

IL-33 BINDING ANTIBODIES

SEQUENCE LISTING SUBMITTED ELECTRONICALLY

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5 and was created on or about October 30, 2024. The sequence listing submitted electronically is part of the specification and is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

The present disclosure relates to the treatment of interleukin 33 (IL-33) mediated diseases, including respiratory diseases. In particular, the present disclosure relates to IL-33
10 binding proteins, including anti-IL-33 antibodies, and their uses in the treatment of IL-33 mediated diseases.

BACKGROUND TO THE INVENTION

IL-33 plays a role in a number of different diseases including, but not limited to, chronic obstructive pulmonary disease (COPD), asthma, bronchitis, bronchiolitis, acute respiratory
15 failure, inflammatory lung diseases, diabetic kidney disease, endometriosis, chronic rhinosinusitis with nasal polyps, food hypersensitivity, peanut allergy, allergic rhinitis, eosinophilic oesophagitis, atopic dermatitis, cystic fibrosis, and chronic urticaria. These serious diseases affect hundreds of millions of people worldwide.

One example of an IL-33 mediated disease is Chronic Obstructive Pulmonary Disease
20 (COPD), which is a lung disease characterized by persistent respiratory symptoms caused by airway or alveoli abnormalities. COPD is one of the top three leading causes of death in the world (Global Initiative for Chronic Obstructive Lung Disease (GOLD): Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease (2024 Report), available at goldcopd.org/wp-content/uploads/2024/02/GOLD-2024_v1.2-11Jan24_WMV.pdf (last accessed November 8, 2024)). It is estimated that globally three million
25 deaths occur annually due to COPD, and it is projected that 5.4 million annual deaths will occur from COPD and related conditions by 2060. COPD is projected to increase globally due to the population aging and continuing exposure to COPD risk factors. Many individuals suffer with

COPD or its complications for years prior to death. Thus, COPD is a global health challenge that requires both prevention and treatment.

COPD is a progressive disease that is not fully reversible. COPD results in periodic exacerbations that may result in long term disability or mortality, reducing the quality of life of those who suffer with the disease. This creates a significant economic burden, with projected costs of \$40 billion per year in the United States alone (GOLD 2024).

To prevent these periodic acute exacerbations, many patients with COPD use long-term bronchodilator maintenance therapy. However, there is a substantial unmet need in COPD patients who continue to exacerbate despite maximal bronchodilator maintenance therapy. In the IMPACT study, despite optimizing patients with dual or triple therapy, almost half of the patients continued to experience exacerbations (Bardsley et al., Respir Med. 2022 Dec;205:107040). Thus, there is a need for new therapies for the treatment and/or prevention of COPD, as well as the reduction of acute exacerbations of COPD (AECOPD).

SUMMARY OF THE INVENTION

In a first aspect of the invention, the present disclosure provides an IL-33 binding protein comprising:

- (i) CDRH1, CDRH2, and CDRH3 from SEQ ID NO:20 and CDRL1, CDRL2, and CDRL3 from SEQ ID NO:25, wherein SEQ ID NO:20 comprises:

QVQLVQSGAEVKKPGASVKVSCKASGYTFISYGMHWVRQAPGQGLEW
MGEINPHGGSTSYAQKF₁GRVTMTRDTSTSTVYMELSSLRSED₁AV
YYCARPSAAYSHYLGX₂DX₃WGRGTLVTVSS; and

wherein SEQ ID NO:25 comprises:

DIQMTQSPSSVSASVGDRVTITCRX₄SQGIX₅WLX₆WYQQKPGKAPK
LLIYAX₇SX₈LQSGVPSRFSGSGSGTDFTLTIS₁SLQPEDFATYYCQQA
X₉VX₁₀PLTFGGG₁TKVEIK; and

wherein:

X₁ = K or Q; X₂ = I or L; X₃ = I, L, or M; X₄ = A or T; X₅ = P or S; X₆ = A or S; X₇ = A or G; X₈ = R or S; X₉ = H or N; and X₁₀ = F or L; or

- (ii) one or more CDR variants of (i), wherein the variant has 1, 2, or 3 amino acid modifications.

In a second aspect of the invention, the present disclosure provides an IL-33 binding protein comprising:

- 5 (i) CDRH1, CDRH2, and CDRH3 from any one of SEQ ID NOs:21-24 and CDRL1, CDRL2, and CDRL3 from any one of SEQ ID NOs:26-28; or
- (ii) one or more CDR variants of (i), wherein the variant has 1, 2, or 3 amino acid modifications.

10 In a third aspect of the invention, the present disclosure provides an IL-33 binding protein comprising a heavy chain (HC) having at least 90% identity to any one of SEQ ID NOs:29-33 and a light chain (LC) having at least 90% identity to any one of SEQ ID NOs:34-37, wherein SEQ ID NO:29 comprises:

15 QVQLVQSGAEVKKPGASVKVSCKASGYTFISYGMHWVRQAPGQGLEWMGEIN
PHGGSTSYAQKFX₁GRVTMTRDTSTSTVYMESSLRSEDTAVYYCARPSAAY
SHYLGX₂DX₃WGRGTLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY
FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN
VNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLYI
TREPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE
20 LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSK
LTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK; and

wherein SEQ ID NO:34 comprises:

25 DIQMTQSPSSVSASVGDRVTITCRX₄SQGISX₅WLX₆WYQQKPGKAPKLLIYA
X₇SX₈LQSGVPSRFRSGSGSDFTLTITSSLPEDFATYYCQQA₉VX₁₀PLTFG
GGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD
NALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSS
PVTKSFNRGEC; and

wherein:

30 X₁ = K or Q; X₂ = I or L; X₃ = I, L, or M; X₄ = A or T;
X₅ = P or S; X₆ = A or S; X₇ = A or G; X₈ = R or S; X₉ =
H or N; and X₁₀ = F or L.

In a fourth aspect of the invention, the present disclosure provides a pharmaceutical composition comprising the IL-33 binding protein as defined in any one of the above aspects or embodiments of the invention and a pharmaceutically acceptable excipient.

5 In a fifth aspect of the invention, the present disclosure provides a method of treating or preventing a disease or condition in a human in need thereof comprising administering to the human a therapeutically effective amount of the IL-33 binding protein of any one of the first three aspects of the invention and corresponding embodiments, or the pharmaceutical composition of the fourth aspect of the invention.

10 In a sixth aspect of the invention, the present disclosure provides an IL-33 binding protein of any one of the first three aspects of the invention and corresponding embodiments, or a pharmaceutical composition of the fourth aspect of the invention, for use in treating or preventing a disease or condition.

15 In a seventh aspect of the invention, the present disclosure provides use of the IL-33 binding protein of any one of the first three aspects of the invention and corresponding embodiments, or a pharmaceutical composition of the fourth aspect of the invention, in the manufacture of a medicament for treating or preventing a disease or condition.

In an eighth aspect of the invention, the present disclosure provides a nucleic acid sequence or plurality of nucleic acid sequences encoding an IL-33 binding protein according to any one of the first three aspects of the invention and corresponding embodiments.

20 In a ninth aspect of the invention, the present disclosure provides nucleic acid sequence or plurality of nucleic acid sequences comprising any one of SEQ ID NOs:59-64 and/or any one of SEQ ID NOs:69-76.

25 In a tenth aspect of the invention, the present disclosure provides a nucleic acid sequence or plurality of nucleic acid sequences comprising any one of SEQ ID NOs:65-68 and/or any one of SEQ ID NOs:77-79.

In an eleventh aspect of the invention, the present disclosure provides a nucleic acid sequence or plurality of nucleic acid sequences comprising SEQ ID NO:66 and/or SEQ ID NO:77.

30 In a twelfth aspect of the invention, the present disclosure provides an expression vector comprising the nucleic acid sequence or plurality of nucleic acid sequences of the eighth, ninth, tenth, or eleventh aspects of the invention.

In a thirteenth aspect of the invention, the present disclosure provides a host cell that comprises the nucleic acid sequence or plurality of nucleic acids of any one of the eighth, ninth, tenth, or eleventh aspects of the invention, or the expression vector of the twelfth aspect of the invention.

5 In a fourteenth aspect of the invention, the present disclosure provides a method of producing an IL-33 binding protein, comprising culturing the host cell as defined in the thirteenth aspect of the invention under conditions suitable for expression of said nucleic acid sequence, plurality of nucleic acid sequences, or vector, whereby a polypeptide comprising the IL-33 binding protein is produced.

10 In a fifteenth aspect of the invention, the present disclosure provides the IL-33 binding protein produced by the method of the fourteenth aspect of the invention.

DESCRIPTION OF FIGURES

Figure 1 illustrates eosinophil superoxide production after stimulation with IL-33 pre-complexed with an IL-33 binding protein.

15 **Figure 2** illustrates CD4⁺ T cell IFN- γ secretion after co-stimulation with IL-2 + IL-12 + IL-33 pre-complexed with an IL-33 binding protein.

Figure 3A illustrates inhibition of HUVEC IL-8 secretion by an IL-33 binding protein pre-complexed with IL-33.

20 **Figure 3B** illustrates inhibition of HUVEC IL-6 secretion by an IL-33 binding protein pre-complexed with IL-33.

Figure 4 illustrates inhibition of basophil β -hexosaminidase release after stimulation with IL-33 pre-complexed with an IL-33 binding protein and cross linked IgE (anti-IgE).

Figure 5 illustrates mouse, rat, and human IL-33 induced cytokine production from mouse mast cells for TNF- α (Panel A), IL-6 (Panel B), IL-13 (Panel C), and IL-18 (Panel D).

25 **Figure 6** illustrates inhibition of 1ng/mL IL-33 induced cytokine production by an IL-33 binding protein for TNF- α (Panel A), IL-6 (Panel B), IL-13 (Panel C), and IL-18 (Panel D).

