Immunol.* 24:249 (1994)), are described in US2005/0014934A1 (Hinton et al.). These antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Polypeptide variants with altered Fc region amino acid sequences and increased or decreased C1q binding capability are described in U.S. Pat. No. 6,194,551B1, WO99/51642. The contents of those patent publications are specifically incorporated herein by reference. See, also, Idusogie et al. *J. Immunol.* 164: 4178-4184 (2000).

7. Vectors, Host Cells, and Recombinant Methods

[0194] For recombinant production of an antibody of the invention, the nucleic acid encoding it is isolated and inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. DNA encoding the antibody is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody). Many vectors are available. The choice of vector depends in part on the host cell to be used. Generally, host cells are of either prokaryotic or eukaryotic (generally mammalian) origin. It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM, IgA, IgD, and

IgE constant regions, and that such constant regions can be obtained from any human or animal species.

Generating Antibodies Using Prokaryotic Host Cells:

a) Vector Construction

[0195] Polynucleotide sequences encoding polypeptide components of the antibody of the invention can be obtained using standard recombinant techniques. Desired polynucleotide sequences may be isolated and sequenced from antibody producing cells such as hybridoma cells. Alternatively, polynucleotides can be synthesized using nucleotide synthesizer or PCR techniques. Once obtained, sequences encoding the polypeptides are inserted into a recombinant vector capable of replicating and expressing heterologous polynucleotides in prokaryotic hosts. Many vectors that are available and known in the art can be used for the purpose of the present invention. Selection of an appropriate vector will depend mainly on the size of the nucleic acids to be inserted into the vector and the particular host cell to be transformed with the vector. Each vector contains various components, depending on its function (amplification or expression of heterologous polynucleotide, or both) and its compatibility with the particular host cell in which it resides. The vector components generally include, but are not limited to: an origin of replication, a selection marker gene, a promoter, a ribosome binding site (RBS), a signal sequence, the heterologous nucleic acid insert and a transcription termination sequence.

[0196] In general, plasmid vectors containing replicon and control sequences which are derived from species compatible with the host cell are used in connection with these hosts. The vector ordinarily carries a replication site, as well as marking sequences which are capable of providing phenotypic selection in transformed cells. For example, *E. coli* is typically transformed using pBR322, a plasmid derived from an *E. coli* species. pBR322 contains genes-encoding ampicillin (Amp) and tetracycline (Tet) resistance and thus provides easy means for identifying transformed cells. pBR322, its derivatives, or other microbial plasmids or bacteriophage may also contain, or be modified to contain, promoters which can be used by the microbial organism for expression of endogenous proteins. Examples of pBR322 derivatives used for expression of particular antibodies are described in detail in Carter et al., U.S. Pat. No. 5,648,237.

[0197] In addition, phage vectors containing replicon and control sequences that are compatible with the host microorganism can be used as transforming vectors in connection with these hosts. For example, bacteriophage such as λ GEM.TM.-11 may be utilized in making a recombinant vector which can be used to transform susceptible host cells such as *E. coli* LE392.

[0198] The expression vector of the invention may comprise two or more promoter-cistron pairs, encoding each of the polypeptide components. A promoter is an untranslated regulatory

sequence located upstream (5') to a cistron that modulates its expression. Prokaryotic promoters typically fall into two classes, inducible and constitutive. Inducible promoter is a promoter that initiates increased levels of transcription of the cistron under its control in response to changes in the culture condition, *e.g.* the presence or absence of a nutrient or a change in temperature.

[0199] A large number of promoters recognized by a variety of potential host cells are well known. The selected promoter can be operably linked to cistron DNA encoding the light or heavy chain by removing the promoter from the source DNA via restriction enzyme digestion and inserting the isolated promoter sequence into the vector of the invention. Both the native promoter sequence and many heterologous promoters may be used to direct amplification and/or expression of the target genes. In some embodiments, heterologous promoters are utilized, as they generally permit greater transcription and higher yields of expressed target gene as compared to the native target polypeptide promoter.

[0200] Promoters suitable for use with prokaryotic hosts include the PhoA promoter, the β -galactamase and lactose promoter systems, a tryptophan (*trp*) promoter system and hybrid promoters such as the *tac* or the *trc* promoter. However, other promoters that are functional in bacteria (such as other known bacterial or phage promoters) are suitable as well. Their nucleotide sequences have been published, thereby enabling a skilled worker operably to ligate them to cistrons encoding the target light and heavy chains (Siebenlist et al. (1980) *Cell* 20: 269) using linkers or adaptors to supply any required restriction sites.

[0201] In one aspect of the invention, each cistron within the recombinant vector comprises a secretion signal sequence component that directs translocation of the expressed polypeptides across a membrane. In general, the signal sequence may be a component of the vector, or it may be a part of the target polypeptide DNA that is inserted into the vector. The signal sequence selected for the purpose of this invention should be one that is recognized and processed (*i.e.* cleaved by a signal peptidase) by the host cell. For prokaryotic host cells that do not recognize and process the signal sequences native to the heterologous polypeptides, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group consisting of the alkaline phosphatase, penicillinase, Ipp, or heat-stable enterotoxin II (STII) leaders, LamB, PhoE, PelB, OmpA and MBP. In one embodiment of the invention, the signal sequences used in both cistrons of the expression system are STII signal sequences or variants thereof.

[0202] In another aspect, the production of the immunoglobulins according to the invention can occur in the cytoplasm of the host cell, and therefore does not require the presence of secretion signal sequences within each cistron. In that regard, immunoglobulin light and heavy chains are expressed, folded and assembled to form functional immunoglobulins within the cytoplasm. Certain host strains (*e.g.*, the *E. coli* *trxB*-strains) provide cytoplasm conditions that

are favorable for disulfide bond formation, thereby permitting proper folding and assembly of expressed protein subunits. Proba and Pluckthun *Gene*, 159:203 (1995).

[0203] Antibodies of the invention can also be produced by using an expression system in which the quantitative ratio of expressed polypeptide components can be modulated in order to maximize the yield of secreted and properly assembled antibodies of the invention. Such modulation is accomplished at least in part by simultaneously modulating translational strengths for the polypeptide components.

[0204] One technique for modulating translational strength is disclosed in Simmons et al., U.S. Pat. No. 5,840,523. It utilizes variants of the translational initiation region (TIR) within a cistron. For a given TIR, a series of amino acid or nucleic acid sequence variants can be created with a range of translational strengths, thereby providing a convenient means by which to adjust this factor for the desired expression level of the specific chain. TIR variants can be generated by conventional mutagenesis techniques that result in codon changes which can alter the amino acid sequence. In certain embodiments, changes in the nucleotide sequence are silent. Alterations in the TIR can include, for example, alterations in the number or spacing of Shine-Dalgarno sequences, along with alterations in the signal sequence. One method for generating mutant signal sequences is the generation of a "codon bank" at the beginning of a coding sequence that does not change the amino acid sequence of the signal sequence (i.e., the changes are silent). This can be accomplished by changing the third nucleotide position of each codon; additionally, some amino acids, such as leucine, serine, and arginine, have multiple first and second positions that can add complexity in making the bank. This method of mutagenesis is described in detail in Yansura et al. (1992) *METHODS: A Companion to Methods in Enzymol.* 4:151-158.

[0205] In one embodiment, a set of vectors is generated with a range of TIR strengths for each cistron therein. This limited set provides a comparison of expression levels of each chain as well as the yield of the desired antibody products under various TIR strength combinations. TIR strengths can be determined by quantifying the expression level of a reporter gene as described in detail in Simmons et al. U.S. Pat. No. 5,840,523. Based on the translational strength comparison, the desired individual TIRs are selected to be combined in the expression vector constructs of the invention.

[0206] Prokaryotic host cells suitable for expressing antibodies of the invention include Archaeobacteria and Eubacteria, such as Gram-negative or Gram-positive organisms. Examples of useful bacteria include *Escherichia* (e.g., *E. coli*), *Bacilli* (e.g., *B. subtilis*), *Enterobacteria*, *Pseudomonas* species (e.g., *P. aeruginosa*), *Salmonella typhimurium*, *Serratia marcescans*, *Klebsiella*, *Proteus*, *Shigella*, *Rhizobia*, *Vitreoscilla*, or *Paracoccus*. In one embodiment, gram-negative cells are used. In one embodiment, *E. coli* cells are used as hosts for the invention.

Examples of *E. coli* strains include strain W3110 (Bachmann, Cellular and Molecular Biology, vol. 2 (Washington, D.C.: American Society for Microbiology, 1987), pp. 1190-1219; ATCC Deposit No. 27,325) and derivatives thereof, including strain 33D3 having genotype W3110 Δ hlyA (Δ tonA) ptr3 lac Iq lacL8 Δ ompT Δ (nmpc-fepE) degP41 kanR (U.S. Pat. No. 5,639,635). Other strains and derivatives thereof, such as *E. coli* 294 (ATCC 31,446), *E. coli* B, *E. coli* 1776 (ATCC 31,537) and *E. coli* RV308(ATCC 31,608) are also suitable. These examples are illustrative rather than limiting. Methods for constructing derivatives of any of the above-mentioned bacteria having defined genotypes are known in the art and described in, for example, Bass et al., Proteins, 8:309-314 (1990). It is generally necessary to select the appropriate bacteria taking into consideration replicability of the replicon in the cells of a bacterium. For example, *E. coli*, *Serratia*, or *Salmonella* species can be suitably used as the host when well known plasmids such as pBR322, pBR325, pACYC177, or pKN410 are used to supply the replicon. Typically the host cell should secrete minimal amounts of proteolytic enzymes, and additional protease inhibitors may desirably be incorporated in the cell culture.

b) Antibody Production

[0207] Host cells are transformed with the above-described expression vectors and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

[0208] Transformation means introducing DNA into the prokaryotic host so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integrant. Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride is generally used for bacterial cells that contain substantial cell-wall barriers. Another method for transformation employs polyethylene glycol/DMSO. Yet another technique used is electroporation.

[0209] Prokaryotic cells used to produce the polypeptides of the invention are grown in media known in the art and suitable for culture of the selected host cells. Examples of suitable media include luria broth (LB) plus necessary nutrient supplements. In some embodiments, the media also contains a selection agent, chosen based on the construction of the expression vector, to selectively permit growth of prokaryotic cells containing the expression vector. For example, ampicillin is added to media for growth of cells expressing ampicillin resistant gene.

[0210] Any necessary supplements besides carbon, nitrogen, and inorganic phosphate sources may also be included at appropriate concentrations introduced alone or as a mixture with another supplement or medium such as a complex nitrogen source. Optionally the culture medium may contain one or more reducing agents selected from the group consisting of glutathione, cysteine, cystamine, thioglycollate, dithioerythritol and dithiothreitol.

[0211] The prokaryotic host cells are cultured at suitable temperatures. In certain embodiments, for *E. coli* growth, growth temperatures range from about 20° C. to about 39° C.; from about 25° C. to about 37° C.; or about 30° C. The pH of the medium may be any pH ranging from about 5 to about 9, depending mainly on the host organism. In certain embodiments, for *E. coli*, the pH is from about 6.8 to about 7.4, or about 7.0.

[0212] If an inducible promoter is used in the expression vector of the invention, protein expression is induced under conditions suitable for the activation of the promoter. In one aspect of the invention, PhoA promoters are used for controlling transcription of the polypeptides. Accordingly, the transformed host cells are cultured in a phosphate-limiting medium for induction. In certain embodiments, the phosphate-limiting medium is the C.R.A.P. medium (see, *e.g.*, Simmons et al., *J. Immunol. Methods* (2002), 263:133-147). A variety of other inducers may be used, according to the vector construct employed, as is known in the art.

[0213] In one embodiment, the expressed polypeptides of the present invention are secreted into and recovered from the periplasm of the host cells. Protein recovery typically involves disrupting the microorganism, generally by such means as osmotic shock, sonication or lysis. Once cells are disrupted, cell debris or whole cells may be removed by centrifugation or filtration. The proteins may be further purified, for example, by affinity resin chromatography. Alternatively, proteins can be transported into the culture media and isolated therein. Cells may be removed from the culture and the culture supernatant being filtered and concentrated for further purification of the proteins produced. The expressed polypeptides can be further isolated and identified using commonly known methods such as polyacrylamide gel electrophoresis (PAGE) and Western blot assay.

[0214] In one aspect of the invention, antibody production is conducted in large quantity by a fermentation process. Various large-scale fed-batch fermentation procedures are available for production of recombinant proteins. Large-scale fermentations have at least 1000 liters of capacity, and in certain embodiments, about 1,000 to 100,000 liters of capacity. These fermentors use agitator impellers to distribute oxygen and nutrients, especially glucose. Small scale fermentation refers generally to fermentation in a fermentor that is no more than approximately 100 liters in volumetric capacity, and can range from about 1 liter to about 100 liters.

[0215] In a fermentation process, induction of protein expression is typically initiated after the cells have been grown under suitable conditions to a desired density, *e.g.*, an OD550 of about 180-220, at which stage the cells are in the early stationary phase. A variety of inducers may be used, according to the vector construct employed, as is known in the art and described above.

Cells may be grown for shorter periods prior to induction. Cells are usually induced for about 12-50 hours, although longer or shorter induction time may be used.

[0216] To improve the production yield and quality of the polypeptides of the invention, various fermentation conditions can be modified. For example, to improve the proper assembly and folding of the secreted antibody polypeptides, additional vectors overexpressing chaperone proteins, such as Dsb proteins (DsbA, DsbB, DsbC, DsbD and or DsbG) or FkpA (a peptidylprolyl cis,trans-isomerase with chaperone activity) can be used to co-transform the host prokaryotic cells. The chaperone proteins have been demonstrated to facilitate the proper folding and solubility of heterologous proteins produced in bacterial host cells. Chen et al. (1999) J. Biol. Chem. 274:19601-19605; Georgiou et al., U.S. Pat. No. 6,083,715; Georgiou et al., U.S. Pat. No. 6,027,888; Bothmann and Pluckthun (2000) J. Biol. Chem. 275:17100-17105; Ramm and Pluckthun (2000) J. Biol. Chem. 275:17106-17113; Arie et al. (2001) Mol. Microbiol. 39:199-210.

[0217] To minimize proteolysis of expressed heterologous proteins (especially those that are proteolytically sensitive), certain host strains deficient for proteolytic enzymes can be used for the present invention. For example, host cell strains may be modified to effect genetic mutation(s) in the genes encoding known bacterial proteases such as Protease III, OmpT, DegP, Tsp, Protease I, Protease Mi, Protease V, Protease VI and combinations thereof. Some *E. coli* protease-deficient strains are available and described in, for example, Joly et al. (1998), supra; Georgiou et al., U.S. Pat. No. 5,264,365; Georgiou et al., U.S. Pat. No. 5,508,192; Hara et al., Microbial Drug Resistance, 2:63-72 (1996).

[0218] In one embodiment, *E. coli* strains deficient for proteolytic enzymes and transformed with plasmids overexpressing one or more chaperone proteins are used as host cells in the expression system of the invention.

c) Antibody Purification

[0219] In one embodiment, the antibody protein produced herein is further purified to obtain preparations that are substantially homogeneous for further assays and uses. Standard protein purification methods known in the art can be employed. The following procedures are exemplary of suitable purification procedures: fractionation on immunoaffinity or ion-exchange columns, ethanol precipitation, reverse phase HPLC, chromatography on silica or on a cation-exchange resin such as DEAE, chromatofocusing, SDS-PAGE, ammonium sulfate precipitation, and gel filtration using, for example, Sephadex G-75.

[0220] In one aspect, Protein A immobilized on a solid phase is used for immunoaffinity purification of the antibody products of the invention. Protein A is a 41 kD cell wall protein from *Staphylococcus aureus* which binds with a high affinity to the Fc region of antibodies.

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

[0221] As the first step of purification, a preparation derived from the cell culture as described above can be applied onto a Protein A immobilized solid phase to allow specific binding of the antibody of interest to Protein A. The solid phase would then be washed to remove contaminants non-specifically bound to the solid phase. Finally the antibody of interest is recovered from the solid phase by elution.

Generating Antibodies Using Eukaryotic Host Cells:

[0222] A vector for use in a eukaryotic host cell generally includes one or more of the following non-limiting components: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence.

a) Signal Sequence Component

[0223] A vector for use in a eukaryotic host cell may also contain a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide of interest. The heterologous signal sequence selected may be one that is recognized and processed (i.e., cleaved by a signal peptidase) by the host cell. In mammalian cell expression, mammalian signal sequences as well as viral secretory leaders, for example, the herpes simplex gD signal, are available. The DNA for such a precursor region is ligated in reading frame to DNA encoding the antibody.

b) Origin of Replication

[0224] Generally, an origin of replication component is not needed for mammalian expression vectors. For example, the SV40 origin may typically be used only because it contains the early promoter.

c) Selection Gene Component

[0225] Expression and cloning vectors may contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, *e.g.*, ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, where relevant, or (c) supply critical nutrients not available from complex media.

[0226] One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene produce a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin, mycophenolic acid and hygromycin.

[0227] Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the antibody nucleic acid, such as DHFR, thymidine kinase, metallothionein-I and -II, primate metallothionein genes, adenosine deaminase, ornithine decarboxylase, etc.

[0228] For example, in some embodiments, cells transformed with the DHFR selection gene are first identified by culturing all of the transformants in a culture medium that contains methotrexate (Mtx), a competitive antagonist of DHFR. In some embodiments, an appropriate host cell when wild-type DHFR is employed is the Chinese hamster ovary (CHO) cell line deficient in DHFR activity (*e.g.*, ATCC CRL-9096).

[0229] Alternatively, host cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding an antibody, wild-type DHFR protein, and another selectable marker such as aminoglycoside 3'-phosphotransferase (APH) can be selected by cell growth in medium containing a selection agent for the selectable marker such as an aminoglycosidic antibiotic, *e.g.*, kanamycin, neomycin, or G418. See U.S. Pat. No. 4,965,199. Host cells may include NS0, CHOK1, CHOK1SV or derivatives, including cell lines deficient in glutamine synthetase (GS). Methods for the use of GS as a selectable marker for mammalian cells are described in U.S. Pat. No. 5,122,464 and U.S. Pat. No. 5,891,693.

d) Promoter Component

[0230] Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to nucleic acid encoding a polypeptide of interest (*e.g.*, an antibody). Promoter sequences are known for eukaryotes. For example, virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CNCAAT region where N may be any nucleotide. At the 3' end of most eukaryotic genes is an AATAAA sequence that may be the signal for addition of the poly A tail to the 3' end of the coding sequence. In certain embodiments, any or all of these sequences may be suitably inserted into eukaryotic expression vectors.

[0231] Transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and Simian Virus 40 (SV40), from heterologous mammalian promoters, *e.g.*, the actin promoter or an immunoglobulin promoter, from heat-shock promoters, provided such promoters are compatible with the host cell systems.

[0232] The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. The immediate early

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

e) Enhancer Element Component

[0233] Transcription of DNA encoding an antibody of this invention by higher eukaryotes is often increased by inserting an enhancer sequence into the vector. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α -fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the human cytomegalovirus early promoter enhancer, the mouse cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, *Nature* 297:17-18 (1982) describing enhancer elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the antibody polypeptide-encoding sequence, but is generally located at a site 5' from the promoter.

f) Transcription Termination Component

[0234] Expression vectors used in eukaryotic host cells may also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding an antibody. One useful transcription termination component is the bovine growth hormone polyadenylation region. See WO94/11026 and the expression vector disclosed therein.

g) Selection and Transformation of Host Cells

[0235] Suitable host cells for cloning or expressing the DNA in the vectors herein include higher eukaryote cells described herein, including vertebrate host cells. Propagation of vertebrate cells in culture (tissue culture) has become a routine procedure. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub et al., *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); mouse sertoli cells (TM4, Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney

cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather et al., *Annals N.Y. Acad. Sci.* 383:44-68 (1982)); MRC 5 cells; FS4 cells; CHOK1 cells, CHOK1SV cells or derivatives and a human hepatoma line (Hep G2).

[0236] Host cells are transformed with the above-described-expression or cloning vectors for antibody production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

h) Culturing the Host Cells

[0237] The host cells used to produce an antibody of this invention may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham et al., *Meth. Enz.* 58:44 (1979), Barnes et al., *Anal. Biochem.* 102:255 (1980), U.S. Pat. No. 4,767,704; 4,657,866; 4,927,762; 4,560,655; or 5,122,469; WO 90/03430; WO 87/00195; or U.S. Pat. Re. 30,985 may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleotides (such as adenosine and thymidine), antibiotics (such as GENTAMYCIN™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

i) Purification of Antibody

[0238] When using recombinant techniques, the antibody can be produced intracellularly, or directly secreted into the medium. If the antibody is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, may be removed, for example, by centrifugation or ultrafiltration. Where the antibody is secreted into the medium, supernatants from such expression systems may be first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. A protease

inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis, and antibiotics may be included to prevent the growth of adventitious contaminants.

[0239] The antibody composition prepared from the cells can be purified using, for example, hydroxylapatite chromatography, gel electrophoresis, dialysis, and affinity chromatography, with affinity chromatography being a convenient technique. The suitability of protein A as an affinity ligand depends on the species and isotype of any immunoglobulin Fc domain that is present in the antibody. Protein A can be used to purify antibodies that are based on human $\gamma 1$, $\gamma 2$, or $\gamma 4$ heavy chains (Lindmark et al., J. Immunol. Methods 62:1-13 (1983)). Protein G is recommended for all mouse isotypes and for human $\gamma 3$ (Guss et al., EMBO J. 5:1567-1575 (1986)). The matrix to which the affinity ligand is attached may be agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrene-divinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the antibody comprises a CH3 domain, the Bakerbond ABXTM resin (J. T. Baker, Phillipsburg, N.J.) is useful for purification. Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on heparin SEPHAROSETM, chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody to be recovered.