Figure 7 illustrates inhibition of 10ng/mL IL-33 induced cytokine production by an IL-33 binding protein for TNF- α (Panel A), IL-6 (Panel B), IL-13 (Panel C), and IL-18 (Panel D).

30 **Figure 8** illustrates inhibition of cyno IL-33 stimulated HEK-BLUE cell activation by cyno PK serum and an IL-33 binding protein for cynomolgus 1 (Panel A), cynomolgus 2 (Panel B),

cynomolgus 3 (Panel C), cynomolgus 4 (Panel D), cynomolgus 5 (Panel E), and cynomolgus 6 (Panel F).

Figure 9 illustrates inhibition of rhu-IL-33 stimulated HEK-BLUE cell activation by cyno PK serum and an IL-33 binding protein for cynomolgus 2 (Panel A), cynomolgus 3 (Panel B),
5 cynomolgus 4 (Panel C), cynomolgus 5 (Panel D), and cynomolgus 6 (Panel E).

Figure 10 illustrates a refined structure of a fAb-IL-33-Nanobody complex (Panel A) and a Cryo-EM map superimposed onto the structure in Panel A (Panel B).

Figure 11 illustrates a comparison of Cryo-EM epitope and paratope in comparison with HDX data.

10 **Figure 12** illustrates a comparison of Cryo-EM structure with crystal structure 4KC3.

Figure 13 illustrates a comparison of Cryo-EM structure with crystal structure 5VI4.

DETAILED DESCRIPTION OF THE INVENTION

Interleukin 33 (IL-33) is an alarmin and a pleiotropic cytokine that is released by epithelium, endothelium, and other cell types following damage or infection (Cayrol and Girard,
15 Cytokine, 2022, 156, 155891). IL-33 promotes inflammation through binding to its receptor (transmembrane ST2) which is present on multiple cells including endothelial cells, type 2 innate lymphoid cells (ILC2s), mast cells, myeloid cells, natural killer (NK) cells, T-cells, NK T-cells, and basophils (Calderon et al., Eur Respir Rev, 2023 32(167), 220144, Erratum in: Eur Respir Rev, 2023, 32(168)). IL-33 promotes the production of cytokines associated with Type 1 (e.g.,
20 interferon gamma, IL-6, IL-8) and Type 2 (e.g., IL-4, IL-5, IL-13) immune responses resulting in further immune cell recruitment to sites of inflammation (Afferni et al., Front Immunol., 2018, 13, 9, 2601; Calderon et al., Eur Respir Rev., 2023, 32(167), 220144, Erratum in: Eur Respir Rev., 2023, 32(168); Yagami et al., J Immunol., 2010, 185(10), 5743-50). Therefore, IL-33 potentially amplifies cytokine/chemokine production, inflammation, and tissue damage. IL-33
25 has also been implicated as a mediator of eosinophil accumulation, maturation, and release from bone marrow by its effects on ILC2s (Johansson et al., Immunology, 2018, 153(2), 268-278; Johnston et al., J Immunol., 2016, 197(9), 3445-3453; Wu YH, et al., Allergy, 2020, 75(4), 818-830).

An increasing realization in the field of immunology is the importance of the role played
30 by mucosal epithelial cells. These cells have an important function as a barrier to the

environment, but they are also intimately associated with resident dendritic cells (DCs) that initiate adaptive immune responses. IL-33 has been shown to be one of the factors released by epithelial cells very early following airway stress that results in cellular damage. Once released, IL-33 has an important role instructing DCs to induce a Type 2 (T2) immune response and is a driving factor in the emerging concept of tissue-specific control of immunity. As IL-33 is upstream of the subsequent immune responses, it plays a role, as an alarmin, in translating this environmental stress to the subsequent innate and adaptive immune responses, being able to induce the full breadth of the T2 response. Antagonism of this response should therefore dampen the entire T2 response rather than individual elements of this response.

In humans, the IL33 gene is located on chromosome 9p24.1, encoding a full-length protein of 270 amino acids with a calculated molecular weight of 30.759 kDa. Under resting conditions, the full-length protein resides in the nucleus where it associates with histone complexes. IL-33 does not possess a signal peptide; therefore, release of IL-33 is thought to require a cell damage event, where the initial release of full-length IL-33-histone complexes occurs. This complex is rapidly cleaved by proteases such as calpain, neutrophil elastase, chymase, and cathepsin-G producing shorter isoforms of IL-33 which are more biologically active than the full-length version of the protein. IL-33 is released from the airway epithelium by all the key environmental stressors thought to have an impact on lung sensitivity in asthmatics, for example, respiratory (particularly viral) infections, allergens, and various pollutants, including smoke and other inhaled particulates.

In the extra-cellular environment, the IL-33 activity is controlled by a rapid oxidation event of the initial bioactive reduced form (Cohen et al., Nature Comms. 2015; 6:8327). This oxidation results from disulfide bonding in the core of the molecule inducing a significant conformational shift which renders the oxidized IL-33 (oxIL-33) form unable to bind ST2 and induce signaling. This is a relatively fast process in plasma where it is expected that the reduced form only has approximately a 90-minute half- life, while the oxidized IL-33 form is thought to be more stable. Studies using immunoassays specific for each form have shown that the predominant measurable form in asthma is the oxidized form where the reduced form is rarely detected, probably due to its fast transition kinetics (Cohen et al., Nature Comms. 2015; 6:8327). This highlights the complexity of the biology of IL-33 as it is likely that in vivo multiple forms of IL-33 exist, including the reduced form, bioactive fragments, and the oxidized form.

Tissue resident immune cells constitute the major targets of IL-33. IL-33 signaling occurs via a heterodimeric receptor composed of ST2 and IL1RacP. Many immune tissue resident cells express ST2 including mast cells, ILC2 cells, eosinophils, macrophages, DCs NK cells, and T-cell populations including Th2 and T-regs. IL-33 released into a tissue environment acts as an alarmin, amplifying key mechanisms that drive the T2 immune cascade that results in asthma immune pathology. For example, IL-33 primed mast cells increase their sensitivity to IgE driven degranulation, eosinophils are highly sensitive to activation by IL-33 which causes immediate degranulation, macrophages are primed towards an M2 phenotype, allergen specific T2 responses are amplified, and ILC2 cells proliferate and secrete large quantities of IL-5 and IL-13 in response to IL-33 (Chan et al., *Frontiers in Immunology*, 2019; 10: Article 364). While the influence of IL-33 is strongly linked to T2 inflammation, it is now clear that the action of IL-33 is not limited to the activation of type-2 immune responses. Indeed, recent studies have revealed important roles of IL-33 in the activation of immune cells involved in type-1 immunity, such as Th1 cells (Komai-Koma et al., 2016, *Immunobiology* 221(3): 412-417). IL-33 is only capable of stimulating IFN- γ production from human CD4⁺ T cells when in combination with IL-12, emphasising that the amplification role of IL-33 is dependent on the context of the inflammatory milieu.

In addition to numerous primary human cell-based functional assays, the important impact of the IL-33 pathway on immune responses has been extensively validated in mouse models of lung inflammation using IL-33 over-expression, administration of recombinant IL-33, IL-33/ST2 deficient mice, or anti-ST2/anti-IL-33 blocking antibodies (e.g., Ravanetti et al., *J Allergy Clin Immunol.* 2019, 143(4):1355-1370). In summary, the data from multiple studies are consistent that blocking the IL-33 pathway dampens lung inflammation and pathology.

DEFINITIONS

The term “about” as used herein means plus or minus 10%.

“Acceptor antibody” refers to an antibody that is heterologous to a donor antibody, which contributes all (or any portion) of the amino acid sequences encoding its heavy and/or light chain framework regions and/or its heavy and/or light chain constant regions to the first immunoglobulin partner. A human antibody may be an acceptor antibody.

“Affinity”, also referred to as “binding affinity”, is the strength of binding at a single

interaction site, i.e., of one molecule, e.g., an antigen binding protein of the invention, to another molecule, e.g., IL-33, at a single binding site. The binding affinity of an antigen binding protein to its target may be determined by equilibrium methods (e.g., enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), or solution equilibrium titration (SET)), or kinetics (e.g., BIACORE analysis). For example, MSD-SET methods described in **Example 2** may be used to measure binding affinity.

“Antibody” is used herein in the broadest sense to refer to molecules with an immunoglobulin-like domain (for example, IgG, IgM, IgA, IgD, or IgE) and includes monoclonal, recombinant, polyclonal, chimeric, human, humanized, multispecific antibodies, including bispecific antibodies, and heteroconjugate antibodies; a single variable domain (e.g., a domain antibody (DAB)), antigen binding antibody fragments, Fab, F(ab')₂, Fv, disulfide linked Fv, single chain Fv, disulfide-linked scFv, diabodies, TANDABS, etc., and modified versions of any of the foregoing (for a summary of alternative “antibody” formats, see Holliger and Hudson, Nature Biotechnology, 2005, Vol. 23, No. 9, 1126-1136). The terms “full”, “whole”, or “intact” antibody are used interchangeably herein and refer to a heterotetrameric glycoprotein with an approximate molecular weight of 150,000 Daltons. An intact antibody is composed of two identical heavy chains (HCs) and two identical light chains (LCs) linked by covalent disulfide bonds. This H₂L₂ structure folds to form three functional domains comprising two antigen-binding fragments, known as ‘Fab’ fragments, and a ‘Fc’ crystallizable fragment. The Fab fragment is composed of the variable domain at the amino-terminus, variable heavy (VH) or variable light (VL), and the constant domain at the carboxyl terminus, CH1 (heavy) and CL (light). The Fc fragment is composed of two domains formed by dimerization of paired CH2 and CH3 regions. The Fc may elicit effector functions by binding to receptors on immune cells or by binding C1q, the first component of the classical complement pathway. The five classes of antibodies IgM, IgA, IgG, IgE, and IgD are defined by distinct heavy chain amino acid sequences, which are called μ , α , γ , ϵ , and δ , respectively, and each heavy chain can pair with either a K or λ light chain. The majority of antibodies in the serum belong to the IgG class; there are four isotypes of human IgG (IgG1, IgG2, IgG3, and IgG4), the sequences of which differ mainly in their hinge region. An antibody that binds to IL-33 may be referred to herein as an “anti-IL-33 antibody” or an “IL-33 antibody”.