[0240] Following any preliminary purification step(s), the mixture comprising the antibody of interest and contaminants may be subjected to further purification, for example, by low pH hydrophobic interaction chromatography using an elution buffer at a pH between about 2.5-4.5, performed at low salt concentrations (*e.g.*, from about 0-0.25M salt).

[0241] In general, various methodologies for preparing antibodies for use in research, testing, and clinical use are well-established in the art, consistent with the above-described methodologies and/or as deemed appropriate by one skilled in the art for a particular antibody of interest.

Production of non-fucosylated antibodies

[0242] Provided herein are methods for preparing antibodies with a reduced degree of fucosylation. For example, methods contemplated herein include, but are not limited to, use of cell lines deficient in protein fucosylation (*e.g.*, Lec13 CHO cells, α -1,6-fucosyltransferase gene knockout CHO cells, cells overexpressing β 1,4-N-acetylglycosaminyltransferase III and further overexpressing Golgi μ -mannosidase II, etc.), and addition of a fucose analog(s) in a cell culture medium used for the production of the antibodies. *See* Ripka et al. Arch. Biochem. Biophys. 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; WO

2004/056312 A1; Yamane-Ohnuki et al. Biotech. Bioeng. 87: 614 (2004); and US Pat. No. 8,574,907. Additional techniques for reducing the fucose content of antibodies include Glymaxx technology described in U.S. Patent Application Publication No. 2012/0214975. Additional techniques for reducing the fucose content of antibodies also include the addition of one or more glycosidase inhibitors in a cell culture medium used for the production of the antibodies.

Glycosidase inhibitors include α -glucosidase I, α -glucosidase II, and α -mannosidase I. In some embodiments, the glycosidase inhibitor is an inhibitor of α -mannosidase I (*e.g.*, kifunensine).

[0243] As used herein, “core fucosylation” refers to addition of fucose (“fucosylation”) to N-acetylglucosamine (“GlcNAc”) at the reducing terminal of an N-linked glycan. Also provided are antibodies produced by such methods and compositions thereof.

[0244] In some embodiments, fucosylation of complex N-glycoside-linked sugar chains bound to the Fc region (or domain) is reduced. As used herein, a “complex N-glycoside-linked sugar chain” is typically bound to asparagine 297 (according to the number of Kabat), although a complex N-glycoside linked sugar chain can also be linked to other asparagine residues. A “complex N-glycoside-linked sugar chain” excludes a high mannose type of sugar chain, in which only mannose is incorporated at the non-reducing terminal of the core structure, but includes 1) a complex type, in which the non-reducing terminal side of the core structure has one or more branches of galactose-N-acetylglucosamine (also referred to as “gal-GlcNAc”) and the non-reducing terminal side of Gal-GlcNAc optionally has a sialic acid, bisecting N-acetylglucosamine or the like; or 2) a hybrid type, in which the non-reducing terminal side of the core structure has both branches of the high mannose N-glycoside-linked sugar chain and complex N-glycoside-linked sugar chain.

[0245] In some embodiments, the “complex N-glycoside-linked sugar chain” includes a complex type in which the non-reducing terminal side of the core structure has zero, one or more branches of galactose-N-acetylglucosamine (also referred to as “gal-GlcNAc”) and the non-reducing terminal side of Gal-GlcNAc optionally further has a structure such as a sialic acid, bisecting N-acetylglucosamine or the like.

[0246] According to the present methods, typically only a minor amount of fucose is incorporated into the complex N-glycoside-linked sugar chain(s). For example, in various embodiments, less than about 60%, less than about 50%, less than about 40%, less than about 30%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, or less than about 1% of the antibody has core fucosylation by fucose in a composition. In some embodiments, substantially none (*i.e.*, less than about 0.5%) of the antibody has core fucosylation by fucose in a composition. In some embodiments, more than about 40%, more than about 50%, more than about 60%, more than about 70%, more than about 80%, more than

about 90%, more than about 91%, more than about 92%, more than about 93%, more than about 94%, more than about 95%, more than about 96%, more than about 97%, more than about 98%, or more than about 99% of the antibody is nonfucosylated in a composition.

[0247] In some embodiments, provided herein is an antibody wherein substantially none (*i.e.*, less than about 0.5%) of the N-glycoside-linked carbohydrate chains contain a fucose residue. In some embodiments, provided herein is an antibody wherein at least one or two of the heavy chains of the antibody is non-fucosylated.

[0248] As described above, a variety of mammalian host-expression vector systems can be utilized to express an antibody. In some embodiments, the culture media is not supplemented with fucose. In some embodiments, an effective amount of a fucose analog is added to the culture media. In this context, an "effective amount" refers to an amount of the analog that is sufficient to decrease fucose incorporation into a complex N-glycoside-linked sugar chain of an antibody by at least about 10%, at least about 20%, at least about 30%, at least about 40% or at least about 50%. In some embodiments, antibodies produced by the instant methods comprise at least about 10%, at least about 20%, at least about 30%, at least about 40% or at least about 50% non-core fucosylated protein (*e.g.*, lacking core fucosylation), as compared with antibodies produced from the host cells cultured in the absence of a fucose analog.

[0249] The content (*e.g.*, the ratio) of sugar chains in which fucose is not bound to N-acetylglucosamine in the reducing end of the sugar chain versus sugar chains in which fucose is bound to N-acetylglucosamine in the reducing end of the sugar chain can be determined, for example, as described in the Examples. Other methods include hydrazinolysis or enzyme digestion (see, *e.g.*, *Biochemical Experimentation Methods 23: Method for Studying Glycoprotein Sugar Chain* (Japan Scientific Societies Press), edited by Reiko Takahashi (1989)), fluorescence labeling or radioisotope labeling of the released sugar chain and then separating the labeled sugar chain by chromatography. Also, the compositions of the released sugar chains can be determined by analyzing the chains by the HPAEC-PAD method (see, *e.g.*, *J. Liq Chromatogr.* 6:1557 (1983)). (See generally U.S. Patent Application Publication No. 2004/0110282.).

B. Compositions of the Invention

[0250] In some aspects, also provided herein are compositions (*e.g.*, pharmaceutical compositions) comprising any of the anti-Siglec-8 antibodies described herein (*e.g.*, an antibody that binds to Siglec-8) or agonists described herein. In some aspects, provided herein is a composition comprising an anti-Siglec-8 antibody described herein, wherein the antibody comprises a Fc region and N-glycoside-linked carbohydrate chains linked to the Fc region, wherein less than about 50% of the N-glycoside-linked carbohydrate chains contain a fucose

residue. In some embodiments, the antibody comprises a Fc region and N-glycoside-linked carbohydrate chains linked to the Fc region, wherein less than about 45%, about 40%, about 35%, about 30%, about 25%, about 20%, or about 15% of the N-glycoside-linked carbohydrate chains contain a fucose residue. In some aspects, provided herein is a composition comprising an anti-Siglec-8 antibody described herein, wherein the antibody comprises a Fc region and N-glycoside-linked carbohydrate chains linked to the Fc region, wherein substantially none of the N-glycoside-linked carbohydrate chains contain a fucose residue.

[0251] Therapeutic formulations are prepared for storage by mixing the active ingredient having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (*Remington: The Science and Practice of Pharmacy*, 20th Ed., Lippincott Williams & Wilkins, Pub., Gennaro Ed., Philadelphia, Pa. 2000). Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers, antioxidants including ascorbic acid, methionine, Vitamin E, sodium metabisulfite; preservatives, isotonicifiers, stabilizers, metal complexes (e.g., Zn-protein complexes); chelating agents such as EDTA and/or non-ionic surfactants.

[0252] Buffers can be used to control the pH in a range which optimizes the therapeutic effectiveness, especially if stability is pH dependent. Buffers can be present at concentrations ranging from about 50 mM to about 250 mM. Suitable buffering agents for use with the present invention include both organic and inorganic acids and salts thereof. For example, citrate, phosphate, succinate, tartrate, fumarate, gluconate, oxalate, lactate, acetate. Additionally, buffers may be comprised of histidine and trimethylamine salts such as Tris.

[0253] Preservatives can be added to prevent microbial growth, and are typically present in a range from about 0.2%-1.0% (w/v). Suitable preservatives for use with the present invention include octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium halides (e.g., chloride, bromide, iodide), benzethonium chloride; thimerosal, phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol, 3-pentanol, and m-cresol.

[0254] Tonicity agents, sometimes known as “stabilizers” can be present to adjust or maintain the tonicity of liquid in a composition. When used with large, charged biomolecules such as proteins and antibodies, they are often termed “stabilizers” because they can interact with the charged groups of the amino acid side chains, thereby lessening the potential for inter and intra-molecular interactions. Tonicity agents can be present in any amount between about 0.1% to about 25% by weight or between about 1 to about 5% by weight, taking into account the relative amounts of the other ingredients. In some embodiments, tonicity agents include polyhydric sugar

alcohols, trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol and mannitol.

[0255] Additional excipients include agents which can serve as one or more of the following: (1) bulking agents, (2) solubility enhancers, (3) stabilizers and (4) agents preventing denaturation or adherence to the container wall. Such excipients include: polyhydric sugar alcohols (enumerated above); amino acids such as alanine, glycine, glutamine, asparagine, histidine, arginine, lysine, ornithine, leucine, 2-phenylalanine, glutamic acid, threonine, etc.; organic sugars or sugar alcohols such as sucrose, lactose, lactitol, trehalose, stachyose, mannose, sorbose, xylose, ribose, ribitol, myoinositol, galactose, galactitol, glycerol, cyclitols (*e.g.*, inositol), polyethylene glycol; sulfur containing reducing agents, such as urea, glutathione, thiocetic acid, sodium thioglycolate, thioglycerol, α -monothioglycerol and sodium thio sulfate; low molecular weight proteins such as human serum albumin, bovine serum albumin, gelatin or other immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; monosaccharides (*e.g.*, xylose, mannose, fructose, glucose; disaccharides (*e.g.*, lactose, maltose, sucrose); trisaccharides such as raffinose; and polysaccharides such as dextrin or dextran.

[0256] Non-ionic surfactants or detergents (also known as “wetting agents”) can be present to help solubilize the therapeutic agent as well as to protect the therapeutic protein against agitation-induced aggregation, which also permits the formulation to be exposed to shear surface stress without causing denaturation of the active therapeutic protein or antibody. Non-ionic surfactants are present in a range of about 0.05 mg/ml to about 1.0 mg/ml or about 0.07 mg/ml to about 0.2 mg/ml. In some embodiments, non-ionic surfactants are present in a range of about 0.001% to about 0.1% w/v or about 0.01% to about 0.1% w/v or about 0.01% to about 0.025% w/v.

[0257] Suitable non-ionic surfactants include polysorbates (20, 40, 60, 65, 80, etc.), polyoxamers (184, 188, etc.), PLURONIC® polyols, TRITON®, polyoxyethylene sorbitan monoethers (TWEEN®-20, TWEEN®-80, etc.), laurmacrogol 400, polyoxyl 40 stearate, polyoxyethylene hydrogenated castor oil 10, 50 and 60, glycerol monostearate, sucrose fatty acid ester, methyl cellulose and carboxymethyl cellulose. Anionic detergents that can be used include sodium lauryl sulfate, dioctyle sodium sulfosuccinate and dioctyl sodium sulfonate. Cationic detergents include benzalkonium chloride or benzethonium chloride.

[0258] In order for the formulations to be used for *in vivo* administration, they must be sterile. The formulation may be rendered sterile by filtration through sterile filtration membranes. The therapeutic compositions herein generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

[0259] The route of administration is in accordance with known and accepted methods, such as by single or multiple bolus or infusion over a long period of time in a suitable manner, *e.g.*, injection or infusion by subcutaneous, intravenous, intraperitoneal, intramuscular, intraarterial, intralesional or intraarticular routes, topical administration, inhalation or by sustained release or extended-release means.

[0260] The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Alternatively, or in addition, the composition may comprise a cytotoxic agent, cytokine or growth inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

III. Articles of Manufacture or Kits

[0261] In another aspect, an article of manufacture or kit is provided which comprises an anti-Siglec-8 antibody described herein (*e.g.*, an antibody that binds human Siglec-8) or an agonist described herein. The article of manufacture or kit may further comprise instructions for use of the antibody or agonist in the methods of the invention. Thus, in certain embodiments, the article of manufacture or kit comprises instructions for the use of an anti-Siglec-8 antibody or agonist that binds to human Siglec-8 in methods for treating or preventing a fibrotic disease (*e.g.*, idiopathic pulmonary fibrosis) and/or a pre-fibrotic disease (*e.g.*, chronic hypersensitivity pneumonitis) in an individual comprising administering to the individual an effective amount of an anti-Siglec-8 antibody or agonist that binds to human Siglec-8.

[0262] In certain embodiments, the article of manufacture comprises a medicament comprising an antibody or agonist that binds to human Siglec-8 and a package insert comprising instructions for administration of the medicament in an individual in need thereof to treat or prevent a fibrotic disease selected from the group consisting of: pulmonary fibrosis, hepatic fibrosis, renal fibrosis, cardiac fibrosis, spleen fibrosis, and ocular fibrosis. In some embodiments, the pulmonary fibrosis is idiopathic pulmonary fibrosis. In some embodiments, the pulmonary fibrosis is associated with chronic obstructive pulmonary disease. In certain embodiments, the article of manufacture comprises a medicament comprising an antibody or agonist that binds to human Siglec-8 and a package insert comprising instructions for administration of the medicament in an individual in need thereof to treat or prevent a fibrotic disease is selected from the group consisting of: mechanical-induced fibrosis, implant-induced fibrosis, radiation-induced fibrosis, drug-induced fibrosis and viral-induced fibrosis. In some embodiments, the mechanical induced fibrosis is ventilator-induced pulmonary fibrosis. In some embodiments, the drug-induced fibrosis is bleomycin-induced pulmonary fibrosis. In certain

embodiments, the article of manufacture comprises a medicament comprising an antibody or agonist that binds to human Siglec-8 and a package insert comprising instructions for administration of the medicament in an individual in need thereof to treat or prevent a fibrotic disease is selected from the group consisting of: cystic fibrosis, cancer-associated fibrosis, atherosclerosis, bone marrow fibrosis, scleroderma, mediastinal fibrosis and retroperitoneal cavity fibrosis. In some embodiments, the package insert further indicates that the treatment is effective in reducing one or more symptom (such as one or more symptom described herein) in the individual with fibrotic disease (*e.g.*, idiopathic pulmonary fibrosis) relative to baseline after administration of the antibody or agonist that binds to human Siglec-8.

[0263] In certain embodiments, the article of manufacture comprises a medicament comprising an antibody or agonist that binds to human Siglec-8 and a package insert comprising instructions for administration of the medicament in an individual in need thereof to treat or prevent a pre-fibrotic disease is selected from the group consisting of: bleomycin-induced pneumonitis, chronic hypersensitivity pneumonitis, polycythemia vera, essential thrombocythemia, age-related macular degeneration, diabetic retinopathy, neovascular glaucoma, retinopathy of prematurity, and proliferative vitreoretinopathy. In some embodiments, the package insert further indicates that the treatment is effective in reducing one or more symptom (such as one or more symptom described herein) in the individual with pre-fibrotic disease (*e.g.*, chronic hypersensitivity pneumonitis) relative to baseline after administration of the antibody or agonist that binds to human Siglec-8. In certain embodiments, the individual is a human.

[0264] The article of manufacture or kit may further comprise a container. Suitable containers include, for example, bottles, vials (*e.g.*, dual chamber vials), syringes (such as single or dual chamber syringes) and test tubes. The container may be formed from a variety of materials such as glass or plastic. The container holds the formulation.

[0265] The article of manufacture or kit may further comprise a label or a package insert, which is on or associated with the container, may indicate directions for reconstitution and/or use of the formulation. The label or package insert may further indicate that the formulation is useful or intended for subcutaneous, intravenous, or other modes of administration for treating or preventing a fibrotic disease (*e.g.*, idiopathic pulmonary fibrosis) and/or a pre-fibrotic disease (*e.g.*, chronic hypersensitivity pneumonitis) in an individual. The container holding the formulation may be a single-use vial or a multi-use vial, which allows for repeat administrations of the reconstituted formulation. The article of manufacture or kit may further comprise a second container comprising a suitable diluent. The article of manufacture or kit may further include

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

[0266] In a specific embodiment, the present invention provides kits for a single dose-administration unit. Such kits comprise a container of an aqueous formulation of therapeutic antibody, including both single or multi-chambered pre-filled syringes. Exemplary pre-filled syringes are available from Vetter GmbH, Ravensburg, Germany.

[0267] The present invention also provides an anti-Siglec-8 antibody described herein (*e.g.*, an antibody that binds human Siglec-8) or an agonist that binds to human Siglec-8 in combination with one or more medicament (*e.g.*, a second medicament) for treating or preventing a fibrotic disease (*e.g.*, idiopathic pulmonary fibrosis) and/or a pre-fibrotic disease (*e.g.*, chronic hypersensitivity pneumonitis) in an individual. In some embodiments, the article of manufacture or kit herein optionally further comprises a container comprising a second medicament, wherein the anti-Siglec-8 antibody or agonist is a first medicament, and which article or kit further comprises instructions on the label or package insert for treating the individual with the second medicament, in an effective amount.

[0268] In another embodiment, provided herein is an article of manufacture or kit comprising the formulations described herein for administration in an auto-injector device. An auto-injector can be described as an injection device that upon activation, will deliver its contents without additional necessary action from the patient or administrator. They are particularly suited for self-medication of therapeutic formulations when the delivery rate must be constant and the time of delivery is greater than a few moments.

[0269] It is understood that the aspects and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

[0270] The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention. It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

EXAMPLES

Example 1: Activity of anti-Siglec-8 antibodies in a mouse model of human fibrosis

[0271] Bleomycin-induced pulmonary fibrosis is an experimental model of human fibrosis. The activity of anti-Siglec-8 antibodies in a bleomycin-induced pulmonary fibrosis model was investigated in a Siglec-8 transgenic mouse in which human Siglec-8 was selectively expressed on the surface of mast cells, eosinophils and basophils. The m2E2 depleting antibody is a murine monoclonal anti-Siglec-8 antibody with a murine IgG2a isotype that kills eosinophils and mast cells by ADCC activity. The m2E2 inhibitory antibody is a murine monoclonal anti-Siglec-8 antibody with a murine IgG1 isotype that kills eosinophils and inhibits mast cell activity.

[0272] Siglec-8 transgenic mice were administered 3 mg/kg of a murine anti-Siglec-8 antibody (m2E2 depleting antibody or m2E2 inhibitory antibody) or murine IgG1 isotype control antibody via intraperitoneal injection for a total of three times. The first administration of the antibody occurred four days before bleomycin administration (Day -4), the second administration was on the same day as bleomycin administration (Day 0), and the third administration was four days after bleomycin administration (Day 4). Oropharyngeal administration of 1.5 U/kg bleomycin occurred four days after the first administration of the indicated anti-Siglec-8 antibody or isotype control antibody. A control group of mice (naive) that were not administered an antibody or bleomycin was used for subsequent analysis. The weight of Siglec-8 transgenic mice with bleomycin-induced lung fibrosis treated with isotype control antibody, m2E2 depleting antibody, or m2E2 inhibitory antibody and of untreated Siglec-8 transgenic mice without bleomycin-induced lung fibrosis was monitored over the course of the study. Bronchoalveolar lavage (BAL) fluid was harvested from the treated mice seven days after administration of bleomycin and processed for collagen, leukocyte, cell differential, and cytokine evaluation. The BAL fluid was centrifuged at 1,000 rpm at 4°C for 5 minutes and the supernatant was transferred for subsequent use. The BAL cell pellet was resuspended in 2 mL of 1X BD Pharm Lyse™ Lysing Buffer (BD Biosciences) to lyse the red blood cells. Phosphate buffered saline (PBS) supplemented with 2% Fetal Bovine Serum (FBS) was added to stop the lysis reaction before centrifuging the cells again at 1,000 rpm. The cells were transferred for subsequent use.

[0273] Leukocytes in the cell pellet sample from BAL fluid were counted using a hemocytometer and the trypan blue exclusion method to monitor dead and live cells. Cytospins were prepared from the cell pellet sample of BAL fluid, differentially stained with Giemsa stain and enumerated under high power magnification for a differential count of nucleated BAL

immune cells (*i.e.*, neutrophils, macrophages, monocytes, lymphocytes, and eosinophils) based on visual cell morphology. Both murine m2E2 depleting antibody and murine m2E2 inhibitory antibody significantly inhibited neutrophil influx into the bronchoalveolar space of Siglec-8 transgenic mice with bleomycin-induced lung fibrosis as compared to Siglec-8 transgenic mice with bleomycin-induced lung fibrosis that received the isotype control antibody (FIG. 1).

[0274] For collagen quantification, a 160 μ l sample of the BAL supernatant was analyzed using the SircolTM Collagen Assay (Biocolor Life Science Assays, United Kingdom). Both murine m2E2 depleting antibody and murine m2E2 inhibitory antibody prevented collagen accumulation in the bronchoalveolar space of Siglec-8 transgenic mice with bleomycin-induced lung fibrosis as compared to Siglec-8 transgenic mice with bleomycin-induced lung fibrosis that received the isotype control antibody (FIG. 2A and FIG. 2B).

[0275] Siglec-8 transgenic mice with bleomycin-induced lung fibrosis experienced weight loss over the course of the study (FIG. 3). However, administration of murine m2E2 depleting antibody or murine m2E2 inhibitory antibody significantly protected Siglec-8 transgenic mice from bleomycin-induced weight loss (FIG. 3). The weight change in anti-Siglec-8 antibody treated mice with bleomycin-induced pulmonary fibrosis was comparable to the weight change in untreated mice without bleomycin-induced lung fibrosis (FIG. 3).

[0276] For cytokine analysis, a 160 μ l sample of the supernatant from the BAL fluid sample was analyzed using a Luminex® Cytokine mouse 32-plex panel (a panel that was designed for quantifying cytokines, chemokines and growth factors in serum, plasma, and tissue culture supernatant; Life Technologies, CA), a latent TGF- β precursor forms panel, and a TGF- β active forms panel for quantification. Both murine m2E2 depleting antibody and murine m2E2 inhibitory antibody inhibited cytokine release in bronchoalveolar fluid of Siglec-8 transgenic mice with bleomycin-induced lung fibrosis as compared to Siglec-8 transgenic mice with bleomycin-induced lung fibrosis that received the isotype control antibody (FIG. 4).

[0277] Following BAL harvest, lungs were inflated by approximately 0.5 mL of 10% neutral buffered formalin (NBF) for histopathological analysis. Microscope fields of lungs from each mouse were given an Ashcroft score (graded on a scale from 0 to 8) by a veterinary pathologist based on the degree of fibrosis in lung specimens (FIG. 5). *See* Ashcroft et al., *J. Clin. Pathol.*, 1998, 41:467-470 for a description of Ashcroft scoring.

Example 2: Anti-Siglec-8 antibody treatment in a mouse model of human fibrosis

[0278] Treatment of Siglec-8 transgenic mice exhibiting bleomycin-induced pulmonary fibrosis with anti-Siglec-8 antibodies was investigated. Siglec-8 transgenic mice selectively expressed human Siglec-8 on the surface of mast cells, eosinophils and basophils. The m2E2

inhibitory antibody is a murine monoclonal anti-Siglec-8 antibody with a murine IgG1 isotype that kills activated eosinophils and inhibits mast cell activity.

[0279] Siglec-8 transgenic mice were administered 3 mg/kg of a murine anti-Siglec-8 antibody (m2E2 inhibitory antibody) or murine IgG1 isotype control antibody via intraperitoneal injection. The first administration of the m2E2 inhibitory antibody or isotype control antibody was given three days after oropharyngeal administration of 1.5 U/kg bleomycin (Day 3). A control group of mice (naive) that were not administered an antibody or bleomycin was used for subsequent analysis. BAL fluid was harvested from the treated mice seven days after administration of bleomycin and processed for collagen, leukocyte, and cell differential evaluation. The BAL fluid was centrifuged at 1,000 rpm at 4°C for 5 minutes and the supernatant was transferred for subsequent use. The BAL cell pellet was resuspended in 2 mL of 1X BD Pharm Lyse™ Lysing Buffer (BD Biosciences) to lyse the red blood cells. Phosphate buffered saline (PBS) supplemented with 2% Fetal Bovine Serum (FBS) was added to stop the lysis reaction before centrifuging the cells again at 1,000 rpm. The cells were transferred for subsequent use.

[0280] Leukocytes in the cell pellet sample from BAL fluid were counted using a hemocytometer and the trypan blue exclusion method to monitor dead and live cells. Cytospins were prepared from the cell pellet sample of BAL fluid, differentially stained with Giemsa stain and enumerated under high power magnification for a differential count of nucleated BAL immune cells (*i.e.*, neutrophils, macrophages, monocytes, lymphocytes, and eosinophils) based on visual cell morphology. Administration of murine m2E2 inhibitory antibody significantly inhibited neutrophil influx into the bronchoalveolar space of Siglec-8 transgenic mice with bleomycin-induced lung fibrosis as compared to Siglec-8 transgenic mice with bleomycin-induced lung fibrosis that received the isotype control antibody (FIG. 6).

[0281] For collagen quantification, a 160 µl sample of the supernatant is analyzed using the Sircol™ Collagen Assay (Biocolor Life Science Assays, United Kingdom).

[0282] For cytokine analysis, a 160 µl sample of the supernatant from the BAL fluid sample is analyzed using a Luminex® Cytokine mouse 32-plex panel (a panel that was designed for quantifying cytokines, chemokines and growth factors in serum, plasma, and tissue culture supernatant; Life Technologies, CA), a latent TGF-β precursor forms panel, and a TGF-β active forms panel for quantification.

[0283] Following BAL harvest, lungs are inflated by approximately 0.5 mL of 10% neutral buffered formalin (NBF) for histopathological analysis. Microscope fields of lungs from each mouse are given an Ashcroft score (graded on a scale from 0 to 8) by a veterinary pathologist

based on the degree of fibrosis in lung specimens. See Ashcroft et al., *J. Clin. Pathol.*, 1998, 41:467-470 for a description of Ashcroft scoring.

[0284] The weight of Siglec-8 transgenic mice with bleomycin-induced lung fibrosis treated with isotype control antibody or m2E2 inhibitory antibody and of untreated Siglec-8 transgenic mice without bleomycin-induced lung fibrosis is monitored over the course of the study.

Example 3: Activity of anti-Siglec-8 antibodies in a humanized mouse model of implant-induced fibrosis

[0285] Immunodeficient mice capable of generating abundant human mast cells after engraftment with human hematopoietic stem cells (HSC) have been described (Tanaka et al., *J Immunol.*, 2012, 188(12):6145-55). The mouse strain designated NSG-SGM3 (The Jackson Laboratory) is a derivative of the nonobese diabetic/severe combined immunodeficiency (NOD SCID) mouse with a deletion of the IL-2 receptor gamma-chain gene (NSG mouse). NSG-SGM3 mice are additionally transgenic for 3 human cytokines (stem cell factor [SCF], IL-3, and GM-CSF) to facilitate engraftment with human hematopoietic stem cells. Upon engraftment of NSG-SGM3 mice, human CD34⁺ cells generate human eosinophils and enhanced numbers of human mast cells. Both cell types in engrafted NSG-SGM3 mice express Siglec-8 at levels comparable to the levels on the corresponding cell types isolated from human peripheral blood and tissues. Thus, these mice provide a model for evaluation of activity of anti-Siglec-8 antibodies *in vivo*.

[0286] In order to evaluate the effect of anti-Siglec-8 antibodies on fibrosis induced by implanted objects, polystyrene beads were implanted in these humanized mice and development of fibrosis was monitored after prophylactic or therapeutic treatment with anti-Siglec-8 antibodies.

Materials and Methods

[0287] Polystyrene beads of 500 μ m mean diameter were purchased from Phosphorex (Hopkinton, MA) and implanted in humanized mice. See Veish et al., *Nat. Mater.*, 2015, 14:643-651 for a description of polystyrene bead implantation in mice. Humanized mice were anesthetized and their abdomens were shaved and sterilized. A 0.5 mm incision was made along the midline of the abdomen and the peritoneal lining was exposed using blunt dissection. The peritoneal wall was then grasped with forceps and a 0.5-1 mm incision was made along the linea alba. Polystyrene beads suspended in PBS were then loaded into a sterile pipette and implanted into the peritoneal cavity through the incision. The incision was closed using 5-0 taper-tipped polydioxanone (PDS II) absorbable sutures and the skin closed over the incision using a wound clip and tissue glue. One day prior to implantation of polystyrene beads (day -1) or seven days

after implantation of polystyrene beads (day 7), 100 µg of anti-Siglec-8 antibody (c2E2 IgG4) or isotype matched control human antibody (hIgG4) was administered via intraperitoneal injection every 4 days (q4d) until termination of the 14 day study. Accordingly, 100 µg of c2E2 IgG4 was administered on day -1, 3, 7 and 11 for the prophylactic treatment study group (n= 5 mice) or administered on day 7 and 11 for the therapeutic treatment study group (n= 5 mice). 100 µg of hIgG4 was administered on day -1, 3, 7 and 11 for the control study group (n= 5 mice). The c2E2 IgG4 antibody is a chimeric monoclonal anti-Siglec-8 antibody with a human IgG4 isotype and murine 2E2 variable domains.

[0288] The mice were euthanized at day 14 of the study. A 5 ml volume of ice cold PBS was first injected in order to perform a peritoneal lavage to rinse out and collect free-floating intraperitoneal cells. An incision was then made along the abdomen skin and peritoneal wall and Krebs buffer was used to wash out polystyrene beads from the abdomen and into petri dishes for collection. After all the beads were washed out or manually retrieved if fibrosed directly to intraperitoneal tissues, the collected beads were transferred into 50 mL conical tubes for downstream processing before imaging. After peritoneal lavage and bead retrieval, remaining fibrosed intraperitoneal tissues were also excised for analysis. The polystyrene beads and tissues recovered from the peritoneal cavity were gently washed using Krebs buffer and transferred to petri dishes for phase contrast microscopy. Collagen quantification was performed on a fraction of peritoneal lavage supernatants (supernatant remaining after removal of beads and tissue) using a Bicolor Sircol Soluble Collagen Assay.

[0289] Alpha smooth muscle actin was quantified using anti-mouse alpha smooth muscle actin antibody (Sigma Aldrich, St. Louis MO). Immunofluorescence imaging was used to determine cell populations attached to beads as described in Veish et al., *Nat. Mater.*, 2015, 14:643-651. Materials retrieved from the mice were fixed overnight using 4% paraformaldehyde at 4°C. Polystyrene beads and the associated cells were washed twice with Krebs buffer, permeabilized for 30 min using a 0.1% Triton X100 solution, and blocked for 1 hour using a 1% bovine serum albumin (BSA) solution. Next, the beads were incubated for 1 hour in an immunostaining cocktail solution consisting of DAPI (500 nM) and anti-mouse alpha smooth muscle actin antibody (1:200 dilution) in BSA. After staining, the beads were washed three times with a 0.1% Tween 20 solution and maintained in a 50% glycerol solution. The beads were then transferred to glass bottom dishes and imaged using a Nikon Eclipse Ti series microscope. Images were further analyzed with Nikon Elements image analysis software.

Results

[0290] Sterile polystyrene beads implanted into the peritoneum of humanized mice for 14 days induced fibrosis as shown by an increase in collagen deposition (FIG. 7) and accumulation of

bead-associated myofibroblasts (FIG. 8). Prophylactic treatment of humanized mice with anti-Siglec-8 antibody one day before bead implantation markedly reduced collagen deposition and accumulation of myofibroblasts on the beads. Anti-Siglec-8 antibody treatment also inhibited collagen deposition and accumulation of myofibroblasts in humanized mice treated with the antibody 7 days after bead implantation.

Example 4: Activity of anti-Siglec-8 antibodies in a mouse model of scleroderma and systemic fibrosis.

[0291] Subcutaneous administration of bleomycin provides an experimental model of scleroderma, including systemic sclerosis. Mice treated with bleomycin via the subcutaneous route develop fibrosis of both the skin and lungs. The activity of anti-Siglec-8 antibodies in a bleomycin-induced cutaneous fibrosis model was investigated in Siglec-8 transgenic mice in which human Siglec-8 is selectively expressed on the surface of mast cells, eosinophils and basophils.

[0292] Siglec-8 transgenic mice were administered 3 mg/kg of murine IgG1 isotype control antibody or murine anti-Siglec-8 antibody (m2E2 IgG1 antibody also known as m2E2 inhibitory antibody) via intraperitoneal injection for a total of six doses in a therapeutic study group (Day +7) or eight doses in a prophylactic study group (Day -1). For the prophylactic study group, the administration of the antibody occurred one day before bleomycin administration (Day -1), and was subsequently administered on Days 3, 7, 11, 15, 19, 23, and 27 after the initial bleomycin administration. For the therapeutic study group, the administration of the antibody occurred on the seventh day after the initial bleomycin administration (Day 7), and was subsequently administered on Days 11, 15, 19, 23, and 27. 0.1 IU bleomycin per mouse was administered subcutaneously on Day 0 and every two days until Day 28 on the back of each mouse. A control group of mice (naïve) that were not administered an antibody or bleomycin was used for subsequent analysis. The lung weight of Siglec-8 transgenic mice with bleomycin-induced cutaneous fibrosis treated with isotype control antibody or m2E2 inhibitory antibody was monitored for thirty days after bleomycin administration (Day 30). Untreated Siglec-8 transgenic mice without bleomycin-induced cutaneous fibrosis were monitored during this 30 day monitoring period. At Day 30, a portion of the lungs and skin from lesions of Siglec-8 transgenic mice were processed for analysis of levels of hydroxyproline, a component of collagen, using a BioVision Kit according to the manufacturer's instructions. The remaining lesional skin was processed for RNA extraction using an RNeasy Kit (Qiagen) and reversed transcribed into cDNA using a High Capacity cDNA Kit (Applied Biosystems) according to the manufacturer's instructions. Scientific photographs of bleomycin-induced cutaneous lesions

were taken fifteen days (Day 15), twenty days (Day 20), twenty-five days (Day 25) and thirty-days (Day 30) after initial bleomycin administration. A visual dermal score was calculated from photographs in the Siglec-8 transgenic mice with bleomycin-induced cutaneous fibrosis treated with isotype control antibody or m2E2 inhibitory antibody and of untreated Siglec-8 transgenic mice without bleomycin-induced cutaneous fibrosis.

[0293] Siglec-8 transgenic mice with bleomycin-induced cutaneous fibrosis experienced increased lung weights after 30 days of the initial bleomycin administration (Day 30). Administration of m2E2 inhibitory antibody significantly protected and inhibited Siglec-8 transgenic mice from lung weight gain compared to Siglec-8 transgenic mice with bleomycin-induced cutaneous fibrosis that received isotype control antibody (FIG. 9). For hydroxyproline quantification, a portion of Siglec-8 transgenic mice lungs and skin were homogenized according to manufacturer's instructions (BioVision). A reduction of hydroxyproline was observed with administration of m2E2 inhibitory antibody in both lungs and skin compared to Siglec-8 transgenic mice with bleomycin-induced cutaneous fibrosis treated with isotype control antibody (FIG. 10A and FIG. 10B). The reduction of hydroxyproline in anti-Siglec-8 antibody treated mice was comparable to the hydroxyproline quantity observed in untreated mice without bleomycin-induced cutaneous fibrosis. For gene expression analysis, cDNA derived from skin of Siglec-8 transgenic mice in bleomycin-induced cutaneous fibrosis was used for quantitative PCR (qPCR) (BioRad) analysis of the pro-fibrotic mediators, interleukin-13 (IL-13) and transforming growth factor beta (TGF β) using pre-designed, gene-specific primers. Murine m2E2 inhibitory antibody significantly inhibited the expression of both IL-13 and TGF β in the bleomycin-induced skin lesions compared to Siglec-8 transgenic mice with bleomycin-induced cutaneous fibrosis that received isotype control antibody (FIG. 11A and FIG. 11B).

[0294] Bleomycin-induced skin lesions were visually quantified for fibrosis and given a score of one through three based on fibrotic severity of the skin lesion (one being less severe, three being most severe). Siglec-8 transgenic mice with bleomycin-induced cutaneous fibrosis treated with m2E2 inhibitory antibody had lower visual dermal scores as compared to Siglec-8 transgenic mice with bleomycin-induced cutaneous fibrosis that received isotype control antibody (FIG. 12).

SEQUENCES

Amino acid sequence of mouse 2E2 heavy chain variable domain

QVQLKESGPGGLVAPSQSLITCTVSGFSLTIYGAHWVRQPPGKGLEWLGVIWAGGSTNY
NSALMSRLSISKDNSKSQVFLKINSLQTDDTALYYCARDGSSPYYYSMEYWGQGTSVT
VSS (SEQ ID NO:1)

Amino acid sequence of 2E2 RHA heavy chain variable domain

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIIYGAHWVRQAPGKGLEWVSVIWAGGSTN
YNSALMSRFTISKDNSKNTVYQLQMNSLRAEDTAVYYCARDGSSPYYYSMEYWGQGTT
VTVSS (SEQ ID NO:2)

Amino acid sequence of 2E2 RHB heavy chain variable domain

EVQLVESGGGLVQPGGSLRLSCAVSGFSLTIYGAHWVRQAPGKGLEWLGVIWAGGSTN
YNSALMSRLSISKDNSKNTVYQLQMNSLRAEDTAVYYCARDGSSPYYYSMEYWGQGTT
VTVSS (SEQ ID NO:3)

Amino acid sequence of 2E2 RHC heavy chain variable domain

EVQLVESGGGLVQPGGSLRLSCAVSGFSLTIYGAHWVRQAPGKGLEWVSVIWAGGSTN
YNSALMSRFTISKDNSKNTVYQLQMNSLRAEDTAVYYCARDGSSPYYYSMEYWGQGTT
VTVSS (SEQ ID NO:4)

Amino acid sequence of 2E2 RHD heavy chain variable domain

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIIYGAHWVRQAPGKGLEWLSVIWAGGSTN
YNSALMSRFTISKDNSKNTVYQLQMNSLRAEDTAVYYCARDGSSPYYYSMEYWGQGTT
VTVSS (SEQ ID NO:5)

Amino acid sequence of 2E2 RHE heavy chain variable domain

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIIYGAHWVRQAPGKGLEWVGVIWAGGST
NYNSALMSRFTISKDNSKNTVYQLQMNSLRAEDTAVYYCARDGSSPYYYSMEYWGQGT
TVTVSS (SEQ ID NO:6)

Amino acid sequence of 2E2 RHF heavy chain variable domain

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIIYGAHWVRQAPGKGLEWVSIVWAGGSTN
YNSALMSRLTISKDNSKNTVYLMNSLRAEDTAVYYCARDGSSPYYYSM EYWGQGT
TVTVSS (SEQ ID NO:7)

Amino acid sequence of 2E2 RHG heavy chain variable domain

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIIYGAHWVRQAPGKGLEWVSIVWAGGSTN
YNSALMSRFSISKDNSKNTVYLMNSLRAEDTAVYYCARDGSSPYYYSM EYWGQGT
TVTVSS (SEQ ID NO:8)

Amino acid sequence of 2E2 RHA2 heavy chain variable domain

QVQLQESGPGLVKPSETLSLTCTVSGGSISIYGAHWIRQPPGKGLEWIGVIWAGGSTN
YNSALMSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARDGSSPYYYSM EYWGQGT
LTVTVSS (SEQ ID NO:9)

Amino acid sequence of 2E2 RHB2 heavy chain variable domain

QVQLQESGPGLVKPSETLSLTCTVSGFSLTIIYGAHWVRQPPGKGLEWLGVIWAGGSTN
YNSALMSRLSISKDNSKNQVSLKLSSVTAADTAVYYCARDGSSPYYYSM EYWGQGT
LTVTVSS (SEQ ID NO:10)

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

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIIYGAHWVRQAPGKGLEWVGVIWAGGST
NYNSALMSRFTISKDNSKNTVYLMNSLRAEDTAVYYCARDGSSPYYYGMEYWGQGT
TVTVTVSS (SEQ ID NO:11)

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

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIIYGAHWVRQAPGKGLEWVGVIWAGGST
NYNSALMSRFTISKDNSKNTVYLMNSLRAEDTAVYYCARDGSSPYYYSM DYWGQGT
TVTVTVSS (SEQ ID NO:12)

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

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIIYGAHWVRQAPGKGLEWVGVIWAGGST
NYNSALMSRFTISKDNSKNTVYLMNSLRAEDTAVYYCARDGSSPYYYSM EVWGQGT
TVTVTVSS (SEQ ID NO:13)

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

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIIYGAHWVRQAPGKGLEWVGVIWAGGST
NYNSALMSRFTISKDNSKNTVYLQMNSLRAEDTAVYYCARDGSSPYYYGMDVWGQG
TTVTVSS (SEQ ID NO:14)

Amino acid sequence of mouse 2E2 light chain variable domain

QHILTQSPAIMASAPGEKVSITCSATSSVSYMHWFQQKPGTSPKLWIYSTSNLASGVPVRF
SGSGSGTSYSLTISRMEAEDAATYQCQRSSYPFTFGSGTKLEIK (SEQ ID NO:15)

Amino acid sequence of 2E2 RKA light chain variable domain

EIVLTQSPATLSLSPGERATLSCSATSSVSYMHWFQQKPGQAPRLLIYSTSNLASGIPARF
SGSGSGTDFTLTISSELPEDFAVYYCQQRSSYPFTFGPGTKLDIK (SEQ ID NO:16)

Amino acid sequence of 2E2 RKB light chain variable domain

EHILTQSPATLSLSPGERATLSCSATSSVSYMHWFQQKPGQAPRLWIYSTSNLASGVPARF
SGSGSGTDYTLTISSELPEDFAVYYCQQRSSYPFTFGPGTKLDIK (SEQ ID NO:17)

Amino acid sequence of 2E2 RKC light chain variable domain

EHILTQSPATLSLSPGERATLSCSATSSVSYMHWFQQKPGQAPRLLIYSTSNLASGIPARFS
GSGSGTDFTLTISSELPEDFAVYYCQQRSSYPFTFGPGTKLDIK (SEQ ID NO:18)

Amino acid sequence of 2E2 RKD light chain variable domain

EIVLTQSPATLSLSPGERATLSCSATSSVSYMHWFQQKPGQAPRLWIYSTSNLASGIPARF
SGSGSGTDFTLTISSELPEDFAVYYCQQRSSYPFTFGPGTKLDIK (SEQ ID NO:19)

Amino acid sequence of 2E2 RKE light chain variable domain

EIVLTQSPATLSLSPGERATLSCSATSSVSYMHWFQQKPGQAPRLLIYSTSNLASGVPAR
FSGSGSGTDFTLTISSELPEDFAVYYCQQRSSYPFTFGPGTKLDIK (SEQ ID NO:20)

Amino acid sequence of 2E2 RKF light chain variable domain

EIVLTQSPATLSLSPGERATLSCSATSSVSYMHWFQQKPGQAPRLLIYSTSNLASGIPARF
SGSGSGTDYTLTISSELPEDFAVYYCQQRSSYPFTFGPGTKLDIK (SEQ ID NO:21)