“Antigen binding protein” as used herein refers to antibodies, antigen binding fragments thereof, and other protein constructs, such as domains, that are capable of binding to an antigen. The term “IL-33 binding protein” as used herein refers to antibodies and other protein constructs, such as domains, that are capable of binding to IL-33. The terms “IL-33 binding protein” and “antigen binding protein” are used interchangeably herein. This does not include the natural cognate ligand or receptor. An IL-33 binding protein can be capable of binding to one or more of a human IL-33, and an IL-33 protein of another organism (e.g., mouse, rat, cow, dog, cat, pig, monkey, etc.). An IL-33 binding protein can be capable of binding to a fragment of, a variant of, or a mutant of IL-33.

“Antigen binding site” refers to a site on an antigen binding protein that is capable of specifically binding to an antigen. This may be a single variable domain, or it may be paired VH/VL domains as can be found on a standard antibody. Single-chain Fv (ScFv) domains can also provide antigen binding sites.

“CDRs” are defined as the complementarity determining region amino acid sequences of an antigen binding protein. These are the hypervariable regions of immunoglobulin heavy and light chains. There are three heavy chain and three light chain CDRs (or CDR regions) in the variable portion of an immunoglobulin. Thus, “CDRs” as used herein refers to all three heavy chain CDRs, all three light chain CDRs, all heavy and light chain CDRs, or at least two CDRs.

“Comprises” or “comprising” as used herein with respect to a SEQ ID NO are understood to include “consists” or “consisting”, respectively.

“Domain” as used herein refers to a folded polypeptide structure that can retain its tertiary structure independent of the rest of the polypeptide. Generally, domains are responsible for discrete functional properties of polypeptides and in many cases may be added, removed, or transferred to other polypeptides without loss of function of the remainder of the protein and/or of the domain.

“Donor antibody” as used herein refers to an antibody that contributes the amino acid sequences of one or more of its variable regions, CDRs, or other functional fragments or analogues thereof to a first immunoglobulin partner. A donor, therefore, provides the altered immunoglobulin coding region and resulting expressed altered antibody with the antigenic specificity and neutralizing activity characteristic of a donor antibody.

“Effector Function” as used herein refers to one or more of antibody-mediated effects

including antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-mediated complement activation including complement-dependent cytotoxicity (CDC), complement-dependent cell-mediated phagocytosis (CDCP), antibody dependent complement-mediated cell lysis (ADCML), and Fc-mediated phagocytosis or antibody-dependent cellular phagocytosis (ADCP).

“Epitope” as used herein refers to that portion of the antigen (i.e., IL-33) that makes contact with a particular binding domain of the antigen binding protein (i.e., IL-33 binding protein), also known as the paratope. An epitope may be linear, conformational, or discontinuous. A conformational or discontinuous epitope comprises amino acid residues that are separated by other sequences, i.e., not in a continuous sequence in the antigen's primary sequence assembled by tertiary folding of the polypeptide chain. Although the residues may be from different regions of the polypeptide chain, they are in close proximity in the three-dimensional structure of the antigen (i.e., IL-33). In the case of multimeric antigens, a conformational or discontinuous epitope may include residues from different peptide chains. Particular residues comprised within an epitope can be determined through computer modelling programs or via three-dimensional structures obtained through methods known in the art, such as X-ray crystallography. Epitope mapping can be carried out using various techniques known to persons skilled in the art as described in publications such as *Methods in Molecular Biology*, including ‘*Epitope Mapping Protocols*’ by Mike Schutkowski and Ulrich Reineke (volume 524, 2009) and ‘*An Introduction to Epitope Mapping*’ by Johan Rockberg and Johan Nilvebrant (volume 1785, 2018). Exemplary methods include peptide-based approaches such as pepscan, whereby a series of overlapping peptides are screened for binding using techniques such as ELISA or by *in vitro* display of large libraries of peptides or protein mutants, e.g., on phage. Detailed epitope information can be determined by structural techniques including X-ray crystallography, solution nuclear magnetic resonance (NMR) spectroscopy, and cryogenic-electron microscopy (cryo-EM). Mutagenesis, such as alanine scanning, is an effective approach whereby loss of binding analysis is used for epitope mapping. Another method is hydrogen/deuterium exchange (HDX) combined with proteolysis and liquid-chromatography mass spectrometry (LC-MS) analysis to characterize discontinuous or conformational epitopes.

The term “forced expiratory volume in one second” or “FEV1” refers to the volume of air exhaled during the first second of air forcibly exhaled from the lungs after the point of maximal inspiration. Methods for measuring FEV1 are known in the art, such as spirometry.

5 The term “forced vital capacity” or “FVC” refers to the volume of air forcibly exhaled after the point of maximal inspiration. Methods for measuring FVC are known in the art, such as spirometry.

“Half-life” (or “ $t_{1/2}$ ”) refers to the time required for the serum concentration of an antigen binding protein to reach half of its original value. The serum half-life of proteins can be measured by pharmacokinetic studies according to the method described by Kim et al., 1994, Eur. J. of Immuno. 24: 542-548. According to this method, radio-labeled protein is injected
10 intravenously into mice and its plasma concentration is periodically measured as a function of time, for example, at about 3 minutes to about 72 hours after the injection. Other methods for pharmacokinetic analysis and determination of the half-life of a molecule will be familiar to those skilled in the art.

15 “Humanized antibody” refers to a type of engineered antibody having CDRs derived from a non-human donor immunoglobulin, the remaining immunoglobulin-derived parts of the molecule being derived from one or more human immunoglobulin(s). In addition, framework support residues may be altered to preserve binding affinity. A suitable human acceptor antibody may be one selected from a conventional database (e.g., the KABAT database, Los Alamos
20 database, and Swiss Protein database), or by homology to the nucleotide and/or amino acid sequences of the donor antibody. A human antibody characterized by a homology to the framework regions of the donor antibody (on an amino acid basis) may be suitable to provide a heavy chain constant region and/or a heavy chain variable framework region for insertion of the donor CDRs. A suitable acceptor antibody capable of donating light chain constant or variable
25 framework regions may be selected in a similar manner. It should be noted that the acceptor antibody heavy and light chains may originate from the same acceptor antibody or different acceptor antibodies.

The term “IL-33 mediated disease” refers to a disorder or condition that is mediated or modulated by IL-33 mechanisms; therefore, IL-33 mediated diseases are diseases or disorders
30 where inhibition of IL-33 would be beneficial. IL-33 and its role in diseases is described in Cayrol, C, Girard, J-P. Immunol Rev. 2018; 281: 154–168; Cayrol C, Girard J-P. Cytokine. 2022

Aug;156:155891; Dwyer GK, et al. Annu Rev Immunol. 2022 Apr 26;40:15-43; Kotsiou OS, et al. Front Immunol. 2018 Oct 24;9:2432; and Yuan C. Int Immunopharmacol. 2022 Jul;108:108887. Examples of IL-33 mediated disorders include, but are not limited to, chronic obstructive pulmonary disease (COPD), asthma, bronchitis, bronchiolitis, acute respiratory failure, inflammatory lung diseases, diabetic kidney disease, endometriosis, chronic rhinosinusitis with nasal polyps, food hypersensitivity, food allergy (e.g., peanut allergy), allergic rhinitis, eosinophilic esophagitis, atopic dermatitis, cystic fibrosis, and chronic urticaria.

The term “neutralizes” as used throughout the present specification means that the biological activity of IL-33 is reduced in the presence of an antigen binding protein as described herein in comparison to the activity of IL-33 in the absence of the antigen binding protein, in vitro or in vivo. Neutralization may be due to one or more of blocking IL-33 binding to its receptor, preventing IL-33 from activating its receptor, down regulating IL-33 or its receptor, or affecting effector functionality. For example, the methods described in **Example 13** through **Example 17** may be used to assess the neutralizing capability of an IL-33 binding protein.

“Percent identity” or “% identity” between a query nucleic acid sequence and a subject nucleic acid sequence is the “Identities” value, expressed as a percentage, that is calculated using a suitable algorithm (e.g., BLASTN, FASTA, Needleman-Wunsch, Smith-Waterman, LALIGN, or GenePAST/KERR) or software (e.g., DNASTAR Lasergene, GenomeQuest, EMBOSS needle, or EMBOSS infoalign), over the entire length of the query sequence after a pair-wise global sequence alignment has been performed using a suitable algorithm (e.g., Needleman-Wunsch or GenePAST/KERR) or software (e.g., DNASTAR Lasergene or GenePAST/KERR). Importantly, a query nucleic acid sequence may be described by a nucleic acid sequence disclosed herein, in particular, in one or more of the claims. “Percent identity” or “% identity” between a query amino acid sequence and a subject amino acid sequence is the “Identities” value, expressed as a percentage, that is calculated using a suitable algorithm (e.g., BLASTP, FASTA, Needleman-Wunsch, Smith-Waterman, LALIGN, or GenePAST/KERR) or software (e.g., DNASTAR Lasergene, GenomeQuest, EMBOSS needle or EMBOSS infoalign), over the entire length of the query sequence after a pair-wise global sequence alignment has been performed using a suitable algorithm (e.g., Needleman-Wunsch or GenePAST/KERR) or software (e.g., DNASTAR Lasergene or GenePAST/KERR). Importantly, a query amino acid sequence may be described by an amino acid sequence disclosed herein, in particular, in one or

more of the claims. The query sequence may be 100% identical to the subject sequence, or it may include up to a certain integer number of amino acid or nucleotide alterations as compared to the subject sequence such that the % identity is less than 100%. For example, the query sequence is at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the subject sequence. In the case of nucleic acid sequences, such alterations include at least one nucleotide residue deletion, substitution, or insertion, wherein said alterations may occur at the 5'- or 3'-terminal positions of the query sequence or anywhere between those terminal positions, interspersed either individually among the nucleotide residues in the query sequence or in one or more contiguous groups within the query sequence. In the case of amino acid sequences, such alterations include at least one amino acid residue deletion, substitution (including conservative and non-conservative substitutions), or insertion, wherein said alterations may occur at the amino- or carboxy-terminal positions of the query sequence or anywhere between those terminal positions, interspersed either individually among the amino acid residues in the query sequence or in one or more contiguous groups within a query sequence. For antibody sequences, the % identity may be determined across the entire length of the query sequence, including the CDRs. Alternatively, the % identity may exclude one or more or all of the CDRs, for example, all of the CDRs are 100% identical to the subject sequence and the % identity variation is in the remaining portion of the query sequence, e.g., the framework sequence, so that the CDR sequences are fixed and intact.