Amino acid sequence of 2E2 RKG light chain variable domain

EIVLTQSPATLSLSPGERATLSCSATSSVSYMHWFQQKPGQAPRLLIYSTSNLASGIPARF
SGSGSGTDFTLTISSELPEDFAVYYCQQRSSYPFTFGPGTKLDIK (SEQ ID NO:22)

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

EIVLTQSPATLSLSPGERATLSCSATSSVSYMHWFQQKPGQAPRLLIYSTSNLASGIPARF
SGSGSGTDFTLTISSELPEDFAVYYCQQRSSYPYTFGPGTKLDIK (SEQ ID NO:23)

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

EIVLTQSPATLSLSPGERATLSCSATSSVSYMHWFQQKPGQAPRLLIYSTSNLASGIPARF
SGSGSGTDYTLTISSELPEDFAVYYCQQRSSYPYTFGPGTKLDIK (SEQ ID NO:24)

Amino acid sequence of HEKA IgG1 heavy chain and HEKF IgG1 heavy chain

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIIYGAHWVRQAPGKGLEWVGVIWAGGST
NYSALMSRFTISKDNSKNTVYLMNSLRAEDTAVYYCARDGSSPYYYSMMEYWGQGT
TVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
AVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPA
PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFEL
YSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:75)

Amino acid sequence of HEKA kappa light chain

EIVLTQSPATLSLSPGERATLSCSATSSVSYMHWFQQKPGQAPRLLIYSTSNLASGIPARF
SGSGSGTDFTLTISSELPEDFAVYYCQQRSSYPFTFGPGTKLDIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLS
KADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:76)

Amino acid sequence of HEKF kappa light chain

EIVLTQSPATLSLSPGERATLSCSATSSVSYMHWFQQKPGQAPRLLIYSTSNLASGIPARF
SGSGSGTDYTLTISSELPEDFAVYYCQQRSSYPFTFGPGTKLDIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLS
KADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:77)

Amino acid sequence of IgG1 heavy chain constant region (IgG4 contains a S228P mutation)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ

YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:78)

Amino acid sequence of IgG4 heavy chain constant region

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSV
FLFPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRW
QEGNVFSCSVMHEALHNHYTQKSLSLSLG (SEQ ID NO:79)

Amino acid sequence of Ig kappa light chain constant region

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
DSKDYSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:80)

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

QVQLKRAAGPGLVAPSSQSLITCTVSGFSLTIYGAHWVRQPPGKGLEWLGVIWAGGSTN
YNSALMSRLSISKDNSKSQVFLKINSLQTDALYYCARDGSSPYYYSMYWGQGTSV
TVSSAKTTPPSVYPLAPGSAQAQNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPA
VLESDLYTLSSSVTVPSRPSETVTCNVAHPASSTKVDKKIVPRDCGCKPCICTVPEVSS
VFIFPPKPKDVLITLTLPKVTCTVVDISKDDPEVQFSWFVDDVEVHTAQTQPREEQFNST
FRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMA
KDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPIMNNTNGSYFVYSKLVNQKSN
WEAGNTFTCSVLHEGLHNHHTEKSLSHSPG (SEQ ID NO:81)

Amino acid sequence of murine 2C4 kappa light chain

EIILTQSPAIMSASPGEKVSITCSATSSVSYMHWFQQKPGTSPKLWIYSTSNLASGVPVRF
SGSGSGTSYSLTISRMEAEADAATYYCQQRSSYPFTFGSGTKLEIKADAAPTVSIFPPSSEQ
LTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDYSTYSMSSTLTLT
KDEYERHNSYTCEATHKTSTSPIVKSFNRNEC (SEQ ID NO:82)

Amino acid sequence of murine 2E2 kappa light chain

QIILTQSPAIMSASPGEKVSITCSATSSVSYMHWFQQKPGTSPKLWIYSTSNLASGVPVRF
SGSGSGTSYSLTISRMEAEADAATYYCQQRSSYPFTFGSGTKLEIKADAAPTVSIFPPSSEQ

LTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDYSTYSMSSTLTLT
KDEYERHNSYTCEATHKTSTSPIVKSFNRNEC (SEQ ID NO:83)

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

QVQLKRASGPGGLVAPSQSLITCTVSGFSLTIYGAHWVRQPPGKGLEWLGVIWAGGSTN
YNSALMSRLSISKDNSKSQVFLKINSLQTDDTALYYCARDGSSPYYYSM EYWGGTSV
TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPE
LLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYS
KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:84)

Amino acid sequence of chimeric 2C4 kappa light chain

EIILTQSPAIMASAPGEKVSITCSATSSVSVMHWFQQKPGTSPKLVWYSTSNLASGVPVRF
SGSGSGTSYSLTISRMEAEADAATYYCQQRSSYPFTFGSGTKLEIKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSTYLSSTLT
SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:85)

Amino acid sequence of chimeric 2E2 kappa light chain

QIILTQSPAIMASAPGEKVSITCSATSSVSVMHWFQQKPGTSPKLVWYSTSNLASGVPVRF
SGSGSGTSYSLTISRMEAEADAATYYCQQRSSYPFTFGSGTKLEIKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSTYLSSTLT
SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:86)

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

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIIYGAHWVRQAPGKGLEWVGVIWAGGST
NYSALMSRFTISKDNSKNTVYLQMNSLRAEDTAVYYCARDGSSPYYYSM EYWGGQT
TVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
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LGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSDQEDPEVQFNWYVDGVEVHNAKTKPR
EEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTL
PPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRL
TVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLG (SEQ ID NO:87)

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

EVQVVESGGDLVKSGGSLKLSCAASGFPFSSYAMSWVRQTPDKRLEWVAIISSGGSYTY
YSDSVKGRFTISRDNAKNTLYLQMSSSLKSEDTAMYYCARHETAQAAWFAYWGQGTLV
TVSA (SEQ ID NO:106)

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

EVQLQQSGAELVRPGASVKLSCTASGFIKDYMYWVKQRPEQGLEWIGRIAPEDGDT
EYAPKFQGKATVTADTSSNTAYLHLSSLTSEDTAVYYCTTEGNYYGSSILDYWGQGT
LTVSS (SEQ ID NO:107)

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

QVQLQQSGAELVKPGASVKISCKASGYAFRSSWMNWVKQRPGKGLEWIGQIYPGDDY
TNYNGKFKGKVTLTADRSSSTAYMQLSSLTSEDSAVYFCARLGPYGPFPADWGQGT
LVSA (SEQ ID NO:108)

Amino acid sequence of mouse 1C3 light chain variable domain

QIVLTQSPAIVSASPGEKVTMTCSASSSVSYMHWYQQKSGTSPKRWIYDTSKLAYGVP
ARFSGSGSGTSYSLTISNLEQEDATYYCQQWSSNPPTFGGGTKLEIK (SEQ ID NO:109)

Amino acid sequence of mouse 1H10 light chain variable domain

DIQMTQTTSSLSASLGDRVTISCRASQDITNYLNWYQQKPDGTVKLLIYFTSRLHSGVPS
RFSGSGSGTDYSLTISNLEQEDATYYCQQGNTLPWTFGGGTKLEIK (SEQ ID NO:110)

Amino acid sequence of mouse 4F11 light chain variable domain

QIVLTQSPAIVSASPGEKVTMTCSASSSVSYMYWYQQRPGSSPRLLIYDTSSLASGVPVR
FSGSGSGTSYSLTISRIESEAANYCQQWNSDPYTFGGGTKLEIK (SEQ ID NO:111)

CLAIMS

WHAT IS CLAIMED IS:

1. A method for treating fibrotic disease in an individual, comprising administering to the individual an effective amount of an antibody that binds to human Siglec-8; wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:6 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:16 or 21; wherein the antibody comprises a heavy chain Fc region comprising a human IgG Fc region; and wherein the fibrotic disease is pulmonary fibrosis, hepatic fibrosis, renal fibrosis, cardiac fibrosis, spleen fibrosis, ocular fibrosis, mechanical-induced fibrosis, implant-induced fibrosis, radiation-induced fibrosis, drug-induced fibrosis, viral-induced fibrosis, cystic fibrosis, cancer-associated fibrosis, atherosclerosis, bone marrow fibrosis, scleroderma, mediastinal fibrosis, retroperitoneal cavity fibrosis, or Dupuytren's contracture.
2. The method of claim 1, wherein the pulmonary fibrosis is idiopathic pulmonary fibrosis.
3. The method of claim 1, wherein the mechanical induced fibrosis is ventilator-induced pulmonary fibrosis.
4. The method of claim 1, wherein the drug-induced fibrosis is bleomycin-induced pulmonary fibrosis.
5. The method of claim 1, wherein the scleroderma is systemic sclerosis.
6. The method of any one of claims 1-5, wherein one or more symptom in the individual with the fibrotic disease is reduced relative to baseline after administration of the antibody or the agonist that binds to human Siglec-8.
7. The method of any one of claims 1, 2 or 6, wherein one or more pulmonary function in the individual with pulmonary fibrosis is increased by at least 5% relative to baseline after administration of the antibody that binds to human Siglec-8.
8. The method of claim 7, wherein the one or more pulmonary function is selected from the group consisting of: vital capacity (VC), residual volume (V), forced expiratory volume (FEV₁), forced vital capacity (FVC), forced expiratory flow (FEF), peak expiratory flow rate (PEFR), inspiratory reserve volume (IRV), functional residual capacity (FRC), inspirator capacity (IC), total lung capacity (TLC), expiratory reserve volume (ERV), tidal volume (TV) and maximum voluntary ventilation (MVV).

9. The method of any one of claims 1-8, wherein one or more pathologic parameter in the individual with fibrotic disease is reduced by at least 5% relative to baseline after administration of the antibody that binds to human Siglec-8.
10. The method of claim 9, wherein the one or more pathologic parameter is selected from the group consisting of: neutrophil influx, number of mast cells, cytokine release, collagen accumulation, fibroblast or myofibroblast infiltration and fibroblastic foci formation.
11. The method of any one of claims 1-10, wherein mast cell activation is reduced in the individual relative to baseline after administration of the antibody that binds to human Siglec-8.
12. The method of any one of claims 1-11, wherein the antibody is a monoclonal antibody.
13. The method of any one of claims 1-12, wherein the antibody is engineered to improve antibody-dependent cell-mediated cytotoxicity (ADCC) activity.
14. The method of claim 13, wherein the antibody comprises at least one amino acid substitution in the Fc region that improves ADCC activity.
15. The method of any one of claims 1-14, wherein at least one or two of the heavy chains of the antibody is non-fucosylated.
16. The method of any one of claims 1-15, wherein the human IgG Fc region comprises a human IgG1 Fc region or a human IgG4 Fc region.
17. The method of any one of claims 1-16, wherein the antibody is a human IgG1 antibody.
18. The method of claim 16, wherein the human IgG4 Fc region comprises the amino acid substitution S228P, and wherein the amino acid residues are numbered according to the EU index as in Kabat.
19. The method of any one of claims 1-15, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:75; and a light chain comprising the amino acid sequence of SEQ ID NO:76 or 77.
20. The method of any one of claims 1-15, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:87; and a light chain comprising the amino acid sequence of SEQ ID NO:76.
21. The method of any one of claims 1-20, wherein the individual is a human.

22. The method of any one of claims 1-21, wherein the antibody is in a pharmaceutical composition comprising the antibody and a pharmaceutically acceptable carrier.
23. The method of claim 22, wherein the antibody comprises N-glycoside-linked carbohydrate chains linked to the Fc region, and wherein less than 50% of the N-glycoside-linked carbohydrate chains of the antibody in the composition contain a fucose residue.
24. The method of claim 23, wherein less than 0.5% of the N-glycoside-linked carbohydrate chains of the antibody in the composition contain a fucose residue.
25. Use of an antibody that binds to human Siglec-8 in the manufacture of a medicament for treating fibrotic disease in an individual; wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:6 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:16 or 21; wherein the antibody comprises a heavy chain Fc region comprising a human IgG Fc region; and wherein the fibrotic disease is pulmonary fibrosis, hepatic fibrosis, renal fibrosis, cardiac fibrosis, spleen fibrosis, ocular fibrosis, mechanical-induced fibrosis, implant-induced fibrosis, radiation-induced fibrosis, drug-induced fibrosis, viral-induced fibrosis, cystic fibrosis, cancer-associated fibrosis, atherosclerosis, bone marrow fibrosis, scleroderma, mediastinal fibrosis, retroperitoneal cavity fibrosis, or Dupuytren's contracture.

FIG. 1

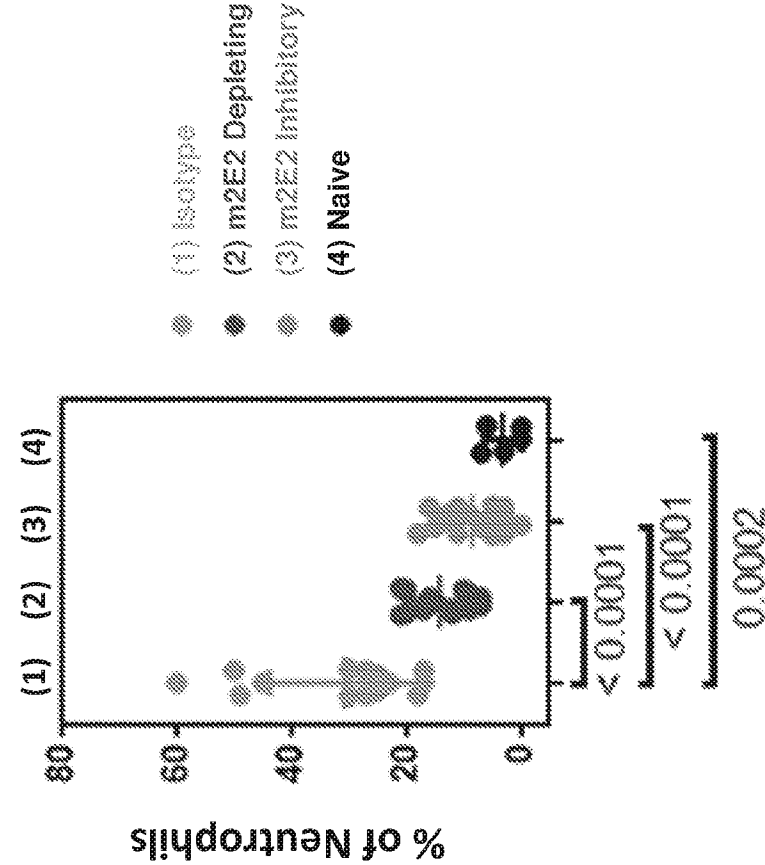


FIG. 2A

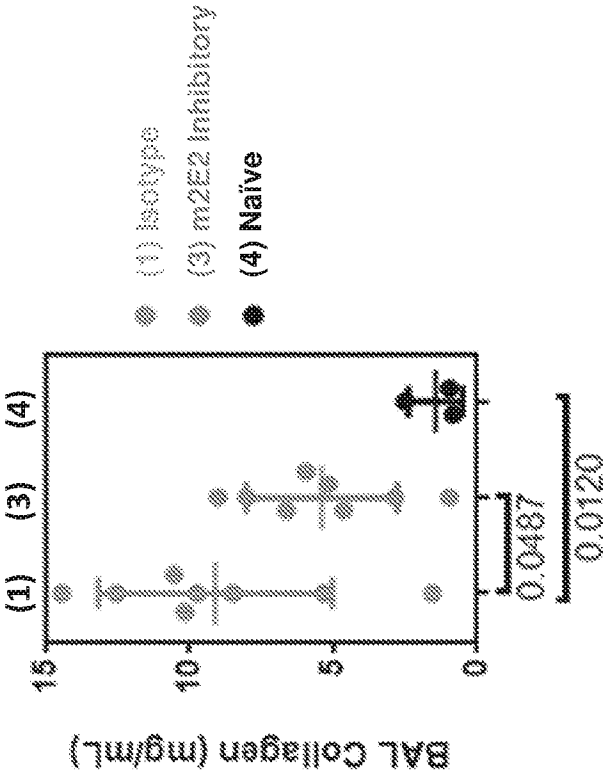


FIG. 2B

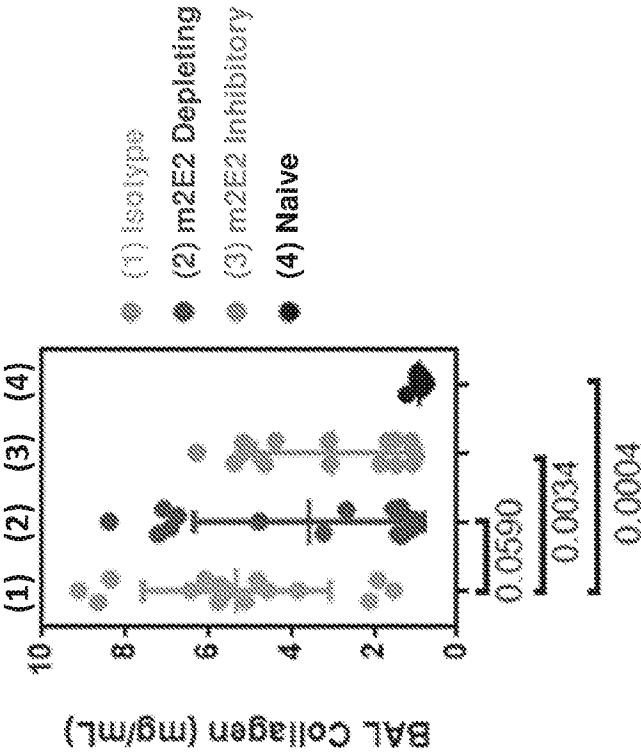
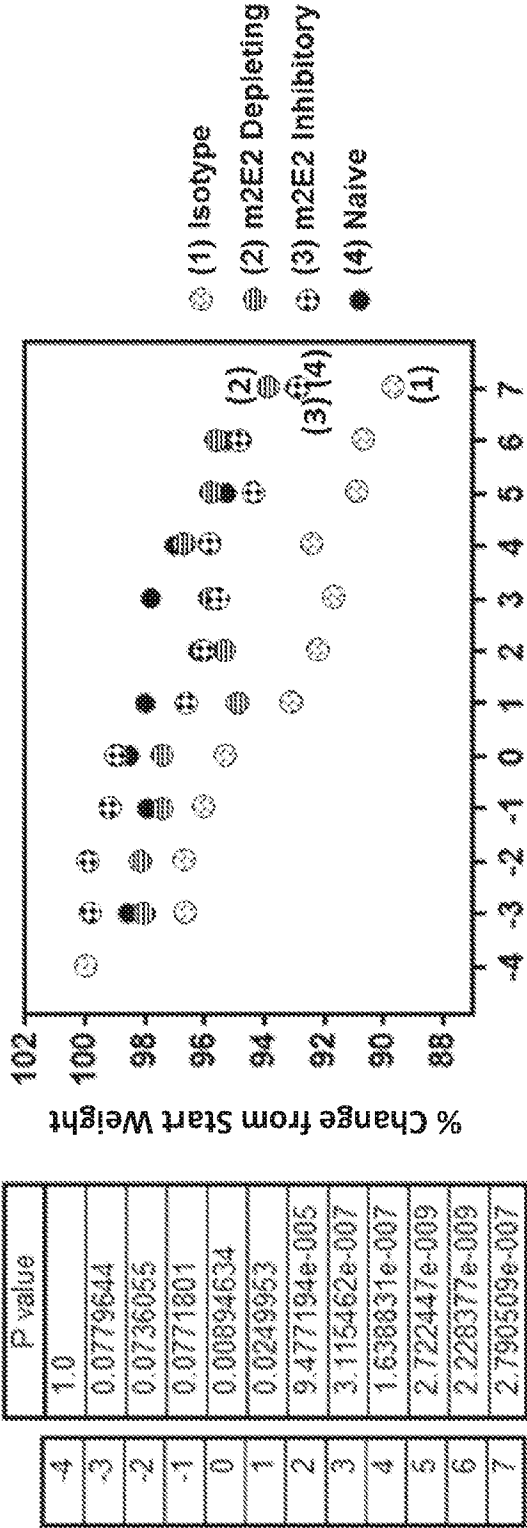


FIG. 3



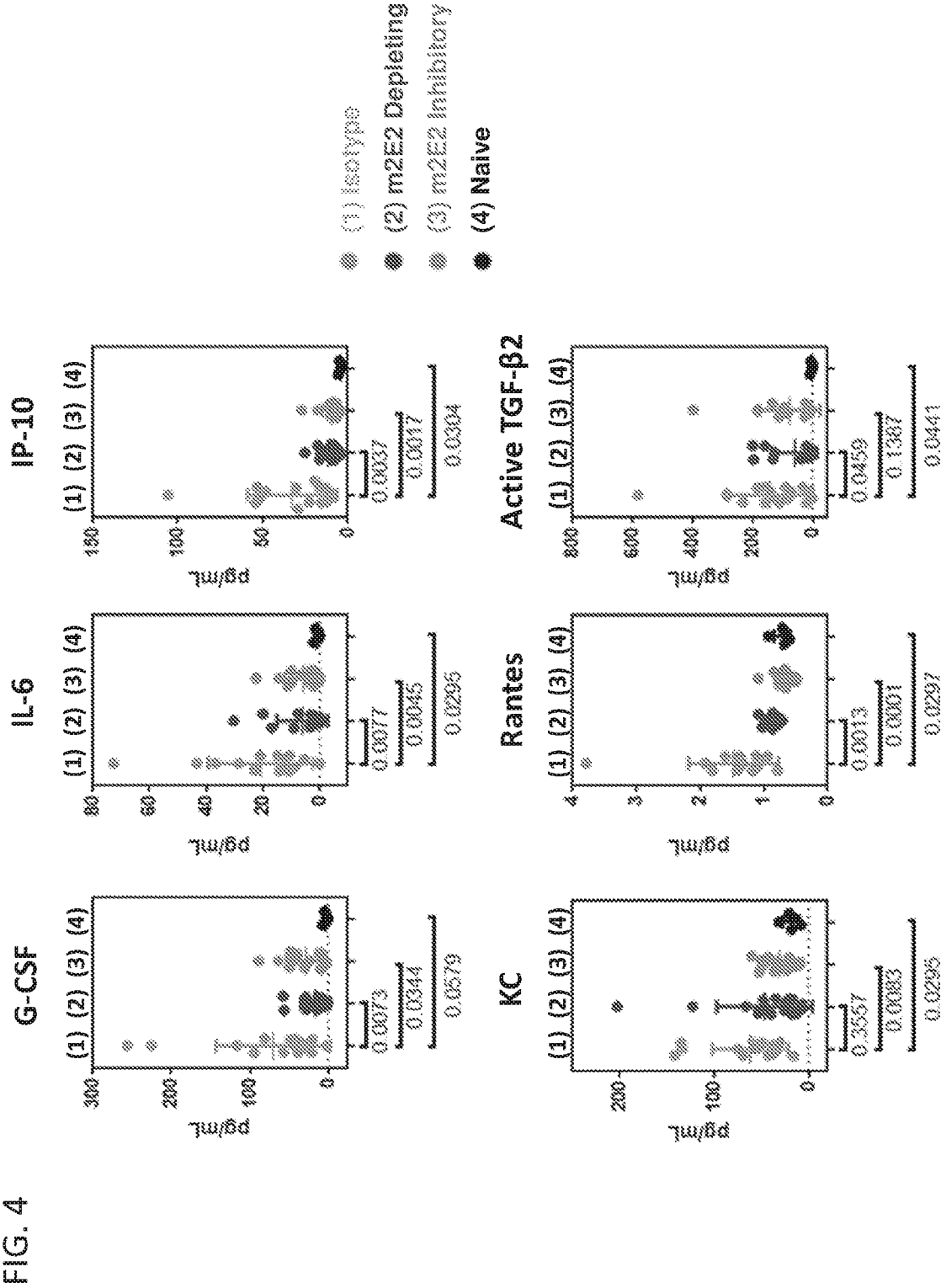


FIG. 5

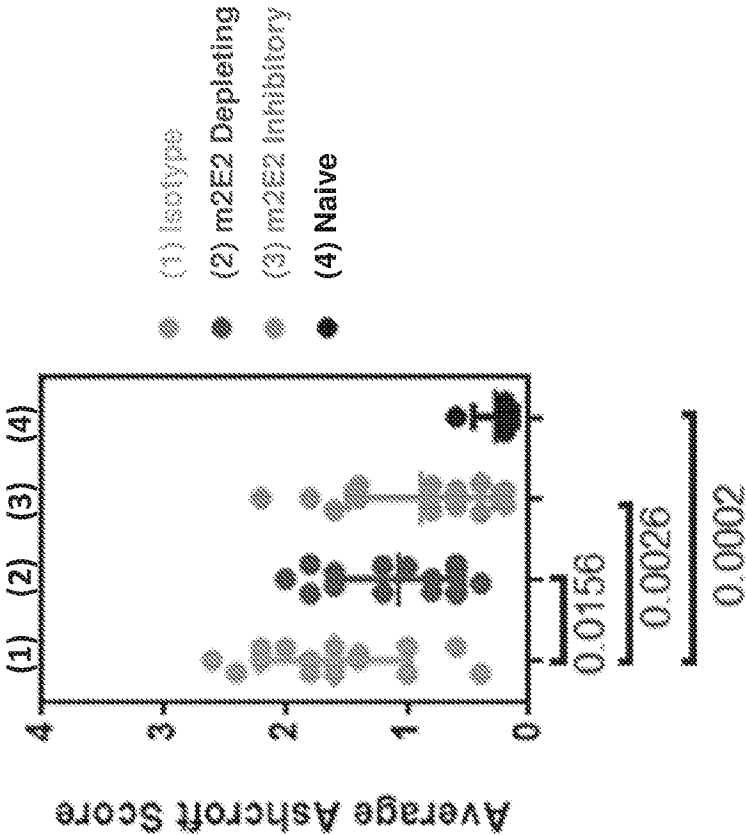


FIG. 6

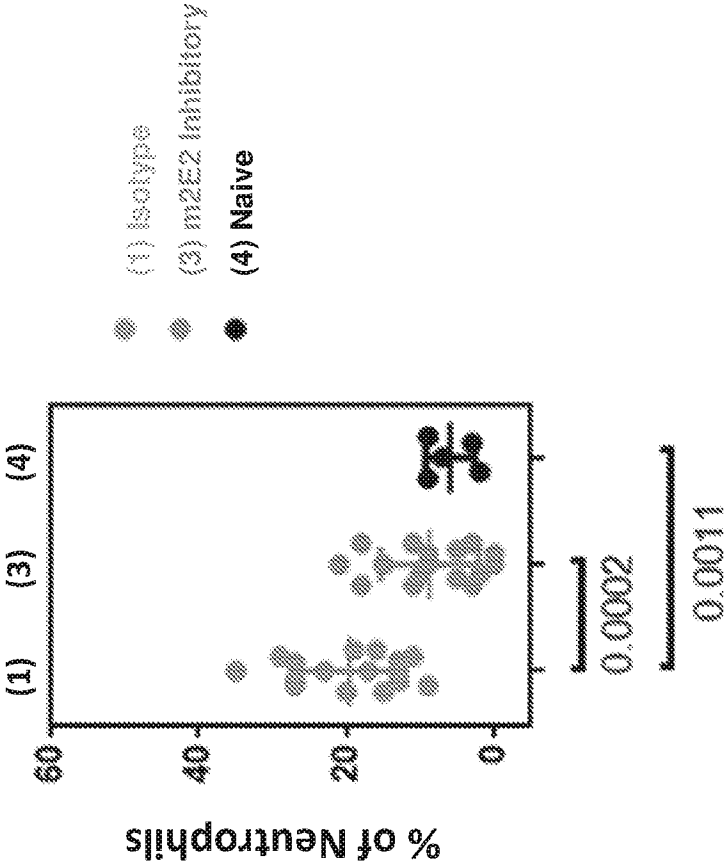


FIG. 7

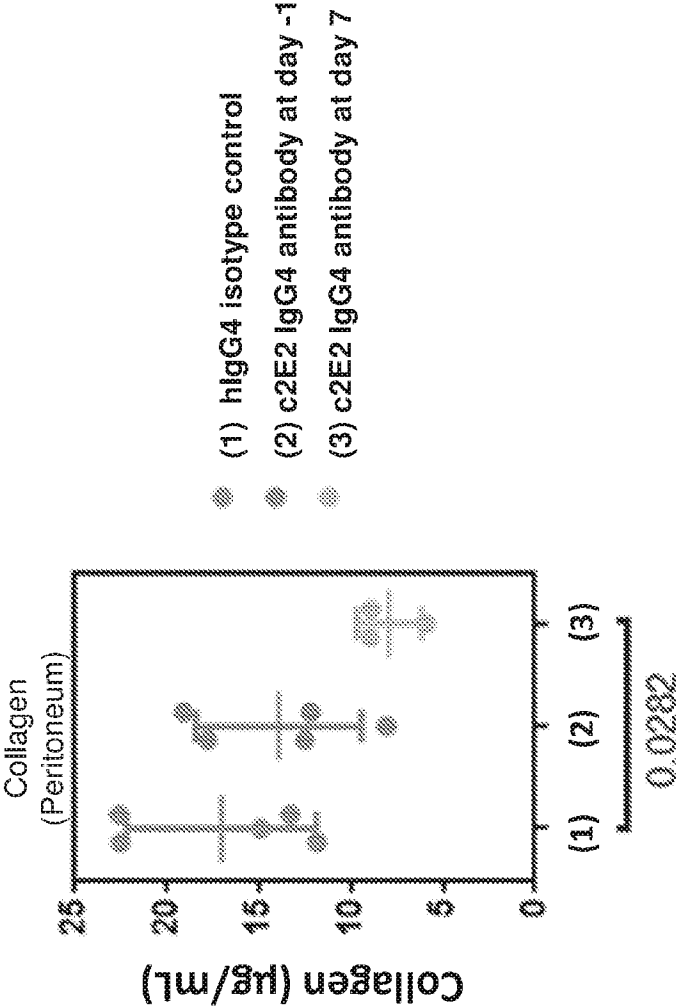


FIG. 8

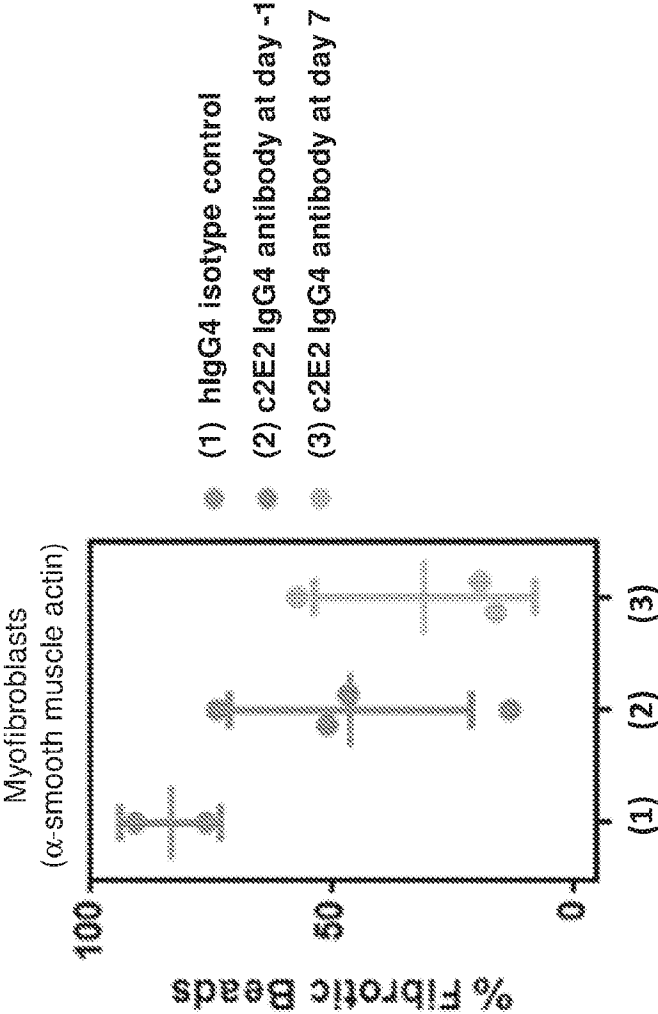


FIG. 9

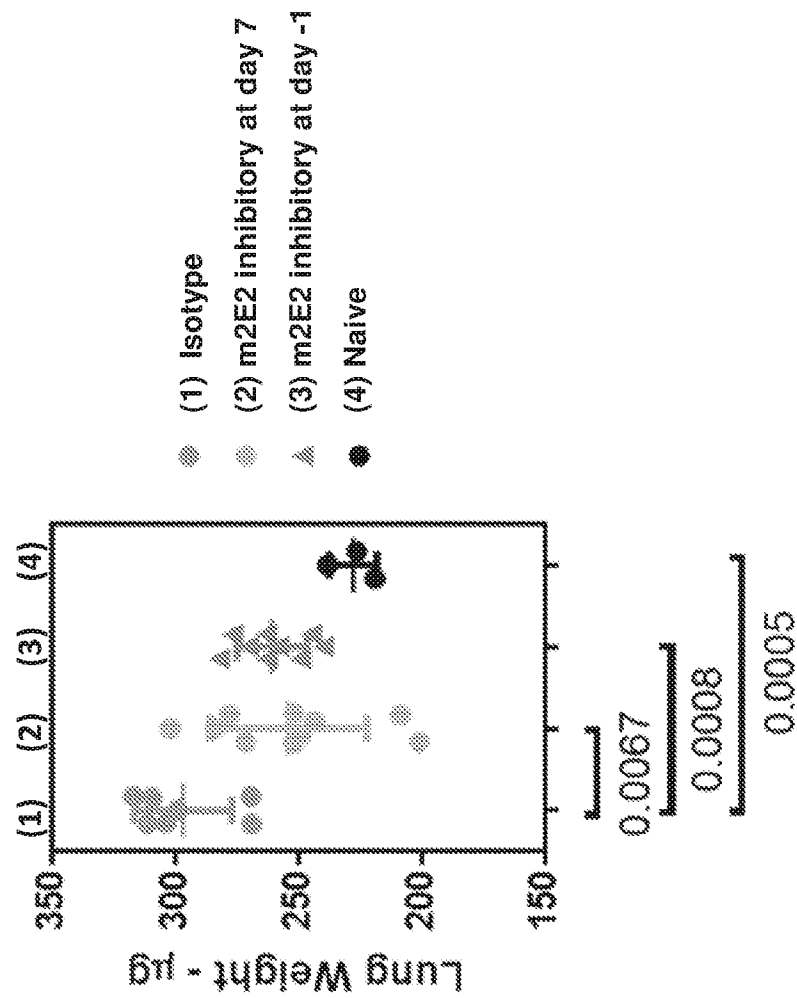


FIG. 10A

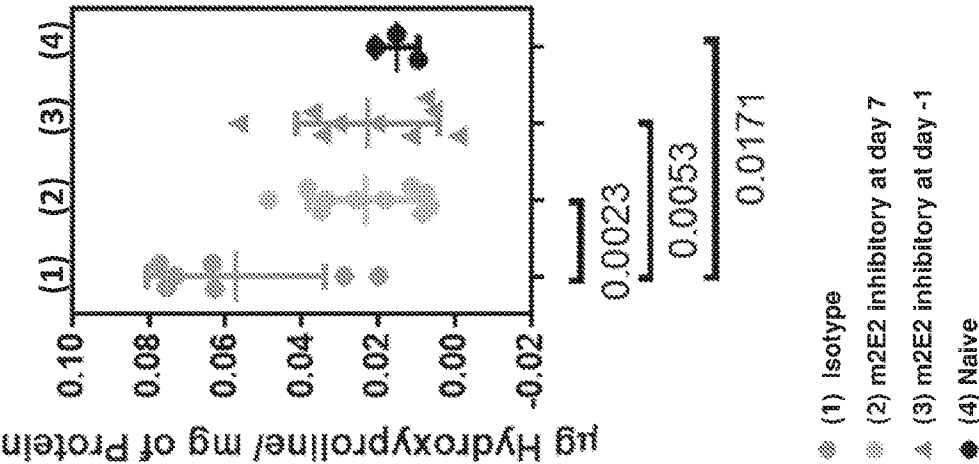
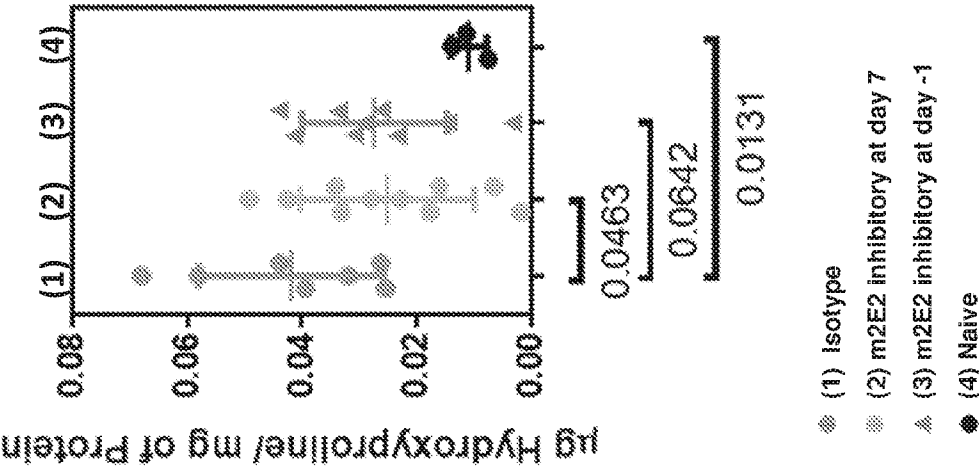
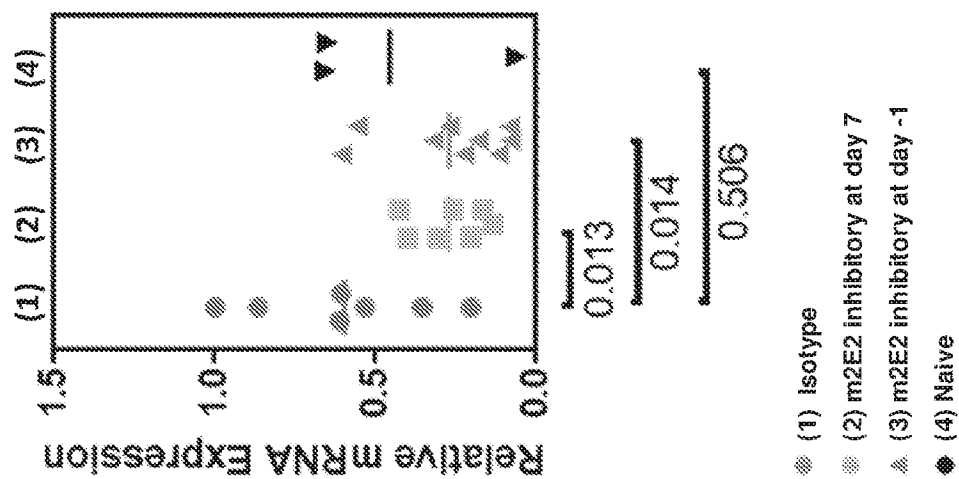
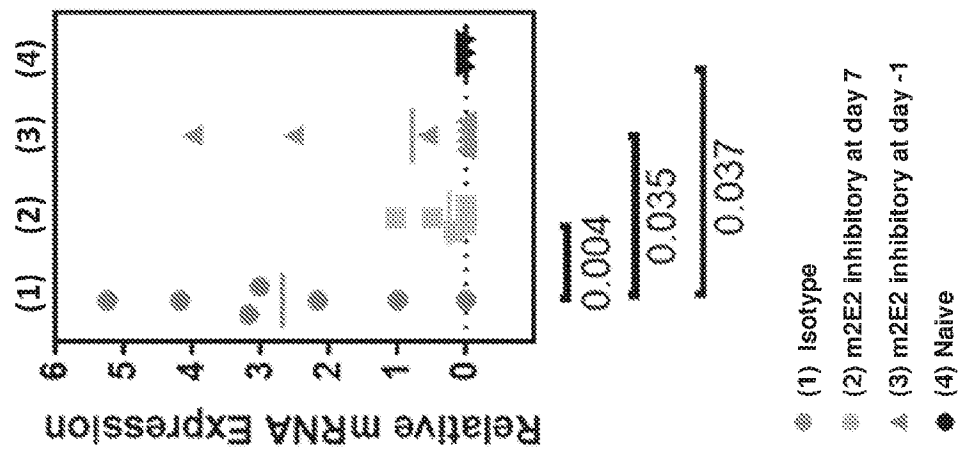


FIG. 10B



LEG. 11A

B
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L

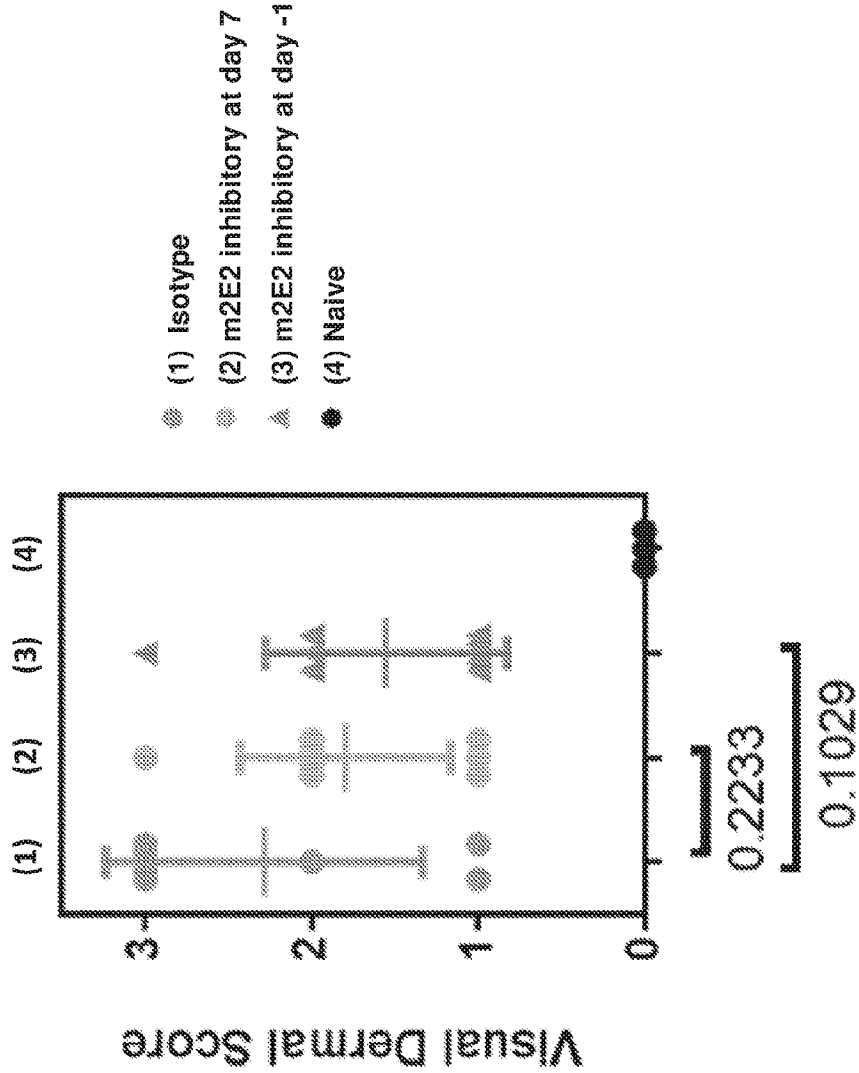


FIG. 12

701712000340SEQLIST
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BEBBINGTON, Christopher, Robert
TOMASEVIC, Nenad
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701712000340SEQLI ST