The term "prevention" refers to avoidance of the stated disease in a subject who is not suffering from the stated disease.

"Protein Scaffold" as used herein includes, but is not limited to, an immunoglobulin (Ig) scaffold, for example, an IgG scaffold, which may be a four chain or two chain antibody, or which may comprise only the Fc region of an antibody, or which may comprise one or more constant regions from an antibody, which constant regions may be of human or primate origin, or which may be an artificial chimera of human and primate constant regions. A protein scaffold may be an Ig scaffold, for example, an IgG or IgA scaffold. An IgG scaffold may comprise some or all the domains of an antibody (i.e., CH1, CH2, CH3, VH, VL). An antigen binding protein may comprise an IgG scaffold selected from IgG1, IgG2, IgG3, IgG4, or IgG4PE. For example, a scaffold may be IgG1. A scaffold may consist of, or comprise, an Fc region of an antibody or a

fragment thereof. A protein scaffold may be a derivative of a scaffold selected from the group consisting of CTLA-4, lipocalin, Protein A derived molecules such as Z-domain of Protein A (Affibody, SpA), A-domain (Avimer/Maxibody); heat shock proteins such as GroEl and GroES; transferrin (trans-body); ankyrin repeat protein (DARPin); peptide aptamer; C-type lectin domain (Tetranectin); human g-crystallin and human ubiquitin (affilins); PDZ domains; scorpion toxin kunitz type domains of human protease inhibitors; and fibronectin/adnectin which has been subjected to protein engineering in order to obtain binding to an antigen, such as IL-33, other than a natural ligand.

“Recombinant host cell” as used herein refers to a cell that comprises a nucleic acid sequence of interest that was isolated prior to its introduction into the cell.

“Single variable domain” as used herein refers to a folded polypeptide domain comprising sequences characteristic of antibody variable domains. It therefore includes complete antibody variable domains such as VH, VHH, and VL and/or modified antibody variable domains, for example, in which one or more loops have been replaced by sequences that are not characteristic of antibody variable domains, or antibody variable domains that have been truncated or comprise N- or C-terminal extensions, as well as folded fragments of variable domains that retain at least the binding activity and specificity of the full-length domain. A single variable domain herein is capable of binding an antigen or epitope independently of a different variable region or domain. A “domain antibody” or “DAB” can be a human “single variable domain”. A single variable domain may be a human single variable domain, but can also be a single variable domains from a non-human species such as rodent (for example, as in WO 00/29004), a nurse shark, or a camelid. Notably, camelid VHHs are immunoglobulin single variable domain polypeptides that are derived from camelid species, such as camel, llama, alpaca, dromedary, and guanaco, which produce heavy chain only antibodies that are naturally devoid of light chains. Such VHH domains may be humanized according to standard techniques available in the art, and such domains can be “single variable domains”.

The terms “individual”, “subject”, and “patient” are used herein interchangeably. The subject may be an animal, in particular a mammal, such as a primate, for example, a marmoset or a monkey. Preferably, the subject is a human.

The term “therapeutically effective amount” refers to the quantity of an IL-33 binding protein or a pharmaceutical composition comprising an IL-33 binding protein which will elicit

the desired biological response in a human body. It may vary depending on the IL-33 binding protein or the pharmaceutical composition comprising the IL-33 binding protein, the disease and its severity, and the age and weight of the subject to be treated.

The term “treatment” refers to ameliorating or stabilizing the specified condition, reducing or eliminating the symptoms of the condition, slowing or eliminating the progression of the condition, and preventing or delaying reoccurrence of the condition in a previously afflicted patient or subject.

EMBODIMENTS OF THE INVENTION

One or more of antigen binding proteins described herein may be an antibody or an antigen binding fragment thereof. An antigen binding protein may be a human antibody or an antigen binding fragment thereof. An antigen binding protein may comprise one of, a plurality of, or all of: a human VH (variable heavy) domain region or a human Heavy Chain (HC) sequence; and/or a human VL (variable light) domain region or a human Light Chain (LC) sequence. An antigen binding protein may be a humanized antibody or an antigen binding fragment thereof. An antigen binding protein may comprise one of, a plurality of, or all of: a humanized VH region or a humanized Heavy Chain (HC) sequence; and/or a humanized VL region or a humanized Light Chain (LC) sequence.

Antibodies provided herein can be fully human antibodies, and can be obtained using a variety of methods, for example, using yeast-based libraries or transgenic animals (e.g., mice) that are capable of producing repertoires of human antibodies. Yeast presenting human antibodies on their surface that bind to an antigen of interest can be selected using FACS (Fluorescence-Activated Cell Sorting) based methods or by capture on beads using labeled antigens. Transgenic animals that have been modified to express human immunoglobulin genes can be immunized with an antigen of interest and antigen-specific human antibodies isolated using B-cell sorting techniques. Human antibodies produced using these techniques can then be characterized for desired properties such as affinity, developability and selectivity. In an embodiment, the antibodies are human antibodies produced using a yeast-based platform.

An antigen binding fragment may be provided by means of arrangement of one or more CDRs on one or more non-antibody protein scaffolds, such as an affibody, a SpA scaffold, an

LDL receptor class A domain, an avimer (see, e.g., U.S. Patent Publication Nos. 2005/0053973, 2005/0089932, 2005/0164301), or an EGF domain.

Throughout this specification, amino acid residues in variable domain sequences and variable domain regions within full-length antigen binding sequences, e.g., within an antibody heavy chain sequence or antibody light chain sequence, are numbered according to the Kabat numbering convention. Similarly, the terms “CDR”, “CDRL1”, “CDRL2”, “CDRL3”, “CDRH1”, “CDRH2”, and “CDRH3” used herein follow the Kabat numbering convention. For further information, see Kabat et al., Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987).

It will be apparent to those skilled in the art that there are alternative numbering conventions for amino acid residues in variable domain sequences and full-length antibody sequences. Throughout this specification, amino acid residues in Fc regions, in antibody sequences or full-length antigen binding protein sequences, are numbered according to the EU index numbering convention.

There are also alternative numbering conventions for CDR sequences, for example, those set out in Chothia et al. (1989) Nature 342: 877-883. The structure and protein folding of the antigen binding protein may mean that other residues are considered part of the CDR sequence and would be understood to be so by a skilled person.

Other numbering conventions for CDR sequences available to a skilled person include “AbM” (University of Bath) and “contact” (University College London) methods.

Table 1 below represents one definition using each numbering convention for each CDR. The Kabat numbering scheme is used in **Table 1** to number the variable domain amino acid sequence. It should be noted that some of the CDR definitions may vary depending on the individual publication used.

Table 1. CDR Numbering

	Kabat CDR	Chothia CDR	AbM CDR	Contact CDR
H1	31-35/35A/35B	26-32/33/34	26-35/35A/35B	30-35/35A/35B
H2	50-65	52-56	50-58	47-58
H3	95-102	95-102	95-102	93-101

L1	24-34	24-34	24-34	30-36
L2	50-56	50-56	50-56	46-55
L3	89-97	89-97	89-97	89-96

Provided herein are IL-33 binding proteins comprising any one or a combination or all of CDRs selected from CDRH1 of SEQ ID NO:20, CDRH2 of SEQ ID NO:20, CDRH3 of SEQ ID NO:20, CDRL1 of SEQ ID NO:25, CDRL2 of SEQ ID NO:25, and CDRL3 of SEQ ID NO:25,

5 wherein SEQ ID NO:20 comprises:

QVQLVQSGAEVKKPGASVKVSCKASGYTFISYGMHWVRQAPGQGLEWMGEINPHGGSTS
YAQKFX₁GRVTMTTRDTSTSTVYMELSSLRSEDTAVYYCARPSAAYSHYLGX₂DX₃WGRGT
LVTVSS; and

wherein SEQ ID NO:25 comprises:

10 DIQMTQSPSSVSASVGDRTITCRX₄SQGIX₅WLX₆WYQQKPGKAPKLLIYAX₇SX₈LQS
GVPSRFGSGSGTDFTLTISSLQPEDFATYYCQQAX₉VX₁₀PLTFGGGTKVEIK; and

wherein:

X₁ = K or Q; X₂ = I or L; X₃ = I, L, or M; X₄ = A or T; X₅ = P
or S; X₆ = A or S; X₇ = A or G; X₈ = R or S; X₉ = H or N; and X₁₀ =
15 F or L. In an embodiment, the antigen binding protein comprises all 6 CDRs. In an
embodiment, the antigen binding protein is an antibody comprising all 6 CDRs.

Provided herein are IL-33 binding proteins comprising any one or a combination or all of CDRs selected from CDRH1 of SEQ ID NO:21, CDRH2 of SEQ ID NO:21, CDRH3 of SEQ ID NO:21, CDRL1 of SEQ ID NO:26, CDRL2 of SEQ ID NO:26, and CDRL3 of SEQ ID NO:26.