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Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	Ala
				85					90					95	
Arg	Asp	Gly	Ser	Ser	Pro	Tyr	Tyr	Tyr	Ser	Met	Glu	Tyr	Trp	Gly	Gln
			100					105					110		
Gly	Thr	Thr	Val	Thr	Val	Ser	Ser								
		115					120								

<210> 3
 <211> 120
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 3

Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly
1				5					10					15	
Ser	Leu	Arg	Leu	Ser	Cys	Ala	Val	Ser	Gly	Phe	Ser	Leu	Thr	Ile	Tyr
			20					25					30		
Gly	Ala	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Leu
		35					40					45			
Gly	Val	Ile	Trp	Ala	Gly	Gly	Ser	Thr	Asn	Tyr	Asn	Ser	Ala	Leu	Met
	50					55					60				
Ser	Arg	Leu	Ser	Ile	Ser	Lys	Asp	Asn	Ser	Lys	Asn	Thr	Val	Tyr	Leu
65					70					75					80
Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	Ala
				85					90					95	
Arg	Asp	Gly	Ser	Ser	Pro	Tyr	Tyr	Tyr	Ser	Met	Glu	Tyr	Trp	Gly	Gln
			100					105					110		
Gly	Thr	Thr	Val	Thr	Val	Ser	Ser								
		115					120								

<210> 4
 <211> 120
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 4

Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly
1				5					10					15	
Ser	Leu	Arg	Leu	Ser	Cys	Ala	Val	Ser	Gly	Phe	Ser	Leu	Thr	Ile	Tyr
			20					25					30		
Gly	Ala	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val
		35					40					45			
Ser	Val	Ile	Trp	Ala	Gly	Gly	Ser	Thr	Asn	Tyr	Asn	Ser	Ala	Leu	Met
	50					55					60				
Ser	Arg	Phe	Thr	Ile	Ser	Lys	Asp	Asn	Ser	Lys	Asn	Thr	Val	Tyr	Leu
65					70					75					80
Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	Ala
				85					90					95	
Arg	Asp	Gly	Ser	Ser	Pro	Tyr	Tyr	Tyr	Ser	Met	Glu	Tyr	Trp	Gly	Gln
			100					105					110		
Gly	Thr	Thr	Val	Thr	Val	Ser	Ser								
		115					120								

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<210> 5
 <211> 120
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 5
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Thr Ile Tyr
 20 25 30
 Gly Ala His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Leu
 35 40 45
 Ser Val Ile Trp Ala Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Met
 50 55 60
 Ser Arg Phe Thr Ile Ser Lys Asp Asn Ser Lys Asn Thr Val Tyr Leu
 65 70 75 80
 Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
 85 90 95
 Arg Asp Gly Ser Ser Pro Tyr Tyr Tyr Ser Met Glu Tyr Trp Gly Gln
 100 105 110
 Gly Thr Thr Val Thr Val Ser Ser
 115 120

<210> 6
 <211> 120
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 6
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Thr Ile Tyr
 20 25 30
 Gly Ala His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Gly Val Ile Trp Ala Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Met
 50 55 60
 Ser Arg Phe Thr Ile Ser Lys Asp Asn Ser Lys Asn Thr Val Tyr Leu
 65 70 75 80
 Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
 85 90 95
 Arg Asp Gly Ser Ser Pro Tyr Tyr Tyr Ser Met Glu Tyr Trp Gly Gln
 100 105 110
 Gly Thr Thr Val Thr Val Ser Ser
 115 120

<210> 7
 <211> 120
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 7
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Thr Ile Tyr
 20 25 30
 Gly Ala His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val

701712000340SEQLIST

Ser	Val	Ile	Trp	Ala	Gly	Gly	Ser	Thr	Asn	Tyr	Asn	Ser	Ala	Leu	Met
50		35				40					45				
Ser	Arg	Leu	Thr	Ile	Ser	Lys	Asp	Asn	Ser	Lys	Asn	Thr	Val	Tyr	Leu
65					70					75					80
Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	Ala
				85					90					95	
Arg	Asp	Gly	Ser	Ser	Pro	Tyr	Tyr	Tyr	Ser	Met	Glu	Tyr	Trp	Gly	Gln
			100					105					110		
Gly	Thr	Thr	Val	Thr	Val	Ser	Ser								
		115					120								

<210> 8
 <211> 120
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 8

Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly
1				5				10						15	
Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Ser	Leu	Thr	Ile	Tyr
			20					25				30			
Gly	Ala	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val
		35				40						45			
Ser	Val	Ile	Trp	Ala	Gly	Gly	Ser	Thr	Asn	Tyr	Asn	Ser	Ala	Leu	Met
	50				55						60				
Ser	Arg	Phe	Ser	Ile	Ser	Lys	Asp	Asn	Ser	Lys	Asn	Thr	Val	Tyr	Leu
65					70					75					80
Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	Ala
				85					90					95	
Arg	Asp	Gly	Ser	Ser	Pro	Tyr	Tyr	Tyr	Ser	Met	Glu	Tyr	Trp	Gly	Gln
			100					105					110		
Gly	Thr	Thr	Val	Thr	Val	Ser	Ser								
		115					120								

<210> 9
 <211> 120
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 9

Gln	Val	Gln	Leu	Gln	Glu	Ser	Gly	Pro	Gly	Leu	Val	Lys	Pro	Ser	Glu
1				5					10					15	
Thr	Leu	Ser	Leu	Thr	Cys	Thr	Val	Ser	Gly	Gly	Ser	Ile	Ser	Ile	Tyr
			20					25				30			
Gly	Ala	His	Trp	Ile	Arg	Gln	Pro	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Ile
		35				40						45			
Gly	Val	Ile	Trp	Ala	Gly	Gly	Ser	Thr	Asn	Tyr	Asn	Ser	Ala	Leu	Met
	50				55						60				
Ser	Arg	Val	Thr	Ile	Ser	Val	Asp	Thr	Ser	Lys	Asn	Gln	Phe	Ser	Leu
65					70					75					80
Lys	Leu	Ser	Ser	Val	Thr	Ala	Ala	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	Ala
				85					90					95	
Arg	Asp	Gly	Ser	Ser	Pro	Tyr	Tyr	Tyr	Ser	Met	Glu	Tyr	Trp	Gly	Gln
			100					105					110		
Gly	Thr	Leu	Val	Thr	Val	Ser	Ser								
		115					120								

<210> 10

701712000340SEQLIST

<211> 120
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 10
 Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
 1 5 10 15
 Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ile Tyr
 20 25 30
 Gly Ala His Trp Val Arg Gln Pro Gly Lys Gly Leu Glu Trp Leu
 35 40 45
 Gly Val Ile Trp Ala Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Met
 50 55 60
 Ser Arg Leu Ser Ile Ser Lys Asp Asn Ser Lys Asn Gln Val Ser Leu
 65 70 75 80
 Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
 85 90 95
 Arg Asp Gly Ser Ser Pro Tyr Tyr Tyr Ser Met Glu Tyr Trp Gly Gln
 100 105 110
 Gly Thr Leu Val Thr Val Ser Ser
 115 120

<210> 11
 <211> 120
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 11
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Thr Ile Tyr
 20 25 30
 Gly Ala His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Gly Val Ile Trp Ala Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Met
 50 55 60
 Ser Arg Phe Thr Ile Ser Lys Asp Asn Ser Lys Asn Thr Val Tyr Leu
 65 70 75 80
 Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
 85 90 95
 Arg Asp Gly Ser Ser Pro Tyr Tyr Tyr Gly Met Glu Tyr Trp Gly Gln
 100 105 110
 Gly Thr Thr Val Thr Val Ser Ser
 115 120

<210> 12
 <211> 120
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 12
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Thr Ile Tyr
 20 25 30
 Gly Ala His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

701712000340SEQLIST

Gly	Val	Ile	Trp	Ala	Gly	Gly	Ser	Thr	Asn	Tyr	Asn	Ser	Ala	Leu	Met
50					55						60				
Ser	Arg	Phe	Thr	Ile	Ser	Lys	Asp	Asn	Ser	Lys	Asn	Thr	Val	Tyr	Leu
65					70					75					80
Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	Ala
				85					90					95	
Arg	Asp	Gly	Ser	Ser	Pro	Tyr	Tyr	Tyr	Ser	Met	Asp	Tyr	Trp	Gly	Gln
			100					105					110		
Gly	Thr	Thr	Val	Thr	Val	Ser	Ser								
		115					120								

<210> 13
 <211> 120
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 13

Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly
1				5					10					15	
Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Ser	Leu	Thr	Ile	Tyr
			20					25					30		
Gly	Ala	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val
		35					40					45			
Gly	Val	Ile	Trp	Ala	Gly	Gly	Ser	Thr	Asn	Tyr	Asn	Ser	Ala	Leu	Met
					55						60				
Ser	Arg	Phe	Thr	Ile	Ser	Lys	Asp	Asn	Ser	Lys	Asn	Thr	Val	Tyr	Leu
65					70					75					80
Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	Ala
				85					90					95	
Arg	Asp	Gly	Ser	Ser	Pro	Tyr	Tyr	Tyr	Ser	Met	Glu	Val	Trp	Gly	Gln
			100					105					110		
Gly	Thr	Thr	Val	Thr	Val	Ser	Ser								
		115					120								

<210> 14
 <211> 120
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 14

Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly
1				5					10					15	
Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Ser	Leu	Thr	Ile	Tyr
			20					25					30		
Gly	Ala	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val
		35					40					45			
Gly	Val	Ile	Trp	Ala	Gly	Gly	Ser	Thr	Asn	Tyr	Asn	Ser	Ala	Leu	Met
					55						60				
Ser	Arg	Phe	Thr	Ile	Ser	Lys	Asp	Asn	Ser	Lys	Asn	Thr	Val	Tyr	Leu
65					70					75					80
Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	Ala
				85					90					95	
Arg	Asp	Gly	Ser	Ser	Pro	Tyr	Tyr	Tyr	Gly	Met	Asp	Val	Trp	Gly	Gln
			100					105					110		
Gly	Thr	Thr	Val	Thr	Val	Ser	Ser								
		115					120								

<210> 15
 <211> 106

<212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 15
 Gln Ile Ile Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly
 1 5 10 15
 Glu Lys Val Ser Ile Thr Cys Ser Ala Thr Ser Ser Val Ser Tyr Met
 20 25 30
 His Trp Phe Gln Gln Lys Pro Gly Thr Ser Pro Lys Leu Trp Ile Tyr
 35 40 45
 Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Val Arg Phe Ser Gly Ser
 50 55 60
 Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Met Glu Ala Glu
 65 70 75 80
 Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Arg Ser Ser Tyr Pro Phe Thr
 85 90 95
 Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
 100 105

<210> 16
 <211> 106
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 16
 Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15
 Glu Arg Ala Thr Leu Ser Cys Ser Ala Thr Ser Ser Val Ser Tyr Met
 20 25 30
 His Trp Phe Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr
 35 40 45
 Ser Thr Ser Asn Leu Ala Ser Gly Ile Pro Ala Arg Phe Ser Gly Ser
 50 55 60
 Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu
 65 70 75 80
 Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Ser Tyr Pro Phe Thr
 85 90 95
 Phe Gly Pro Gly Thr Lys Leu Asp Ile Lys
 100 105

<210> 17
 <211> 106
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 17
 Glu Ile Ile Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15
 Glu Arg Ala Thr Leu Ser Cys Ser Ala Thr Ser Ser Val Ser Tyr Met
 20 25 30
 His Trp Phe Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Trp Ile Tyr
 35 40 45
 Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
 50 55 60
 Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu
 65 70 75 80
 Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Ser Tyr Pro Phe Thr

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Phe Gly Pro Gly Thr Lys Leu Asp Ile Lys 95
 100 85 90 105

<210> 18
 <211> 106
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 18
 Glu Ile Ile Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15
 Glu Arg Ala Thr Leu Ser Cys Ser Ala Thr Ser Ser Val Ser Tyr Met
 20 25 30
 His Trp Phe Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Ile Tyr
 35 40 45
 Ser Thr Ser Asn Leu Ala Ser Gly Ile Pro Ala Arg Phe Ser Gly Ser
 50 55 60
 Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu
 65 70 75 80
 Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Ser Tyr Pro Phe Thr
 85 90 95
 Phe Gly Pro Gly Thr Lys Leu Asp Ile Lys
 100 105

<210> 19
 <211> 106
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 19
 Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15
 Glu Arg Ala Thr Leu Ser Cys Ser Ala Thr Ser Ser Val Ser Tyr Met
 20 25 30
 His Trp Phe Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Trp Ile Tyr
 35 40 45
 Ser Thr Ser Asn Leu Ala Ser Gly Ile Pro Ala Arg Phe Ser Gly Ser
 50 55 60
 Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu
 65 70 75 80
 Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Ser Tyr Pro Phe Thr
 85 90 95
 Phe Gly Pro Gly Thr Lys Leu Asp Ile Lys
 100 105

<210> 20
 <211> 106
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 20
 Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15
 Glu Arg Ala Thr Leu Ser Cys Ser Ala Thr Ser Ser Val Ser Tyr Met
 20 25 30

701712000340SEQLIST

His	Trp	Phe	Gln	Gln	Lys	Pro	Gly	Gln	Ala	Pro	Arg	Leu	Leu	Ile	Tyr
		35					40				45				
Ser	Thr	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro	Ala	Arg	Phe	Ser	Gly	Ser
	50					55					60				
Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Glu	Pro	Glu
65					70					75				80	
Asp	Phe	Ala	Val	Tyr	Tyr	Cys	Gln	Gln	Arg	Ser	Ser	Tyr	Pro	Phe	Thr
				85					90					95	
Phe	Gly	Pro	Gly	Thr	Lys	Leu	Asp	Ile	Lys						
			100					105							

<210> 21
 <211> 106
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 21

Glu	Ile	Val	Leu	Thr	Gln	Ser	Pro	Ala	Thr	Leu	Ser	Leu	Ser	Pro	Gly
1				5					10					15	
Glu	Arg	Ala	Thr	Leu	Ser	Cys	Ser	Ala	Thr	Ser	Ser	Val	Ser	Tyr	Met
			20					25					30		
His	Trp	Phe	Gln	Gln	Lys	Pro	Gly	Gln	Ala	Pro	Arg	Leu	Leu	Ile	Tyr
		35					40					45			
Ser	Thr	Ser	Asn	Leu	Ala	Ser	Gly	Ile	Pro	Ala	Arg	Phe	Ser	Gly	Ser
	50					55					60				
Gly	Ser	Gly	Thr	Asp	Tyr	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Glu	Pro	Glu
65					70					75				80	
Asp	Phe	Ala	Val	Tyr	Tyr	Cys	Gln	Gln	Arg	Ser	Ser	Tyr	Pro	Phe	Thr
				85					90					95	
Phe	Gly	Pro	Gly	Thr	Lys	Leu	Asp	Ile	Lys						
			100					105							

<210> 22
 <211> 106
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 22

Glu	Ile	Val	Leu	Thr	Gln	Ser	Pro	Ala	Thr	Leu	Ser	Leu	Ser	Pro	Gly
1				5					10					15	
Glu	Arg	Ala	Thr	Leu	Ser	Cys	Ser	Ala	Thr	Ser	Ser	Val	Ser	Tyr	Met
			20					25					30		
His	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln	Ala	Pro	Arg	Leu	Leu	Ile	Tyr
		35					40					45			
Ser	Thr	Ser	Asn	Leu	Ala	Ser	Gly	Ile	Pro	Ala	Arg	Phe	Ser	Gly	Ser
	50					55					60				
Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Glu	Pro	Glu
65					70					75				80	
Asp	Phe	Ala	Val	Tyr	Tyr	Cys	Gln	Gln	Arg	Ser	Ser	Tyr	Pro	Phe	Thr
				85					90					95	
Phe	Gly	Pro	Gly	Thr	Lys	Leu	Asp	Ile	Lys						
			100					105							

<210> 23
 <211> 106
 <212> PRT
 <213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 23

Glu	Ile	Val	Leu	Thr	Gln	Ser	Pro	Ala	Thr	Leu	Ser	Leu	Ser	Pro	Gly
1				5					10					15	
Glu	Arg	Ala	Thr	Leu	Ser	Cys	Ser	Ala	Thr	Ser	Ser	Val	Ser	Tyr	Met
			20					25					30		
His	Trp	Phe	Gln	Gln	Lys	Pro	Gly	Gln	Ala	Pro	Arg	Leu	Leu	Ile	Tyr
		35					40					45			
Ser	Thr	Ser	Asn	Leu	Ala	Ser	Gly	Ile	Pro	Ala	Arg	Phe	Ser	Gly	Ser
		50				55					60				
Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Glu	Pro	Glu
65				70					75					80	
Asp	Phe	Ala	Val	Tyr	Tyr	Cys	Gln	Gln	Arg	Ser	Ser	Tyr	Pro	Tyr	Thr
			85						90					95	
Phe	Gly	Pro	Gly	Thr	Lys	Leu	Asp	Ile	Lys						
			100					105							

<210> 24

<211> 106

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 24

Glu	Ile	Val	Leu	Thr	Gln	Ser	Pro	Ala	Thr	Leu	Ser	Leu	Ser	Pro	Gly
1				5					10					15	
Glu	Arg	Ala	Thr	Leu	Ser	Cys	Ser	Ala	Thr	Ser	Ser	Val	Ser	Tyr	Met
			20					25					30		
His	Trp	Phe	Gln	Gln	Lys	Pro	Gly	Gln	Ala	Pro	Arg	Leu	Leu	Ile	Tyr
		35					40					45			
Ser	Thr	Ser	Asn	Leu	Ala	Ser	Gly	Ile	Pro	Ala	Arg	Phe	Ser	Gly	Ser
		50				55					60				
Gly	Ser	Gly	Thr	Asp	Tyr	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Glu	Pro	Glu
65				70					75					80	
Asp	Phe	Ala	Val	Tyr	Tyr	Cys	Gln	Gln	Arg	Ser	Ser	Tyr	Pro	Tyr	Thr
			85						90					95	
Phe	Gly	Pro	Gly	Thr	Lys	Leu	Asp	Ile	Lys						
			100					105							

<210> 25

<211> 30

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 25

Gln	Val	Gln	Leu	Lys	Glu	Ser	Gly	Pro	Gly	Leu	Val	Ala	Pro	Ser	Gln
1				5					10					15	
Ser	Leu	Ser	Ile	Thr	Cys	Thr	Val	Ser	Gly	Phe	Ser	Leu	Thr		
			20					25					30		

<210> 26

<211> 30

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 26

701712000340SEQLIST

Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly
1				5				10						15	
Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Ser	Leu	Thr		
			20					25					30		

<210> 27
 <211> 30
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 27

Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly
1				5				10						15	
Ser	Leu	Arg	Leu	Ser	Cys	Ala	Val	Ser	Gly	Phe	Ser	Leu	Thr		
			20					25					30		

<210> 28
 <211> 30
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 28

Gln	Val	Gln	Leu	Gln	Glu	Ser	Gly	Pro	Gly	Leu	Val	Lys	Pro	Ser	Glu
1				5				10						15	
Thr	Leu	Ser	Leu	Thr	Cys	Thr	Val	Ser	Gly	Gly	Ser	Ile	Ser		
			20					25					30		

<210> 29
 <211> 30
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 29

Gln	Val	Gln	Leu	Gln	Glu	Ser	Gly	Pro	Gly	Leu	Val	Lys	Pro	Ser	Glu
1				5				10						15	
Thr	Leu	Ser	Leu	Thr	Cys	Thr	Val	Ser	Gly	Phe	Ser	Leu	Thr		
			20					25					30		

<210> 30
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 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 30

Trp	Val	Arg	Gln	Pro	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Leu	Gly		
1				5				10							

<210> 31
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 <212> PRT
 <213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 31

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser
 1 5 10

<210> 32

<211> 14

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 32

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Leu Gly
 1 5 10

<210> 33

<211> 14

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 33

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Leu Ser
 1 5 10

<210> 34

<211> 14

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 34

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Gly
 1 5 10

<210> 35

<211> 14

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 35

Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile Gly
 1 5 10

<210> 36

<211> 14

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

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<400> 36

Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu Gly
1 5 10

<210> 37

<211> 32

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 37

Arg Leu Ser Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Phe Leu Lys
1 5 10 15
Ile Asn Ser Leu Gln Thr Asp Asp Thr Ala Leu Tyr Tyr Cys Ala Arg
20 25 30

<210> 38

<211> 32

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 38

Arg Phe Thr Ile Ser Lys Asp Asn Ser Lys Asn Thr Val Tyr Leu Gln
1 5 10 15
Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
20 25 30

<210> 39

<211> 32

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 39

Arg Leu Ser Ile Ser Lys Asp Asn Ser Lys Asn Thr Val Tyr Leu Gln
1 5 10 15
Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
20 25 30

<210> 40

<211> 32

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 40

Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Asn Thr Val Tyr Leu Gln
1 5 10 15
Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
20 25 30

<210> 41

<211> 32

<212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 41
 Arg Phe Ser Ile Ser Lys Asp Asn Ser Lys Asn Thr Val Tyr Leu Gln
 1 5 10 15
 Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
 20 25 30

<210> 42
 <211> 32
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 42
 Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu Lys
 1 5 10 15
 Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg
 20 25 30

<210> 43
 <211> 32
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 43
 Arg Leu Ser Ile Ser Lys Asp Asn Ser Lys Asn Gln Val Ser Leu Lys
 1 5 10 15
 Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg
 20 25 30