20 In an embodiment, the antigen binding protein comprises all 6 CDRs. In an embodiment, the
antigen binding protein is an antibody comprising all 6 CDRs.

Provided herein are IL-33 binding proteins comprising any one or a combination or all of CDRs selected from CDRH1 of SEQ ID NO:22, CDRH2 of SEQ ID NO:22, CDRH3 of SEQ ID NO:22, CDRL1 of SEQ ID NO:26, CDRL2 of SEQ ID NO:26, and CDRL3 of SEQ ID NO:26.

25 In an embodiment, the antigen binding protein comprises all 6 CDRs. In an embodiment, the
antigen binding protein is an antibody comprising all 6 CDRs.

Provided herein are IL-33 binding proteins comprising any one or a combination or all of CDRs selected from CDRH1 of SEQ ID NO:23, CDRH2 of SEQ ID NO:23, CDRH3 of SEQ ID NO:23, CDRL1 of SEQ ID NO:27, CDRL2 of SEQ ID NO:27, and CDRL3 of SEQ ID NO:27.

In an embodiment, the antigen binding protein comprises all 6 CDRs. In an embodiment, the antigen binding protein is an antibody comprising all 6 CDRs.

Provided herein are IL-33 binding proteins comprising any one or a combination or all of CDRs selected from CDRH1 of SEQ ID NO:24, CDRH2 of SEQ ID NO:24, CDRH3 of SEQ ID NO:24, CDRL1 of SEQ ID NO:28, CDRL2 of SEQ ID NO:28, and CDRL3 of SEQ ID NO:28.

In an embodiment, the antigen binding protein comprises all 6 CDRs. In an embodiment, the antigen binding protein is an antibody comprising all 6 CDRs.

In one embodiment, an IL-33 binding protein described herein comprises any one or a combination or all of CDRs selected from CDRH1 of SEQ ID NO:1, CDRH2 of SEQ ID NO:2, CDRH3 of SEQ ID NO:5, CDRL1 of SEQ ID NO:9, CDRL2 of SEQ ID NO:13, and CDRL3 of SEQ ID NO:17, wherein SEQ ID NO:2 comprises:

EINPHGGSTSYAQKFX₁G;

wherein SEQ ID NO:5 comprises:

PSAAYSHYLGX₂DX₃;

wherein SEQ ID NO:9 comprises:

RX₄SQGISX₅WLX₆;

wherein SEQ ID NO:13 comprises:

AX₇SX₈LQS;

wherein SEQ ID NO:17 comprises:

QQAX₉VX₁₀PLT; and

wherein:

X₁ = K or Q; X₂ = I or L; X₃ = I, L, or M; X₄ = A or T; X₅ = P or S; X₆ = A or S; X₇ = A or G; X₈ = R or S; X₉ = H or N; and X₁₀ = F or L.

In an embodiment, an IL-33 binding protein described herein comprises the following 6 CDRs: CDRH1 of SEQ ID NO:1, CDRH2 of SEQ ID NO:2, CDRH3 of SEQ ID NO:5, CDRL1 of SEQ ID NO:9, CDRL2 of SEQ ID NO:13, and CDRL3 of SEQ ID NO:17. In an embodiment, the antigen binding protein is an antibody.

In one embodiment, an IL-33 binding protein described herein comprises any one or a combination or all of CDRs selected from CDRH1 of SEQ ID NO:1, CDRH2 of SEQ ID NO:4, CDRH3 of SEQ ID NO:7, CDRL1 of SEQ ID NO:10, CDRL2 of SEQ ID NO:14, and CDRL3 of SEQ ID NO:18. In an embodiment, an IL-33 binding protein described herein comprises the following 6 CDRs: CDRH1 of SEQ ID NO:1, CDRH2 of SEQ ID NO:4, CDRH3 of SEQ ID NO:7, CDRL1 of SEQ ID NO:10, CDRL2 of SEQ ID NO:14, and CDRL3 of SEQ ID NO:18. In an embodiment, the antigen binding protein is an antibody.

In one embodiment, an IL-33 binding protein described herein comprises any one or a combination or all of CDRs selected from CDRH1 of SEQ ID NO:1, CDRH2 of SEQ ID NO:3, CDRH3 of SEQ ID NO:7, CDRL1 of SEQ ID NO:12, CDRL2 of SEQ ID NO:16, and CDRL3 of SEQ ID NO:19. In an embodiment, an IL-33 binding protein described herein comprises the following 6 CDRs: CDRH1 of SEQ ID NO:1, CDRH2 of SEQ ID NO:3, CDRH3 of SEQ ID NO:7, CDRL1 of SEQ ID NO:12, CDRL2 of SEQ ID NO:16, and CDRL3 of SEQ ID NO:19. In an embodiment, the antigen binding protein is an antibody.

In one embodiment, an IL-33 binding protein described herein comprises any one or a combination or all of CDRs selected from CDRH1 of SEQ ID NO:1, CDRH2 of SEQ ID NO:3, CDRH3 of SEQ ID NO:6, CDRL1 of SEQ ID NO:10, CDRL2 of SEQ ID NO:14, and CDRL3 of SEQ ID NO:18. In an embodiment, an IL-33 binding protein described herein comprises the following 6 CDRs: CDRH1 of SEQ ID NO:1, CDRH2 of SEQ ID NO:3, CDRH3 of SEQ ID NO:6, CDRL1 of SEQ ID NO:10, CDRL2 of SEQ ID NO:14, and CDRL3 of SEQ ID NO:18. In an embodiment, the antigen binding protein is an antibody.

In one embodiment, an IL-33 binding protein described herein comprises any one or a combination or all of CDRs selected from CDRH1 of SEQ ID NO:1, CDRH2 of SEQ ID NO:3, CDRH3 of SEQ ID NO:8, CDRL1 of SEQ ID NO:11, CDRL2 of SEQ ID NO:15, and CDRL3 of SEQ ID NO:18. In an embodiment, an IL-33 binding protein described herein comprises the following 6 CDRs: CDRH1 of SEQ ID NO:1, CDRH2 of SEQ ID NO:3, CDRH3 of SEQ ID NO:8, CDRL1 of SEQ ID NO:11, CDRL2 of SEQ ID NO:15, and CDRL3 of SEQ ID NO:18. In an embodiment, the antigen binding protein is an antibody.

CDRs of an IL-33 binding protein provided herein can be modified by one or by more than one amino acid substitution, deletion, or addition, wherein the variant IL-33 binding protein

substantially retains the biological characteristics of the unmodified protein, such as inhibiting the binding of IL-33 to the ST2 receptor.

It will be appreciated that each of CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, or CDRL3 may be modified alone or in combination with any other CDR, in any permutation or combination. A CDR may be modified by the substitution, deletion, or addition of up to 3 amino acids, for example, 1 or 2 amino acids, for example, 1 amino acid. Each modification of a CDR, VH, VL, or other protein provided herein can be a conservative substitution. A modification can be a conservative substitution, for example, as shown in **Table 2A** or in **Table 2B** below.

Table 2A. *Examples of conservative substitutions by side chain type*

10

Side chain	Members
Hydrophobic	Met, Ala, Val, Leu, Ile
Neutral hydrophilic	Cys, Ser, Thr
Acidic	Asp, Glu
Basic	Asn, Gln, His, Lys, Arg
Residues that influence chain orientation	Gly, Pro
Aromatic	Trp, Tyr, Phe

Table 2B. *Examples of conservative substitutions by amino acid*

Amino Acid	Conservative Substitution
A	D, E, G, S, T
C	G, R, S, W, Y
D	A, E, G, H, N, V, Y
E	A, D, G, K, Q, V
F	I, L, Y
G	A, C, D, E, R
H	D, L, N, P, Q, R, Y
I	F, L, M, N, V
K	E, M, N, Q, R, T

L	F, H, I, M, P, Q, R, V, W
M	I, K, L, R, T, V
N	D, H, I, K, S, T, Y
P	H, L, Q, R, S
Q	E, H, L, L, P, R
R	C, G, H, K, L, M, P, Q, T, W
S	A, C, N, P, T, W, Y
T	A, K, M, N, R, S
V	D, E, I, L, M
W	C, L, R, S
Y	C, D, F, H, N, S

For example, in a variant CDR, one or more flanking residues that comprise the CDR as part of alternative definition(s), e.g., Kabat or Chothia, may be substituted with a conservative amino acid residue.

5 Such antigen binding proteins comprising variant CDRs as described above may be referred to herein as “functional CDR variants”.

IL-33 binding proteins described herein may comprise:

- a. (i) any one or a combination of CDRs selected from CDRH1, CDRH2, and/or CDRH3 from SEQ ID NO:20 and/or CDRL1, CDRL2, and/or CDRL3 from
- 10 SEQ ID NO:25, wherein SEQ ID NO:20 comprises:

QVQLVQSGAEVKKPGASVKVSCKASGYTFISYGMHWVRQAPGQGLEW
MGEINPHGGSTSYAQKFX₁GRVTMTRDTSTSTVYMELSSLRSED
TAVYYCARPSAAYSHYLGX₂DX₃WGRGTLVTVSS; and

wherein SEQ ID NO:25 comprises:

15 DIQMTQSPSSVSASVGDRVTITCRX₄SQGIX₅WLX₆WYQQKPGKAPK
LLIYAX₇SX₈LQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQA
X₉VX₁₀PLTFGGGTKVEIK; and

wherein:

$X_1 = K \text{ or } Q$; $X_2 = I \text{ or } L$; $X_3 = I, L, \text{ or } M$; $X_4 = A \text{ or } T$; $X_5 = P \text{ or } S$; $X_6 = A \text{ or } S$; $X_7 = A \text{ or } G$; $X_8 = R \text{ or } S$; $X_9 = H \text{ or } N$; and $X_{10} = F \text{ or } L$; or

- (ii) a CDR variant of (i), wherein the variant has 1, 2, or 3 amino acid modifications; or
- b. a VH region comprising a sequence at least 80% identical to the sequence of SEQ ID NO:20 and/or a VL region comprising a sequence at least 80% identical to the sequence of SEQ ID NO:25.

IL-33 binding proteins described herein may comprise:

- (i) CDRH1, CDRH2, and/or CDRH3 from any one of SEQ ID NOs:21-24 and/or CDRL1, CDRL2, and/or CDRL3 from SEQ ID NO:26-28; or
- (ii) one or more CDR variants of (i), wherein the variant has 1, 2, or 3 amino acid modifications.

IL-33 binding proteins described herein may comprise:

- a. (i) any one or a combination of CDRs selected from CDRH1, CDRH2, and/or CDRH3 from SEQ ID NO:21 and/or CDRL1, CDRL2, and/or CDRL3 from SEQ ID NO:26; or
- (ii) a CDR variant of (i), wherein the variant has 1, 2, or 3 amino acid modifications; or
- b. a VH region comprising a sequence at least 80% identical to the sequence of SEQ ID NO:21 and/or a VL region comprising a sequence at least 80% identical to the sequence of SEQ ID NO:26.

IL-33 binding proteins described herein may comprise:

- a. (i) any one or a combination of CDRs selected from CDRH1, CDRH2, and/or CDRH3 from SEQ ID NO:22 and/or CDRL1, CDRL2, and/or CDRL3 from SEQ ID NO:26; or
- (ii) a CDR variant of (i), wherein the variant has 1, 2, or 3 amino acid modifications; or
- b. a VH region comprising a sequence at least 80% identical to the sequence of SEQ ID NO:22 and/or a VL region comprising a sequence at least 80% identical to the sequence of SEQ ID NO:26.

IL-33 binding proteins described herein may comprise:

- a. (i) any one or a combination of CDRs selected from CDRH1, CDRH2, and/or CDRH3 from SEQ ID NO:23 and/or CDRL1, CDRL2, and/or CDRL3 from SEQ ID NO:27; or
- (ii) a CDR variant of (i), wherein the variant has 1, 2, or 3 amino acid modifications; or
- b. a VH region comprising a sequence at least 80% identical to the sequence of SEQ ID NO:23 and/or a VL region comprising a sequence at least 80% identical to the sequence of SEQ ID NO:27.

IL-33 binding proteins described herein may comprise:

- a. (i) any one or a combination of CDRs selected from CDRH1, CDRH2, and/or CDRH3 from SEQ ID NO:24 and/or CDRL1, CDRL2, and/or CDRL3 from SEQ ID NO:28; or
- (ii) a CDR variant of (i), wherein the variant has 1, 2, or 3 amino acid modifications; or
- b. a VH region comprising a sequence at least 80% identical to the sequence of SEQ ID NO:24 and/or a VL region comprising a sequence at least 80% identical to the sequence of SEQ ID NO:28.

In one embodiment, IL-33 binding proteins described herein may comprise:

- a. any one or a combination of CDRs selected from CDRH1 of SEQ ID NO:1, CDRH2 of SEQ ID NO:2, CDRH3 of SEQ ID NO:5, CDRL1 of SEQ ID NO:9, CDRL2 of SEQ ID NO:13, and CDRL3 of SEQ ID NO:17, wherein SEQ ID NO:2 comprises:

EINPHGGSTSYAQKFX₁G;

wherein SEQ ID NO:5 comprises:

PSAAYSHYLGX₂DX₃;

wherein SEQ ID NO:9 comprises:

RX₄SQGISX₅WLX₆;

wherein SEQ ID NO:13 comprises:

AX₇SX₈LQS;

wherein SEQ ID NO:17 comprises:

QQAX₉VX₁₀PLT; and

wherein:

X₁ = K or Q; X₂ = I or L; X₃ = I, L, or M; X₄ = A or
T; X₅ = P or S; X₆ = A or S; X₇ = A or G; X₈ = R or
S; X₉ = H or N; and X₁₀ = F or L; or

b. a CDR variant of (a), wherein the variant has 1, 2, or 3 amino acid modifications.

In an embodiment, the antigen binding protein is an antibody.

In an embodiment, an IL-33 binding protein described herein comprises the following 6
CDRs or a variant of any one or more thereof: CDRH1 of SEQ ID NO:1, CDRH2 of SEQ ID
NO:2, CDRH3 of SEQ ID NO:5, CDRL1 of SEQ ID NO:9, CDRL2 of SEQ ID NO:13, and
CDRL3 of SEQ ID NO:17, wherein SEQ ID NO:2 comprises:

EINPHGGSTSYAQKFX₁G;

wherein SEQ ID NO:5 comprises:

PSAAYSHYLGX₂DX₃;

wherein SEQ ID NO:9 comprises:

RX₄SQGIX₅WLX₆;

wherein SEQ ID NO:13 comprises:

AX₇SX₈LQS;

wherein SEQ ID NO:17 comprises:

QQAX₉VX₁₀PLT; and

wherein:

X₁ = K or Q; X₂ = I or L; X₃ = I, L, or M; X₄ = A or T; X₅ = P
or S; X₆ = A or S; X₇ = A or G; X₈ = R or S; X₉ = H or N; and
X₁₀ = F or L.

In one embodiment, the variant has 1, 2, or 3 amino acid modifications. In an embodiment, the
antigen binding protein is an antibody.

In one embodiment, IL-33 binding proteins described herein may comprise:

a. any one or a combination of CDRs selected from CDRH1 of SEQ ID NO:1,
CDRH2 of SEQ ID NO:4, CDRH3 of SEQ ID NO:7, CDRL1 of SEQ ID NO:10,
CDRL2 of SEQ ID NO:14, and CDRL3 of SEQ ID NO:18; or

b. a CDR variant of (a), wherein the variant has 1, 2, or 3 amino acid modifications.

In an embodiment, the antigen binding protein is an antibody.

In an embodiment, an IL-33 binding protein described herein comprises the following 6 CDRs or a variant of any one or more thereof: CDRH1 of SEQ ID NO:1, CDRH2 of SEQ ID NO:4, CDRH3 of SEQ ID NO:7, CDRL1 of SEQ ID NO:10, CDRL2 of SEQ ID NO:14, and
5 CDRL3 of SEQ ID NO:18. In one embodiment, the variant has 1, 2, or 3 amino acid modifications. In an embodiment, the antigen binding protein is an antibody.

In one embodiment, IL-33 binding proteins described herein may comprise:

- a. any one or a combination of CDRs selected from CDRH1 of SEQ ID NO:1, CDRH2 of SEQ ID NO:3, CDRH3 of SEQ ID NO:7, CDRL1 of SEQ ID NO:12,
10 CDRL2 of SEQ ID NO:16, and CDRL3 of SEQ ID NO:19; or
- b. a CDR variant of (a), wherein the variant has 1, 2, or 3 amino acid modifications.

In an embodiment, the antigen binding protein is an antibody.

In an embodiment, an IL-33 binding protein described herein comprises the following 6 CDRs or a variant of any one or more thereof: CDRH1 of SEQ ID NO:1, CDRH2 of SEQ ID NO:3, CDRH3 of SEQ ID NO:7, CDRL1 of SEQ ID NO:12, CDRL2 of SEQ ID NO:16, and
15 CDRL3 of SEQ ID NO:19. In one embodiment, the variant has 1, 2, or 3 amino acid modifications. In an embodiment, the antigen binding protein is an antibody.

In one embodiment, IL-33 binding proteins described herein may comprise:

- a. any one or a combination of CDRs selected from CDRH1 of SEQ ID NO:1, CDRH2 of SEQ ID NO:3, CDRH3 of SEQ ID NO:6, CDRL1 of SEQ ID NO:10, CDRL2 of SEQ ID NO:14, and CDRL3 of SEQ ID NO:18; or
20
- b. a CDR variant of (a), wherein the variant has 1, 2, or 3 amino acid modifications.

In an embodiment, the antigen binding protein is an antibody.

In an embodiment, an IL-33 binding protein described herein comprises the following 6 CDRs or a variant of any one or more thereof: CDRH1 of SEQ ID NO:1, CDRH2 of SEQ ID NO:3, CDRH3 of SEQ ID NO:6, CDRL1 of SEQ ID NO:10, CDRL2 of SEQ ID NO:14, and
25 CDRL3 of SEQ ID NO:18. In one embodiment, the variant has 1, 2, or 3 amino acid modifications. In an embodiment, the antigen binding protein is an antibody.

In one embodiment, IL-33 binding proteins described herein may comprise:

- a. any one or a combination of CDRs selected from CDRH1 of SEQ ID NO:1, CDRH2 of SEQ ID NO:3, CDRH3 of SEQ ID NO:8, CDRL1 of SEQ ID NO:11, CDRL2 of SEQ ID NO:15, and CDRL3 of SEQ ID NO:18; or
- b. a CDR variant of (a), wherein the variant has 1, 2, or 3 amino acid modifications.

5 In an embodiment, the antigen binding protein is an antibody.

In an embodiment, an IL-33 binding protein described herein comprises the following 6 CDRs or a variant of any one or more thereof: CDRH1 of SEQ ID NO:1, CDRH2 of SEQ ID NO:3, CDRH3 of SEQ ID NO:8, CDRL1 of SEQ ID NO:11, CDRL2 of SEQ ID NO:15, and CDRL3 of SEQ ID NO:18. In one embodiment, the variant has 1, 2, or 3 amino acid
10 modifications. In an embodiment, the antigen binding protein is an antibody.