<210> 44
 <211> 11
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 44
 Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser
 1 5 10

<210> 45
 <211> 11
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 45
 Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
 1 5 10

<210> 46
 <211> 11
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 46
 Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
 1 5 10

<210> 47
 <211> 23
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 47
 Gln Ile Ile Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly
 1 5 10 15
 Glu Lys Val Ser Ile Thr Cys
 20

<210> 48
 <211> 23
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 48
 Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15
 Glu Arg Ala Thr Leu Ser Cys
 20

<210> 49
 <211> 23
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 49
 Glu Ile Ile Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15
 Glu Arg Ala Thr Leu Ser Cys
 20

<210> 50
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 50

701712000340SEQLIST

Trp Phe Gln Gln Lys Pro Gly Thr Ser Pro Lys Leu Trp Ile Tyr
1 5 10 15

<210> 51
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 51
Trp Phe Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr
1 5 10 15

<210> 52
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 52
Trp Phe Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Trp Ile Tyr
1 5 10 15

<210> 53
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 53
Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr
1 5 10 15

<210> 54
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 54
Gly Val Pro Val Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser
1 5 10 15
Leu Thr Ile Ser Arg Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys
20 25 30

<210> 55
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 55
Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr

701712000340SEQLIST

1 5 10 15
 Leu Thr Ile Ser Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys
 20 25 30

<210> 56
 <211> 32
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 56
 Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr
 1 5 10 15
 Leu Thr Ile Ser Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys
 20 25 30

<210> 57
 <211> 32
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 57
 Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
 1 5 10 15
 Leu Thr Ile Ser Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys
 20 25 30

<210> 58
 <211> 32
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 58
 Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr
 1 5 10 15
 Leu Thr Ile Ser Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys
 20 25 30

<210> 59
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 59
 Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
 1 5 10

<210> 60
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 60

Phe Gly Pro Gly Thr Lys Leu Asp Ile Lys
1 5 10

<210> 61

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 61

Ile Tyr Gly Ala His
1 5

<210> 62

<211> 16

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 62

Val Ile Trp Ala Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Met Ser
1 5 10 15

<210> 63

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 63

Asp Gly Ser Ser Pro Tyr Tyr Tyr Ser Met Glu Tyr
1 5 10

<210> 64

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 64

Ser Ala Thr Ser Ser Val Ser Tyr Met His
1 5 10

<210> 65

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 65

Ser Thr Ser Asn Leu Ala Ser
1 5

<210> 66

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 66

Gln Gln Arg Ser Ser Tyr Pro Phe Thr
1 5

<210> 67

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 67

Asp Gly Ser Ser Pro Tyr Tyr Tyr Gly Met Glu Tyr
1 5 10

<210> 68

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 68

Asp Gly Ser Ser Pro Tyr Tyr Tyr Ser Met Asp Tyr
1 5 10

<210> 69

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 69

Asp Gly Ser Ser Pro Tyr Tyr Tyr Ser Met Glu Val
1 5 10

<210> 70

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 70

Asp Gly Ser Ser Pro Tyr Tyr Tyr Gly Met Asp Val
1 5 10

<210> 71
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 71
 Gln Gln Arg Ser Ser Tyr Pro Tyr Thr
 1 5

<210> 72
 <211> 474
 <212> PRT
 <213> Homo sapiens

<400> 72
 Gly Tyr Leu Leu Gln Val Gln Glu Leu Val Thr Val Gln Glu Gly Leu
 1 5 10 15
 Cys Val His Val Pro Cys Ser Phe Ser Tyr Pro Gln Asp Gly Trp Thr
 20 25 30
 Asp Ser Asp Pro Val His Gly Tyr Trp Phe Arg Ala Gly Asp Arg Pro
 35 40 45
 Tyr Gln Asp Ala Pro Val Ala Thr Asn Asn Pro Asp Arg Glu Val Gln
 50 55 60
 Ala Glu Thr Gln Gly Arg Phe Gln Leu Leu Gly Asp Ile Trp Ser Asn
 65 70 75
 Asp Cys Ser Leu Ser Ile Arg Asp Ala Arg Lys Arg Asp Lys Gly Ser
 85 90 95
 Tyr Phe Phe Arg Leu Glu Arg Gly Ser Met Lys Trp Ser Tyr Lys Ser
 100 105 110
 Gln Leu Asn Tyr Lys Thr Lys Gln Leu Ser Val Phe Val Thr Ala Leu
 115 120 125
 Thr His Arg Pro Asp Ile Leu Ile Leu Gly Thr Leu Glu Ser Gly His
 130 135 140
 Ser Arg Asn Leu Thr Cys Ser Val Pro Trp Ala Cys Lys Gln Gly Thr
 145 150 155
 Pro Pro Met Ile Ser Trp Ile Gly Ala Ser Val Ser Ser Pro Gly Pro
 165 170 175
 Thr Thr Ala Arg Ser Ser Val Leu Thr Leu Thr Pro Lys Pro Gln Asp
 180 185 190
 His Gly Thr Ser Leu Thr Cys Gln Val Thr Leu Pro Gly Thr Gly Val
 195 200 205
 Thr Thr Thr Ser Thr Val Arg Leu Asp Val Ser Tyr Pro Pro Trp Asn
 210 215 220
 Leu Thr Met Thr Val Phe Gln Gly Asp Ala Thr Ala Ser Thr Ala Leu
 225 230 235 240
 Gly Asn Gly Ser Ser Leu Ser Val Leu Glu Gly Gln Ser Leu Arg Leu
 245 250 255
 Val Cys Ala Val Asn Ser Asn Pro Pro Ala Arg Leu Ser Trp Thr Arg
 260 265 270
 Gly Ser Leu Thr Leu Cys Pro Ser Arg Ser Ser Asn Pro Gly Leu Leu
 275 280 285
 Glu Leu Pro Arg Val His Val Arg Asp Glu Gly Glu Phe Thr Cys Arg
 290 295 300
 Ala Gln Asn Ala Gln Gly Ser Gln His Ile Ser Leu Ser Leu Ser Leu
 305 310 315 320
 Gln Asn Glu Gly Thr Gly Thr Ser Arg Pro Val Ser Gln Val Thr Leu
 325 330 335
 Ala Ala Val Gly Gly Ala Gly Ala Thr Ala Leu Ala Phe Leu Ser Phe
 340 345 350
 Cys Ile Ile Phe Ile Ile Val Arg Ser Cys Arg Lys Lys Ser Ala Arg
 355 360 365
 Pro Ala Ala Gly Val Gly Asp Thr Gly Met Glu Asp Ala Lys Ala Ile

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370	Arg	Gly	Ser	Ala	Ser	Gln	375	Gly	Pro	Leu	Thr	Glu	380	Ser	Trp	Lys	Asp	Gly
385	Asn	Pro	Leu	Lys	Lys	Pro	390	Pro	Pro	Ala	Val	395	Ala	Pro	Ser	Ser	Gly	400
	Glu	Gly	Glu	Leu	His	Tyr	405	Ala	Thr	Leu	410	Ser	Phe	His	Lys	Val	Lys	415
	Gln	Asp	Pro	Gln	Gly	Gln	420	Glu	Ala	Thr	425	Asp	Ser	Glu	Tyr	430	Ser	Glu
	Lys	Ile	His	Lys	Arg	Glu	435	Thr	Ala	Glu	440	Thr	Gln	Ala	445	Cys	Leu	Arg
	His	Asn	Pro	Ser	Ser	Lys	450	Glu	Val	Arg	455	Gly			460			Asn
465						470												

<210> 73
 <211> 474
 <212> PRT
 <213> Homo sapiens

<400> 73	Gly	Tyr	Leu	Leu	Gln	Val	Gln	Glu	Leu	Val	Thr	Val	Gln	Glu	Gly	Leu
1	Cys	Val	His	Val	Pro	Cys	Ser	Phe	Ser	Tyr	Pro	Gln	Asp	Gly	Trp	Thr
	Asp	Ser	Asp	Pro	Val	His	Gly	Tyr	Trp	Phe	Arg	Ala	Gly	Asp	Arg	Pro
	Tyr	Gln	Asp	Ala	Pro	Val	Ala	Thr	Asn	Asn	Pro	Asp	Arg	Glu	Val	Gln
	Ala	Glu	Thr	Gln	Gly	Arg	Phe	Gln	Leu	Leu	Gly	Asp	Ile	Trp	Ser	Asn
65	Asp	Cys	Ser	Leu	Ser	Ile	Arg	Asp	Ala	Arg	Lys	Arg	Asp	Lys	Gly	Ser
	Tyr	Phe	Phe	Arg	Leu	Glu	Arg	Gly	Ser	Met	Lys	Trp	Ser	Tyr	Lys	Ser
	Gln	Leu	Asn	Tyr	Lys	Thr	Lys	Gln	Leu	Ser	Val	Phe	Val	Thr	Ala	Leu
	Thr	His	Arg	Pro	Asp	Ile	Leu	Ile	Leu	Gly	Thr	Leu	Glu	Ser	Gly	His
	Pro	Arg	Asn	Leu	Thr	Cys	Ser	Val	Pro	Trp	Ala	Cys	Lys	Gln	Gly	Thr
145	Pro	Pro	Met	Ile	Ser	Trp	Ile	Gly	Ala	Ser	Val	Ser	Ser	Pro	Gly	Pro
	Thr	Thr	Ala	Arg	Ser	Ser	Val	Leu	Thr	Leu	Thr	Pro	Lys	Pro	Gln	Asp
	His	Gly	Thr	Ser	Leu	Thr	Cys	Gln	Val	Thr	Leu	Pro	Gly	Thr	Gly	Val
	Thr	Thr	Thr	Ser	Thr	Val	Arg	Leu	Asp	Val	Ser	Tyr	Pro	Pro	Trp	Asn
	Leu	Thr	Met	Thr	Val	Phe	Gln	Gly	Asp	Ala	Thr	Ala	Ser	Thr	Ala	Leu
225	Gly	Asn	Gly	Ser	Ser	Leu	Ser	Val	Leu	Glu	Gly	Gln	Ser	Leu	Arg	Leu
	Val	Cys	Ala	Val	Asn	Ser	Asn	Pro	Pro	Ala	Arg	Leu	Ser	Trp	Thr	Arg
	Gly	Ser	Leu	Thr	Leu	Cys	Pro	Ser	Arg	Ser	Ser	Asn	Pro	Gly	Leu	Leu
	Glu	Leu	Pro	Arg	Val	His	Val	Arg	Asp	Glu	Gly	Glu	Phe	Thr	Cys	Arg
	Ala	Gln	Asn	Ala	Gln	Gly	Ser	Gln	His	Ile	Ser	Leu	Ser	Leu	Ser	Leu
305	Gln	Asn	Glu	Gly	Thr	Gly	Thr	Ser	Arg	Pro	Val	Ser	Gln	Val	Thr	Leu
	Ala	Ala	Val	Gly	Gly	Ala	Gly	Ala	Thr	Ala	Leu	Ala	Phe	Leu	Ser	Phe
	Cys	Ile	Ile	Phe	Ile	Ile	Val	Arg	Ser	Cys	Arg	Lys	Lys	Ser	Ala	Arg
	Pro	Ala	Ala	Gly	Val	Gly	Asp	Thr	Gly	Met	Glu	Asp	Ala	Lys	Ala	Ile

701712000340SEQLI ST

370	Arg	Gly	Ser	Ala	Ser	Gln	375	Gly	Pro	Leu	Thr	Glu	380	Ser	Trp	Lys	Asp	Gly	
385	Asn	Pro	Leu	Lys	Lys	Pro	390	Pro	Pro	Ala	Val	395	Ala	Pro	Ser	Ser	Gly	400	Glu
	Glu	Gly	Glu	Leu	His	Tyr	405	Ala	Thr	Leu	410	Ser	Phe	His	Lys	Val	415	Lys	Pro
	Gln	Asp	Pro	Gln	Gly	Gln	420	Glu	Ala	Thr	425	Asp	Ser	Glu	Tyr	430	Ser	Glu	Ile
	Lys	Ile	His	Lys	Arg	Glu	435	Thr	Ala	Glu	440	Thr	Gln	Ala	Cys	Leu	Arg	Asn	
	His	Asn	Pro	Ser	Ser	Lys	445	Glu	Val	Arg	450	Gly							
465						470													

<210> 74

<211> 573

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 74

1	Gly	Tyr	Leu	Leu	Gln	5	Val	Gln	Glu	10	Leu	Val	Thr	Val	Gln	15	Gly	Leu
	Cys	Val	His	Val	Pro	20	Cys	Ser	Phe	25	Ser	Tyr	Pro	Gln	30	Asp	Gly	Trp
	Asp	Ser	Asp	Pro	Val	35	His	Gly	Tyr	40	Trp	Phe	Arg	Ala	45	Gly	Asp	Arg
	Tyr	Gln	Asp	Ala	Pro	50	Val	Ala	Thr	55	Asn	Asn	Pro	Asp	60	Arg	Glu	Val
	Ala	Glu	Thr	Gln	Gly	65	Arg	Phe	Gln	70	Leu	Leu	Gly	Asp	75	Ile	Trp	Ser
	Asp	Cys	Ser	Leu	Ser	85	Ile	Arg	Asp	90	Ala	Arg	Lys	Arg	95	Asp	Lys	Gly
	Tyr	Phe	Phe	Arg	Leu	100	Glu	Arg	Gly	105	Ser	Met	Lys	Trp	110	Ser	Tyr	Lys
	Gln	Leu	Asn	Tyr	Lys	115	Thr	Lys	Gln	120	Leu	Ser	Val	Phe	125	Val	Thr	Ala
	Thr	His	Arg	Pro	Asp	130	Ile	Leu	Ile	135	Leu	Gly	Thr	Leu	140	Glu	Ser	Gly
	Ser	Arg	Asn	Leu	Thr	145	Cys	Ser	Val	150	Pro	Trp	Ala	Cys	155	Lys	Gln	Gly
	Pro	Pro	Met	Ile	Ser	165	Trp	Ile	Gly	170	Ala	Ser	Val	Ser	175	Ser	Pro	Gly
	Thr	Thr	Ala	Arg	Ser	180	Ser	Val	Leu	185	Thr	Leu	Thr	Pro	190	Lys	Pro	Gln
	His	Gly	Thr	Ser	Leu	195	Thr	Cys	Gln	200	Val	Thr	Leu	Pro	205	Gly	Thr	Gly
	Thr	Thr	Thr	Ser	Thr	210	Val	Arg	Leu	215	Asp	Val	Ser	Tyr	220	Pro	Pro	Trp
	Leu	Thr	Met	Thr	Val	225	Phe	Gln	Gly	230	Asp	Ala	Thr	Ala	235	Ser	Thr	Ala
	Gly	Asn	Gly	Ser	Ser	245	Leu	Ser	Val	250	Leu	Glu	Gly	Gln	255	Ser	Leu	Arg
	Val	Cys	Ala	Val	Asn	260	Ser	Asn	Pro	265	Pro	Ala	Arg	Leu	270	Ser	Trp	Thr
	Gly	Ser	Leu	Thr	Leu	275	Cys	Pro	Ser	280	Arg	Ser	Ser	Asn	285	Pro	Gly	Leu
	Glu	Leu	Pro	Arg	Val	290	His	Val	Arg	295	Asp	Glu	Gly	Glu	300	Phe	Thr	Cys
	Ala	Gln	Asn	Ala	Gln	305	Gly	Ser	Gln	310	His	Ile	Ser	Leu	315	Ser	Leu	Ser
	Gln	Asn	Glu	Gly	Thr	325	Gly	Thr	Ser	330	Arg	Pro	Val	Ser	335	Gln	Val	Thr
	Ala	Ala	Val	Gly	Gly	340	Ile	Glu	Gly	345	Arg	Ser	Asp	Lys	350	Thr	His	Cys

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Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu
		355					360					365			
Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu
	370					375					380				
Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys
	385				390					395					400
Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys
				405					410					415	
Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu
			420					425					430		
Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys
		435					440					445			
Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys
	450					455					460				
Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser
	465				470					475					480
Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys
				485					490					495	
Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln
			500					505					510		
Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly
		515					520					525			
Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln
	530					535				540					
Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn
	545				550					555					560
His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys			
				565					570						

<210> 75
 <211> 449
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 75

Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly
1				5					10					15	
Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Ser	Leu	Thr	Ile	Tyr
			20					25					30		
Gly	Ala	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val
		35					40					45			
Gly	Val	Ile	Trp	Ala	Gly	Gly	Ser	Thr	Asn	Tyr	Asn	Ser	Ala	Leu	Met
	50				55					60					
Ser	Arg	Phe	Thr	Ile	Ser	Lys	Asp	Asn	Ser	Lys	Asn	Thr	Val	Tyr	Leu
	65				70					75				80	
Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	Ala
			85					90					95		
Arg	Asp	Gly	Ser	Ser	Pro	Tyr	Tyr	Tyr	Ser	Met	Glu	Tyr	Trp	Gly	Gln
			100					105					110		
Gly	Thr	Thr	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val
		115					120					125			
Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala
	130					135				140					
Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser
	145				150					155					160
Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val
				165					170					175	
Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro
			180					185					190		
Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys
		195					200					205			
Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	Lys	Ser	Cys	Asp
	210					215					220				
Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly

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225	Pro	Ser	Val	Phe	230	Leu	235	Pro	Pro	Lys	235	Lys	Asp	Thr	Leu	240	Met	Ile
				245	Phe	Pro					250	Val	Val	Asp	Val	255	Ser	His
	Ser	Arg	Thr	260	Pro	Val	Thr	Cys	Val	265	Val	Val	Asp	Val	270	Glu	Val	His
	Asp	Pro	Glu	275	Val	Lys	Phe	Asn	Trp	280	Tyr	Val	Asp	Gly	285	Val		
	Asn	Ala	Lys	290	Thr	Lys	Pro	Arg	Glu	295	Glu	Gln	Tyr	Asn	300	Ser	Thr	Tyr
	Val	Val	Ser	305	Val	Leu	Thr	Val	Leu	310	His	Gln	Asp	Trp	315	Leu	Asn	Gly
	Glu	Tyr	Lys	325	Cys	Lys	Val	Ser	Asn	330	Lys	Ala	Leu	Pro	335	Ala	Pro	Ile
	Lys	Thr	Ile	340	Ser	Lys	Ala	Lys	Gly	345	Gln	Pro	Arg	Glu	350	Pro	Gln	Val
	Thr	Leu	Pro	355	Pro	Ser	Arg	Glu	Glu	360	Met	Thr	Lys	Asn	365	Gln	Val	Ser
	Thr	Cys	Leu	370	Val	Lys	Gly	Phe	Tyr	375	Pro	Ser	Asp	Ile	380	Ala	Val	Glu
	Glu	Ser	Asn	385	Gly	Gln	Pro	Glu	Asn	390	Asn	Tyr	Lys	Thr	395	Thr	Pro	Pro
	Leu	Asp	Ser	405	Asp	Gly	Ser	Phe	Phe	410	Leu	Tyr	Ser	Lys	415	Leu	Thr	Val
	Lys	Ser	Arg	420	Trp	Gln	Gln	Gly	Asn	425	Val	Phe	Ser	Cys	430	Ser	Val	Met
	Glu	Ala	Leu	435	His	Asn	His	Tyr	Thr	440	Gln	Lys	Ser	Leu	445	Ser	Leu	Ser
	Gly																	

<210> 76

<211> 213

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 76

Glu	Ile	Val	Leu	Thr	Gln	Ser	Pro	Ala	Thr	Leu	Ser	Leu	Ser	Pro	Gly
1				5					10					15	
Glu	Arg	Ala	Thr	Leu	Ser	Cys	Ser	Ala	Thr	Ser	Ser	Val	Ser	Tyr	Met
			20					25					30		
His	Trp	Phe	Gln	Gln	Lys	Pro	Gly	Gln	Ala	Pro	Arg	Leu	Leu	Ile	Tyr
		35					40					45			
Ser	Thr	Ser	Asn	Leu	Ala	Ser	Gly	Ile	Pro	Ala	Arg	Phe	Ser	Gly	Ser
		50				55				60					
Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Glu	Pro	Glu
65				70					75					80	
Asp	Phe	Ala	Val	Tyr	Tyr	Cys	Gln	Gln	Arg	Ser	Ser	Tyr	Pro	Phe	Thr
			85						90				95		
Phe	Gly	Pro	Gly	Thr	Lys	Leu	Asp	Ile	Lys	Arg	Thr	Val	Ala	Ala	Pro
		100						105					110		
Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	Leu	Lys	Ser	Gly	Thr
		115					120					125			
Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	Pro	Arg	Glu	Ala	Lys
		130				135					140				
Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser	Gly	Asn	Ser	Gln	Glu
145					150				155					160	
Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr	Tyr	Ser	Leu	Ser	Ser
			165						170					175	
Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys	His	Lys	Val	Tyr	Ala
			180					185					190		
Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro	Val	Thr	Lys	Ser	Phe
		195					200					205			
Asn	Arg	Gly	Glu	Cys											
		210													