In one embodiment, antigen binding proteins of the present disclosure show cross-reactivity between human IL-33 and IL-33 from another species, such as cynomolgus IL-33 or rhesus IL-33. An antigen binding protein described herein may specifically bind human IL-33 and cynomolgus IL-33. Such cross-reactivity can be exploited during preclinical research,
15 e.g., in one or more non-human primate systems such as rhesus monkey or cynomolgus monkey. Such preclinical research can be performed before the antigen binding protein is tested in humans. Such cross-reactivity can be exploited to make one or more side-by-side comparisons of using an antigen binding protein herein. In one embodiment, cross reactivity between other species used in disease models such as dog or mouse is also envisaged. Optionally, the binding
20 affinity of the antigen binding protein for cynomolgus IL-33 and the binding affinity for human IL-33 differ by no more than a factor of 2, 5, 10, 50, or 100. In one embodiment, the binding affinity of the antigen binding protein for cynomolgus IL-33 and the binding affinity for human IL-33 differ by no more than a factor of 10.

In an embodiment, the equilibrium dissociation constant (KD) of the antigen binding
25 protein-IL-33 interaction is 100 nM or less, 10 nM or less, 2 nM or less, or 1 nM or less. Alternatively, the KD may be between 1 pM and 500 pM or between 500 pM and 1 nM. In one embodiment, the KD is less than or equal to 500 pM, less than or equal to 400 pM, less than or equal to 300 pM, less than or equal to 200 pM, less than or equal to 100 pM, less than or equal to 75 pM, less than or equal to 50 pM, less than or equal to 40 pM, less than or equal to 30 pM, less
30 than or equal to 25 pM, less than or equal to 20 pM, less than or equal to 10 pM, or less than or equal to 5 pM. In one embodiment, the KD is between 1 pM and 10 pM (e.g., between 1.8 pM

and 6 pM). In another embodiment, the K_D is between 0.01 pM and 100 pM (e.g., between 0.1 pM and 45 pM). In one embodiment, the affinity of the IL-33 binding protein for human IL-33 is less than or equal to 5 pM (e.g., 3.3 pM) at 25°C. In one embodiment, the affinity of the IL-33 binding protein for human IL-33 is less than or equal to 15 pM (e.g., 13.5 pM) at 37°C. In one
5 embodiment, the affinity of the IL-33 binding protein for cynomolgus IL-33 at 25°C is less than or equal to 30 pM (e.g., 27.5 pM). In one embodiment, the affinity of the IL-33 binding protein for cynomolgus IL-33 at 25°C is less than or equal to 60 pM at 37°C (e.g., 56.5 pM). For antigen binding proteins herein, a smaller K_D numerical value corresponds with stronger binding to an antigen (e.g., IL-33). The reciprocal of K_D (i.e., $1/K_D$) is the equilibrium association constant
10 (KA) having units M^{-1} . For antigen binding proteins herein, a larger KA numerical value corresponds with stronger binding to an antigen (e.g., IL-33).

In one embodiment, the IL-33 binding protein does not bind to human IL-1 α and/or human IL-1 β . In one embodiment, the IL-33 binding protein does not bind to human IL-1 α and human IL-1 β .

15 In one embodiment, the IL-33 binding protein does not bind to human oxidized IL-33 and/or cynomolgus oxidized IL-33. In one embodiment, the IL-33 binding protein does not bind to human oxidized IL-33 and cynomolgus oxidized IL-33.

In one embodiment, the IL-33 binding protein binds to hFc γ RI with a K_D of less than 25 nM (e.g., 24.3 nM). In one embodiment, the IL-33 binding protein binds to hFc γ RIIa (H131)
20 with a K_D of less than or equal to 600 nM (e.g., 574.0 nM). In one embodiment, the IL-33 binding protein binds to hFc γ RIIa (R131) with a K_D of less than or equal to 525 nM (e.g., 502.0 nM). In one embodiment, the IL-33 binding protein binds to hFc γ RIIb with a K_D of less than or equal to 5250 nM (e.g., 5220.0 nM). In one embodiment, the IL-33 binding protein binds to hFc γ RIIIa (V158) with a K_D of less than or equal to 225 nM (e.g., 215.0 nM). In one
25 embodiment, the IL-33 binding protein binds to hFc γ RIIIa (F158) with a K_D of less than or equal to 1000 nM (e.g., 987.0 nM). In one embodiment, the IL-33 binding protein binds to cFc γ RIIa with a K_D of less than or equal to 2150 nM (e.g., 2110.0 nM). In one embodiment, the IL-33 binding protein binds to cFc γ RIIb with a K_D of less than or equal to 1125 nM (e.g., 1100.0 nM). In one embodiment, the IL-33 binding protein binds to cFc γ RIIIa with a K_D of less than or
30 equal to 125 nM (e.g., 119.0 nM).

In one embodiment, the IL-33 binding protein binds to human recombinant neonatal receptor (FcRn) with a K_D of less than or equal to 30 nM or less than or equal to 25 nM (e.g., 25.0 nM) at pH 6.0. In one embodiment, the IL-33 binding protein binds to human recombinant neonatal receptor (FcRn) with a K_D of less than or equal to 1250 nM (e.g., 1230 nM) at pH 7.4.

5 In one embodiment, the IL-33 binding protein binds to cynomolgus recombinant neonatal receptor (FcRn) with a K_D of less than or equal to 30 nM (e.g., 24.7 nM) at pH 6.0. In one embodiment, the IL-33 binding protein binds to cynomolgus recombinant neonatal receptor (FcRn) with a K_D of less than or equal to 2600 nM or less than or equal to 2575 nM (e.g., 2560 nM) at pH 7.4.

10 In one embodiment, the IL-33 binding protein binds to human C1q with a K_D of less than or equal to 225 nM (e.g., 213 nM).

The dissociation rate constant (kd) or “off-rate” describes the stability of the antigen binding protein-IL-33 complex, i.e., the fraction of complexes that decay per second. For example, a kd of 0.01 s^{-1} equates to 1% of the complexes decaying per second. In an
 15 embodiment, the dissociation rate constant (kd) is $1 \times 10^{-3}\text{ s}^{-1}$ or less, $1 \times 10^{-4}\text{ s}^{-1}$ or less, $1 \times 10^{-5}\text{ s}^{-1}$ or less, or $1 \times 10^{-6}\text{ s}^{-1}$ or less. The kd may be between $1 \times 10^{-5}\text{ s}^{-1}$ and $1 \times 10^{-4}\text{ s}^{-1}$ or between $1 \times 10^{-4}\text{ s}^{-1}$ and $1 \times 10^{-3}\text{ s}^{-1}$.

The association rate constant (ka) or “on-rate” describes the rate of antigen binding protein-antigen (e.g., IL-33) complex formation. The ka of the antigen binding protein-IL-33
 20 interaction may be about $1.5 \times 10^5\text{ M}^{-1}\text{s}^{-1}$. Alternatively, the ka may be between $1 \times 10^6\text{ M}^{-1}\text{s}^{-1}$ and $1 \times 10^5\text{ M}^{-1}\text{s}^{-1}$. Alternatively, the ka may be between $1 \times 10^5\text{ M}^{-1}\text{s}^{-1}$ and $5 \times 10^5\text{ M}^{-1}\text{s}^{-1}$ or between $1 \times 10^5\text{ M}^{-1}\text{s}^{-1}$ and $8 \times 10^5\text{ M}^{-1}\text{s}^{-1}$.

An IL-33 binding protein described herein can be neutralizing. For example, the methods described in **Example 13** through **Example 17** may be used to assess the neutralizing capability
 25 of an IL-33 binding protein. The reduction or inhibition in biological activity may be partial or total. A neutralizing antigen binding protein may neutralize the activity of IL-33 by at least 20%, at least 30%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or
 30 100% relative to IL-33 activity in the absence of the antigen binding protein. Neutralization may

be determined or measured using one or more assays known to the skilled person or as described herein.

In one embodiment, an IL-33 binding protein described herein inhibits IL-33 induced superoxide generation from isolated eosinophils. In one embodiment, the IL-33 binding protein described herein inhibits IL-33 induced superoxide generation from isolated eosinophils with an
5 IC_{50} of less than or equal to 100 pM, less than or equal to 75 pM, less than or equal to 50 pM, less than or equal to 40 pM, less than or equal to 30 pM, less than or equal to 25 pM, or less than or equal to 20 pM (e.g., 19.65 pM). In one embodiment, the IL-33 binding protein described herein inhibits IL-33 induced superoxide generation from isolated eosinophils with a pIC_{50} of
10 less than or equal to 11 or less than or equal to 10.75 (e.g., 10.73).

In one embodiment, an IL-33 binding protein described herein inhibits IL-33 induced IFN- γ secretion from CD4+ T cells. In one embodiment, the IL-33 binding protein described herein inhibits IL-33 induced IFN- γ secretion from CD4+ T cells with an IC_{50} of less than or equal to 1000 pM, less than or equal to 900 pM, less than or equal to 800 pM, or less than or equal to 700 pM (e.g., 675.14). In one embodiment, the IL-33 binding protein described herein
15 inhibits IL-33 induced IFN- γ secretion from CD4+ T cells with a pIC_{50} of less than or equal to 9.5 or less than or equal to 9.25 (e.g., 9.2).

In one embodiment, an IL-33 binding protein described herein inhibits IL-33 induced IL-8 and/or IL-6 secretion from human umbilical vein endothelial cells (HUVECs). In one
20 embodiment, an IL-33 binding protein described herein inhibits IL-33 induced IL-8 and IL-6 secretion from human umbilical vein endothelial cells (HUVECs).

In one embodiment, an IL-33 binding protein described herein inhibits IL-33 induced IL-8 secretion from human umbilical vein endothelial cells (HUVECs). In one embodiment, the IL-33 binding protein described herein inhibits IL-33 induced IL-8 secretion from HUVECs with an
25 IC_{50} of less than or equal to 500 pM, less than or equal to 475 pM, less than or equal to 450 pM, less than or equal to 425 pM, or less than or equal to 400 pM (e.g., 389.90 pM). In one embodiment, the IL-33 binding protein described herein inhibits IL-33 induced IL-8 secretion from HUVECs with a pIC_{50} of less than or equal to 9.75 or less than or equal to 9.5 (e.g., 9.42).