701712000340SEQLI ST

<210> 77
 <211> 213
 <212> PRT
 <213> Arti f i c i a l Sequence

<220>
 <223> Syntheti c Construct

<400> 77
 Gl u I l e Val Leu Thr G l n Ser Pro Al a Thr Leu Ser Leu Ser Pro Gl y
 1 5 10 15
 Gl u Arg Al a Thr Leu Ser Cys Ser Al a Thr Ser Ser Val Ser Tyr Met
 20 25 30
 Hi s Trp Phe G l n G l n Lys Pro Gly G l n Al a Pro Arg Leu Leu I l e Tyr
 35 40 45
 Ser Thr Ser Asn Leu Al a Ser Gly I l e Pro Al a Arg Phe Ser Gly Ser
 50 55 60
 Gly Ser Gly Thr Asp Tyr Thr Leu Thr I l e Ser Ser Leu Gl u Pro Gl u
 65 70 75 80
 Asp Phe Al a Val Tyr Tyr Cys G l n G l n Arg Ser Ser Tyr Pro Phe Thr
 85 90 95
 Phe Gly Pro Gly Thr Lys Leu Asp I l e Lys Arg Thr Val Al a Al a Pro
 100 105 110
 Ser Val Phe I l e Phe Pro Pro Ser Asp Gl u G l n Leu Lys Ser Gly Thr
 115 120 125
 Al a Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Gl u Al a Lys
 130 135 140
 Val G l n Trp Lys Val Asp Asn Al a Leu G l n Ser Gly Asn Ser G l n Gl u
 145 150 155 160
 Ser Val Thr Gl u G l n Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
 165 170 175
 Thr Leu Thr Leu Ser Lys Al a Asp Tyr Gl u Lys Hi s Lys Val Tyr Al a
 180 185 190
 Cys Gl u Val Thr Hi s G l n Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
 195 200 205
 Asn Arg Gly Gl u Cys
 210

<210> 78
 <211> 329
 <212> PRT
 <213> Homo sapi ens

<400> 78
 Al a Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Al a Pro Ser Ser Lys
 1 5 10 15
 Ser Thr Ser Gly Gly Thr Al a Al a Leu Gly Cys Leu Val Lys Asp Tyr
 20 25 30
 Phe Pro Gl u Pro Val Thr Val Ser Trp Asn Ser Gly Al a Leu Thr Ser
 35 40 45
 Gly Val Hi s Thr Phe Pro Al a Val Leu G l n Ser Ser Gly Leu Tyr Ser
 50 55 60
 Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr G l n Thr
 65 70 75 80
 Tyr I l e Cys Asn Val Asn Hi s Lys Pro Ser Asn Thr Lys Val Asp Lys
 85 90 95
 Arg Val Gl u Pro Lys Ser Cys Asp Lys Thr Hi s Thr Cys Pro Pro Cys
 100 105 110
 Pro Al a Pro Gl u Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
 115 120 125
 Lys Pro Lys Asp Thr Leu Met I l e Ser Arg Thr Pro Gl u Val Thr Cys
 130 135 140
 Val Val Val Asp Val Ser Hi s Gl u Asp Pro Gl u Val Lys Phe Asn Trp
 145 150 155 160
 Tyr Val Asp Gly Val Gl u Val Hi s Asn Al a Lys Thr Lys Pro Arg Gl u

701712000340SEQLI ST

				165					170					175		
Gl u	Gl n	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	
			180					185					190			
Hi s	Gl n	Asp	Trp	Leu	Asn	Gl y	Lys	Gl u	Tyr	Lys	Cys	Lys	Val	Ser	Asn	
		195					200					205				
Lys	Al a	Leu	Pro	Al a	Pro	I l e	Gl u	Lys	Thr	I l e	Ser	Lys	Al a	Lys	Gl y	
	210					215					220					
Gl n	Pro	Arg	Gl u	Pro	Gl n	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Gl u	Gl u	
225					230					235					240	
Met	Thr	Lys	Asn	Gl n	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gl y	Phe	Tyr	
			245						250					255		
Pro	Ser	Asp	I l e	Al a	Val	Gl u	Trp	Gl u	Ser	Asn	Gl y	Gl n	Pro	Gl u	Asn	
		260						265					270			
Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gl y	Ser	Phe	Phe	
		275					280					285				
Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gl n	Gl n	Gl y	Asn	
	290					295					300					
Val	Phe	Ser	Cys	Ser	Val	Met	Hi s	Gl u	Al a	Leu	Hi s	Asn	Hi s	Tyr	Thr	
305					310					315					320	
Gl n	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gl y								
				325												

<210> 79
 <211> 326
 <212> PRT
 <213> Homo sapi ens

<400> 79

Al a	Ser	Thr	Lys	Gl y	Pro	Ser	Val	Phe	Pro	Leu	Al a	Pro	Cys	Ser	Arg	
1				5				10					15			
Ser	Thr	Ser	Gl u	Ser	Thr	Al a	Al a	Leu	Gl y	Cys	Leu	Val	Lys	Asp	Tyr	
			20					25					30			
Phe	Pro	Gl u	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gl y	Al a	Leu	Thr	Ser	
		35				40						45				
Gl y	Val	Hi s	Thr	Phe	Pro	Al a	Val	Leu	Gl n	Ser	Ser	Gl y	Leu	Tyr	Ser	
	50					55					60					
Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gl y	Thr	Lys	Thr	
65					70					75					80	
Tyr	Thr	Cys	Asn	Val	Asp	Hi s	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	
			85						90					95		
Arg	Val	Gl u	Ser	Lys	Tyr	Gl y	Pro	Pro	Cys	Pro	Pro	Cys	Pro	Al a	Pro	
			100					105					110			
Gl u	Phe	Leu	Gl y	Gl y	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	
		115					120					125				
Asp	Thr	Leu	Met	I l e	Ser	Arg	Thr	Pro	Gl u	Val	Thr	Cys	Val	Val	Val	
	130					135					140					
Asp	Val	Ser	Gl n	Gl u	Asp	Pro	Gl u	Val	Gl n	Phe	Asn	Trp	Tyr	Val	Asp	
145					150					155					160	
Gl y	Val	Gl u	Val	Hi s	Asn	Al a	Lys	Thr	Lys	Pro	Arg	Gl u	Gl u	Gl n	Phe	
				165					170					175		
Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	Hi s	Gl n	Asp	
		180						185					190			
Trp	Leu	Asn	Gl y	Lys	Gl u	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Gl y	Leu	
		195					200					205				
Pro	Ser	Ser	I l e	Gl u	Lys	Thr	I l e	Ser	Lys	Al a	Lys	Gl y	Gl n	Pro	Arg	
	210					215					220					
Gl u	Pro	Gl n	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Gl n	Gl u	Gl u	Met	Thr	Lys	
225					230					235					240	
Asn	Gl n	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gl y	Phe	Tyr	Pro	Ser	Asp	
				245					250					255		
I l e	Al a	Val	Gl u	Trp	Gl u	Ser	Asn	Gl y	Gl n	Pro	Gl u	Asn	Asn	Tyr	Lys	
		260						265					270			
Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gl y	Ser	Phe	Phe	Leu	Tyr	Ser	
		275					280					285				
Arg	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gl n	Gl u	Gl y	Asn	Val	Phe	Ser	
	290					295					300					
Cys	Ser	Val	Met	Hi s	Gl u	Al a	Leu	Hi s	Asn	Hi s	Tyr	Thr	Gl n	Lys	Ser	

305
Leu Ser Leu Ser Leu Gly
325

315

320

<210> 80
<211> 107
<212> PRT
<213> Homo sapiens

<400> 80
Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
1 5 10 15
Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
20 25 30
Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
35 40 45
Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
50 55 60
Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
65 70 75 80
Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
85 90 95
Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
100 105

<210> 81
<211> 444
<212> PRT
<213> Mus musculus

<400> 81
Gln Val Gln Leu Lys Arg Ala Ser Gly Pro Gly Leu Val Ala Pro Ser
1 5 10 15
Gln Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ile
20 25 30
Tyr Gly Ala His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp
35 40 45
Leu Gly Val Ile Trp Ala Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu
50 55 60
Met Ser Arg Leu Ser Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Phe
65 70 75 80
Leu Lys Ile Asn Ser Leu Gln Thr Asp Asp Thr Ala Leu Tyr Tyr Cys
85 90 95
Ala Arg Asp Gly Ser Ser Pro Tyr Tyr Tyr Ser Met Glu Tyr Trp Gly
100 105 110
Gln Gly Thr Ser Val Thr Val Ser Ser Ala Lys Thr Thr Pro Pro Ser
115 120 125
Val Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn Ser Met Val
130 135 140
Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Val
145 150 155 160
Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala
165 170 175
Val Leu Glu Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Pro
180 185 190
Ser Ser Pro Arg Pro Ser Glu Thr Val Thr Cys Asn Val Ala His Pro
195 200 205
Ala Ser Ser Thr Lys Val Asp Lys Lys Ile Val Pro Arg Asp Cys Gly
210 215 220
Cys Lys Pro Cys Ile Cys Thr Val Pro Glu Val Ser Ser Val Phe Ile
225 230 235 240
Phe Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr Pro Lys
245 250 255
Val Thr Cys Val Val Val Asp Ile Ser Lys Asp Asp Pro Glu Val Gln
260 265 270
Phe Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala Gln Thr Gln

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Pro	Arg	275	Glu	Glu	Gln	Phe	Asn	280	Ser	Thr	Phe	Arg	Ser	285	Val	Ser	Glu	Leu
Pro	Ile	290	Met	His	Gln	Asp	Trp	295	Leu	Asn	Gly	Lys	Glu	300	Phe	Lys	Cys	Arg
305	Val	Asn	Ser	Ala	Ala	310	Phe	Pro	Ala	Pro	Ile	Glu	Lys	315	Thr	Ile	Ser	Lys
Thr	Lys	Gly	Arg	325	Pro	Lys	Ala	Pro	Gln	330	Val	Tyr	Thr	Ile	Pro	Pro	Pro	Pro
Lys	Glu	Gln	Met	Ala	Lys	Asp	Lys	345	Val	Ser	Leu	Thr	Cys	350	Met	Ile	Thr	Thr
Asp	Phe	355	Phe	Pro	Glu	Asp	Ile	360	Thr	Val	Glu	Trp	Gln	365	Trp	Asn	Gly	Gln
Pro	Ala	Glu	Asn	Tyr	Lys	375	Asn	Thr	Gln	Pro	Ile	Met	Asn	380	Thr	Asn	Gly	400
385	Ser	Tyr	Phe	Val	Tyr	390	Ser	Lys	Leu	Asn	Val	Gln	Lys	395	Ser	Asn	Trp	Glu
Ala	Gly	Asn	Thr	Phe	Thr	405	Cys	Ser	Val	410	Leu	His	Glu	Gly	Leu	His	Asn	415
His	His	Thr	Glu	Lys	Ser	Leu	Ser	425	His	Ser	Pro	Gly		430				
		435						440										

<210> 82
 <211> 212
 <212> PRT
 <213> Mus musculus

<400> 82

Glu	Ile	Ile	Leu	Thr	Gln	Ser	Pro	Ala	Ile	Met	Ser	Ala	Ser	Pro	Gly
1				5					10					15	
Glu	Lys	Val	Ser	Ile	Thr	Cys	Ser	Ala	Thr	Ser	Ser	Val	Ser	Tyr	Met
			20					25					30		
His	Trp	Phe	Gln	Gln	Lys	Pro	Gly	Thr	Ser	Pro	Lys	Leu	Trp	Ile	Tyr
		35					40					45			
Ser	Thr	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro	Val	Arg	Phe	Ser	Gly	Ser
		50				55					60				
Gly	Ser	Gly	Thr	Ser	Tyr	Ser	Leu	Thr	Ile	Ser	Arg	Met	Glu	Ala	Glu
65					70					75					80
Asp	Ala	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Arg	Ser	Ser	Tyr	Pro	Phe	Thr
				85					90					95	
Phe	Gly	Ser	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Ala	Asp	Ala	Ala	Pro	Thr
			100					105					110		
Val	Ser	Ile	Phe	Pro	Pro	Ser	Ser	Glu	Gln	Leu	Thr	Ser	Gly	Gly	Ala
		115					120					125			
Ser	Val	Val	Cys	Phe	Leu	Asn	Asn	Phe	Tyr	Pro	Lys	Asp	Ile	Asn	Val
		130				135					140				
Lys	Trp	Lys	Ile	Asp	Gly	Ser	Glu	Arg	Gln	Asn	Gly	Val	Leu	Asn	Ser
145					150					155					160
Trp	Thr	Asp	Gln	Asp	Ser	Lys	Asp	Ser	Thr	Tyr	Ser	Met	Ser	Ser	Thr
				165					170					175	
Leu	Thr	Leu	Thr	Lys	Asp	Glu	Tyr	Glu	Arg	His	Asn	Ser	Tyr	Thr	Cys
			180					185					190		
Glu	Ala	Thr	His	Lys	Thr	Ser	Thr	Ser	Pro	Ile	Val	Lys	Ser	Phe	Asn
		195					200					205			
Arg	Asn	Glu	Cys												
		210													

<210> 83
 <211> 212
 <212> PRT
 <213> Mus musculus

<400> 83

Gln	Ile	Ile	Leu	Thr	Gln	Ser	Pro	Ala	Ile	Met	Ser	Ala	Ser	Pro	Gly
1				5					10					15	
Glu	Lys	Val	Ser	Ile	Thr	Cys	Ser	Ala	Thr	Ser	Ser	Val	Ser	Tyr	Met

701712000340SEQLIST

Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His
			260					265					270		
Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val
		275					280					285			
His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr
	290					295					300				
Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly
305					310					315					320
Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile
				325					330					335	
Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val
		340						345					350		
Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	
		355					360				365				
Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu
	370					375					380				
Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro
385					390					395					400
Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val
				405					410					415	
Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met
			420					425					430		
His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser
		435					440					445			
Pro	Gly														
	450														

<210> 85
 <211> 213
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 85

Glu	Ile	Ile	Leu	Thr	Gln	Ser	Pro	Ala	Ile	Met	Ser	Ala	Ser	Pro	Gly
1				5					10					15	
Glu	Lys	Val	Ser	Ile	Thr	Cys	Ser	Ala	Thr	Ser	Ser	Val	Ser	Tyr	Met
			20					25					30		
His	Trp	Phe	Gln	Gln	Lys	Pro	Gly	Thr	Ser	Pro	Lys	Leu	Trp	Ile	Tyr
		35					40					45			
Ser	Thr	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro	Val	Arg	Phe	Ser	Gly	Ser
	50					55					60				
Gly	Ser	Gly	Thr	Ser	Tyr	Ser	Leu	Thr	Ile	Ser	Arg	Met	Glu	Ala	Glu
65					70				75					80	
Asp	Ala	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Arg	Ser	Ser	Tyr	Pro	Phe	Thr
				85					90					95	
Phe	Gly	Ser	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg	Thr	Val	Ala	Ala	Pro
			100					105					110		
Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	Leu	Lys	Ser	Gly	Thr
		115					120					125			
Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	Pro	Arg	Glu	Ala	Lys
	130					135					140				
Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser	Gly	Asn	Ser	Gln	Glu
145					150					155				160	
Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr	Tyr	Ser	Leu	Ser	Ser
				165					170					175	
Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys	His	Lys	Val	Tyr	Ala
			180					185					190		
Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro	Val	Thr	Lys	Ser	Phe
		195					200					205			
Asn	Arg	Gly	Glu	Cys											
	210														

<210> 86

<211> 213
<212> PRT
<213> Arti fi ci al Sequence

<220>
<223> Synthetic Construct

[illegible]

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<210> 87
<211> 446
<212> PRT
<213> Arti fi ci al Sequence
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<220>
<223> Synthetic Construct

<400>	87															
Glu 1	Val	Gln	Leu	Val 5	Glu	Ser	Gly	Gly	Gly 10	Leu	Val	Gln	Pro	Gly 15	Gly	
Ser	Leu	Arg	Leu 20	Ser	Cys	Ala	Ala	Ser 25	Gly	Phe	Ser	Leu	Thr 30	Ile	Tyr	
Gly	Ala	His 35	Trp	Val	Arg	Gln	Ala 40	Pro	Gly	Lys	Gly	Leu 45	Glu	Trp	Val	
Gly	Val 50	Ile	Trp	Ala	Gly	Gly 55	Ser	Thr	Asn	Tyr	Asn 60	Ser	Ala	Leu	Met	
Ser 65	Arg	Phe	Thr	Ile	Ser 70	Lys	Asp	Asn	Ser	Lys 75	Asn	Thr	Val	Tyr	Leu 80	
Gln	Met	Asn	Ser	Leu 85	Arg	Ala	Glu	Asp	Thr 90	Ala	Val	Tyr	Tyr	Cys 95	Ala	
Arg	Asp	Gly	Ser 100	Ser	Pro	Tyr	Tyr	Tyr 105	Ser	Met	Glu	Tyr	Trp 110	Gly	Gln	
Gly	Thr	Thr 115	Val	Thr	Val	Ser	Ser 120	Ala	Ser	Thr	Lys	Gly 125	Pro	Ser	Val	
Phe	Pro 130	Leu	Ala	Pro	Cys	Ser 135	Arg	Ser	Thr	Ser	Glu 140	Ser	Thr	Ala	Ala	
Leu 145	Gly	Cys	Leu	Val	Lys 150	Asp	Tyr	Phe	Pro	Glu 155	Pro	Val	Thr	Val	Ser 160	
Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	

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Leu	Gln	Ser	Ser	165 Gly	Leu	Tyr	Ser	Leu	170 Ser	Ser	Val	Val	Thr	175 Val	Pro
Ser	Ser	Ser	Leu	180 Gly	Thr	Lys	Thr	Tyr	185 Thr	Cys	Asn	Val	190 Asp	His	Lys
Pro	Ser	Asn	Thr	195 Lys	Val	Asp	Lys	Arg	200 Val	Glu	Ser	205 Lys	Tyr	Gly	Pro
Pro	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Phe	Leu	Gly	Gly	Pro	Ser	Val
225 Phe	Leu	Phe	Pro	230 Pro	Lys	Pro	Lys	Asp	235 Thr	Leu	Met	Ile	Ser	Arg	Thr
Pro	Glu	Val	Thr	245 Cys	Val	Val	Val	Asp	250 Val	Ser	Gln	Glu	Asp	255 Pro	Glu
Val	Gln	Phe	Asn	260 Trp	Tyr	Val	Asp	Gly	265 Val	Glu	Val	His	Asn	Ala	Lys
Thr	Lys	Pro	Arg	275 Glu	Glu	Gln	Phe	Asn	280 Ser	Thr	Tyr	Arg	Val	Val	Ser
Val	Leu	Thr	Val	290 Leu	His	Gln	Asp	Trp	295 Leu	Asn	Gly	Lys	Glu	Tyr	Lys
305 Cys	Lys	Val	Ser	310 Asn	Lys	Gly	Leu	Pro	315 Ser	Ser	Ile	Glu	Lys	Thr	Ile
Ser	Lys	Ala	Lys	325 Gly	Gln	Pro	Arg	Glu	330 Pro	Gln	Val	Tyr	Thr	Leu	Pro
Pro	Ser	Gln	Glu	340 Glu	Met	Thr	Lys	Asn	345 Gln	Val	Ser	Leu	Thr	Cys	Leu
Val	Lys	Gly	Phe	355 Tyr	Pro	Ser	Asp	Ile	360 Ala	Val	Glu	Trp	Glu	Ser	Asn
Gly	Gln	Pro	Glu	370 Asn	Asn	Tyr	Lys	Thr	375 Thr	Pro	Pro	Val	Leu	Asp	Ser
385 Asp	Gly	Ser	Phe	390 Phe	Leu	Tyr	Ser	Arg	395 Leu	Thr	Val	Asp	Lys	Ser	Arg
Trp	Gln	Glu	Gly	405 Asn	Val	Phe	Ser	Cys	410 Ser	Val	Met	His	Glu	Ala	Leu
His	Asn	His	Tyr	420 Thr	Gln	Lys	Ser	Leu	425 Ser	Leu	Ser	Leu	Gly		
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 Ala Met Ser Trp Val Arg Gln Thr Pro Asp Lys Arg Leu Trp Val
 35 40 45
 Ala Ile Ile Ser Ser Gly Gly Ser Tyr Thr Tyr Tyr Ser Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr Tyr Cys
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 Ala Arg His Glu Thr Ala Gln Ala Ala Trp Phe Ala Tyr Trp Gly Gln
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 Gly Thr Leu Val Thr Val Ser Ala
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 35 40 45
 Gly Arg Ile Ala Pro Glu Asp Gly Asp Thr Glu Tyr Ala Pro Lys Phe
 50 55 60
 Gln Gly Lys Ala Thr Val Thr Ala Asp Thr Ser Ser Asn Thr Ala Tyr
 65 70 75 80
 Leu His Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Thr Thr Glu Gly Asn Tyr Tyr Gly Ser Ser Ile Leu Asp Tyr Trp Gly
 100 105 110
 Gln Gly Thr Thr Leu Thr Val Ser Ser
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 20 25 30
 Trp Met Asn Trp Val Lys Gln Arg Pro Gly Lys Gly Leu Glu Trp Ile
 35 40 45
 Gly Gln Ile Tyr Pro Gly Asp Asp Tyr Thr Asn Tyr Asn Gly Lys Phe
 50 55 60
 Lys Gly Lys Val Thr Leu Thr Ala Asp Arg Ser Ser Thr Ala Tyr
 65 70 75 80
 Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
 85 90 95
 Ala Arg Leu Gly Pro Tyr Gly Pro Phe Ala Asp Trp Gly Gln Gly Thr
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 Leu Val Thr Val Ser Ala
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 His Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr
 35 40 45
 Asp Thr Ser Lys Leu Ala Tyr Gly Val Pro Ala Arg Phe Ser Gly Ser
 50 55 60
 Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu
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 Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
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 20 25 30
 Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Ile
 35 40 45
 Tyr Phe Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60
 Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu Gln
 65 70 75 80
 Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Trp
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 Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
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 20 25 30
 Tyr Trp Tyr Gln Gln Arg Pro Gly Ser Ser Pro Arg Leu Leu Ile Tyr
 35 40 45
 Asp Thr Ser Ser Leu Ala Ser Gly Val Pro Val Arg Phe Ser Gly Ser
 50 55 60
 Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Ile Glu Ser Glu
 65 70 75 80
 Asp Ala Ala Asn Tyr Tyr Cys Gln Gln Trp Asn Ser Asp Pro Tyr Thr
 85 90 95
 Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
 100 105