In one embodiment, an IL-33 binding protein described herein inhibits IL-33 induced IL-6 secretion from human umbilical vein endothelial cells (HUVECs). In one embodiment, the IL-33 binding protein described herein inhibits IL-33 induced IL-6 secretion from HUVECs with an
30

IC₅₀ of less than or equal to 300 pM, less than or equal to 275 pM, less than or equal to 250 pM, or less than or equal to 225 pM (e.g., 217.30). In one embodiment, the IL-33 binding protein described herein inhibits IL-33 induced IL-6 secretion from HUVECs with a pIC₅₀ of less than or equal to 10 or less than or equal to 9.75 (e.g., 9.42).

5 In one embodiment, an IL-33 binding protein described herein inhibits IL-33 induced β -hexosaminidase release from basophils. In one embodiment, the IL-33 binding protein described herein inhibits IL-33 induced β -hexosaminidase release from basophils with an IC₅₀ of less than or equal to 10 nM, less than or equal to 7.75 nM, less than or equal to 5 nM, less than or equal to 4 nM, less than or equal to 3 nM, less than or equal to 2.75 nM, less than or equal to 2.5 nM, or
10 less than or equal to 2.25 nM (e.g., 2.03 nM). In one embodiment, the IL-33 binding protein described herein inhibits IL-33 induced β -hexosaminidase release from basophils with a pIC₅₀ of less than or equal to 9.25 or less than or equal to 9 (e.g., 8.78).

In one embodiment, the affinity of IL-33 for the ST2 receptor is not affected when IL-33 is complexed with an IL-33 binding protein described herein.

15 In one embodiment, an IL-33 binding protein described herein pre-complexed with IL-33 demonstrates concentration dependent inhibition of IL-33/IL-12 stimulated IFN- γ release in whole blood. In one embodiment, the IL-33 binding protein described herein pre-complexed with IL-33 demonstrates concentration dependent inhibition of IL-33/IL-12 stimulated IFN- γ release in whole blood with an IC₅₀ value of less than or equal to 4 nM (e.g., 3.90 nM) for IL-33
20 concentrations of 100ng/mL. In one embodiment, the IL-33 binding protein described herein pre-complexed with IL-33 demonstrates concentration dependent inhibition of IL-33/IL-12 stimulated IFN- γ release in whole blood with an IC₅₀ value of less than or equal to 3.50 or less than or equal to 3.25 (e.g., 3.25 nM) for IL-33 concentrations of 30ng/mL. In one embodiment, the IL-33 binding protein described herein pre-complexed with IL-33 demonstrates concentration
25 dependent inhibition of IL-33/IL-12 stimulated IFN- γ release in whole blood with an IC₅₀ value of less than or equal to 2.5 nM or less than or equal to 2 nM (e.g., 1.95 nM) for IL-33 concentrations of 10ng/mL. In one embodiment, the IL-33 binding protein described herein pre-complexed with IL-33 demonstrates concentration dependent inhibition of IL-33/IL-12 stimulated IFN- γ release in whole blood with a pIC₅₀ value of less than or equal to 9, less than
30 or equal to 8.75, or less than or equal to 8.5 (e.g., 8.48) for IL-33 concentrations of 100ng/mL. In one embodiment, the IL-33 binding protein described herein pre-complexed with IL-33

demonstrates concentration dependent inhibition of IL-33/IL-12 stimulated IFN- γ release in whole blood with a pIC50 value of less than or equal to 9 or less than or equal to 8.75 (e.g., 8.53) for IL-33 concentrations of 30ng/mL. In one embodiment, the IL-33 binding protein described herein pre-complexed with IL-33 demonstrates concentration dependent inhibition of IL-33/IL-12 stimulated IFN- γ release in whole blood with a pIC50 value of less than or equal to 9.25 or less than or equal to 9 (e.g., 8.80) for IL-33 concentrations of 10ng/mL.

In one embodiment, an IL-33 binding protein described herein that is not pre-complexed with IL-33 also demonstrates concentration dependent inhibition of IL-33/IL-12 stimulated IFN- γ release in whole blood. In one embodiment, the IL-33 binding protein described herein that is not pre-complexed with IL-33 demonstrates concentration dependent inhibition of IL-33/IL-12 stimulated IFN- γ release in whole blood with an IC50 value of less than or equal to 2 nM or less than or equal to 1.75 nM (e.g., 1.57 nM) for IL-33 concentrations of 30ng/mL. In one embodiment, the IL-33 binding protein described herein that is not pre-complexed with IL-33 demonstrates concentration dependent inhibition of IL-33/IL-12 stimulated IFN- γ release in whole blood with an IC50 value of less than or equal to 1 nM, less than or equal to 0.75 nM, or less than or equal to 0.5 nM (e.g., 0.37 nM) for IL-33 concentrations of 10ng/mL. In one embodiment, the IL-33 binding protein described herein that is not pre-complexed with IL-33 demonstrates concentration dependent inhibition of IL-33/IL-12 stimulated IFN- γ release in whole blood with a pIC50 value of less than or equal to 9.25 or less than or equal to 9 (e.g., 8.89) for IL-33 concentrations of 30ng/mL. In one embodiment, the IL-33 binding protein described herein that is not pre-complexed with IL-33 demonstrates concentration dependent inhibition of IL-33/IL-12 stimulated IFN- γ release in whole blood with a pIC50 value of less than or equal to 10 or less than or equal to 9.75 (e.g., 9.51) for IL-33 concentrations of 10ng/mL.

In one embodiment, an IL-33 binding protein described herein can completely block production of human IL-33 stimulated IL-6, TNF- α , IL-13, and/or IL-18. In one embodiment, an IL-33 binding protein described herein can completely block production of human IL-33 stimulated IL-6, TNF- α , IL-13, and IL-18.

In one embodiment, an IL-33 binding protein described herein can completely block production of human IL-33 stimulated IL-6. In one embodiment, the IL-33 binding protein herein can completely block production of human IL-33 stimulated IL-6 with an IC50 value of less than or equal to 50 pM, less than or equal to 40 pM, or less than or equal to 35 pM (e.g.,

31.21 pM) at an IL-33 concentration of 1ng/mL. In one embodiment, the IL-33 binding protein herein can completely block production of human IL-33 stimulated IL-6 with a pIC₅₀ value of less than or equal to 11 or less than or equal to 10.75 (e.g., 10.51) at an IL-33 concentration of 1ng/mL. In one embodiment, the IL-33 binding protein herein can completely block production of human IL-33 stimulated IL-6 with an IC₅₀ value of less than or equal to 600 pM or less than or equal to 575 pM (e.g., 566 pM) at an IL-33 concentration of 10ng/mL. In one embodiment, the IL-33 binding protein herein can completely block production of human IL-33 stimulated IL-6 with a pIC₅₀ value of less than or equal to 9.5 or less than or equal to 9.25 (e.g., 9.25) at an IL-33 concentration of 10ng/mL.

In one embodiment, an IL-33 binding protein described herein can completely block production of human IL-33 stimulated TNF- α . In one embodiment, the IL-33 binding protein herein can completely block production of human IL-33 stimulated TNF- α with an IC₅₀ value of less than or equal to 50 pM or less than or equal to 40 pM (e.g., 37.35 pM) at an IL-33 concentration of 1ng/mL. In one embodiment, the IL-33 binding protein herein can completely block production of human IL-33 stimulated TNF- α with a pIC₅₀ value of less than or equal to 11, less than or equal to 10.75, or less than or equal to 10.5 (e.g., 10.43) at an IL-33 concentration of 1ng/mL. In one embodiment, the IL-33 binding protein herein can completely block production of human IL-33 stimulated TNF- α with an IC₅₀ value of less than or equal to 600 pM or less than or equal to 590 pM (e.g., 581 pM) at an IL-33 concentration of 10ng/mL. In one embodiment, the IL-33 binding protein herein can completely block production of human IL-33 stimulated TNF- α with a pIC₅₀ value of less than or equal to 9.5 or less than or equal to 9.25 (e.g., 9.24) at an IL-33 concentration of 10ng/mL.

In one embodiment, an IL-33 binding protein described herein can completely block production of human IL-33 stimulated IL-13. In one embodiment, the IL-33 binding protein herein can completely block production of human IL-33 stimulated IL-13 with an IC₅₀ value of less than or equal to 50 pM, less than or equal to 40 pM, or less than or equal to 30 pM (e.g., 28.01 pM) at an IL-33 concentration of 1ng/mL. In one embodiment, the IL-33 binding protein herein can completely block production of human IL-33 stimulated IL-13 with a pIC₅₀ value of less than or equal to 1, less than or equal to 0.75, or less than or equal to 0.6 (e.g., 0.55) at an IL-33 concentration of 1ng/mL. In one embodiment, the IL-33 binding protein herein can completely block production of human IL-33 stimulated IL-13 with an IC₅₀ value of less than or

equal to 600 pM or less than or equal to 575 pM (e.g., 567 pM) at an IL-33 concentration of 10ng/mL. In one embodiment, the IL-33 binding protein herein can completely block production of human IL-33 stimulated IL-13 with a pIC50 value of less than or equal to 9.5 or less than or equal to 9.25 (e.g., 9.25) at an IL-33 concentration of 10ng/mL.

- 5 In one embodiment, an IL-33 binding protein described herein can completely block production of human IL-33 stimulated IL-18. In one embodiment, the IL-33 binding protein herein can completely block production of human IL-33 stimulated IL-18 with an IC50 value of less than or equal to 100 pM, less than or equal to 90 pM, less than or equal to 80 pM, or less than or equal to 70 pM (e.g., 61.26 pM) at an IL-33 concentration of 1ng/mL. In one
- 10 embodiment, the IL-33 binding protein herein can completely block production of human IL-33 stimulated IL-18 with a pIC50 value of less than or equal to 11, less than or equal to 10.75, less than or equal to 10.5, or less than or equal to 10.25 (e.g., 10.21) at an IL-33 concentration of 1ng/mL. In one embodiment, the IL-33 binding protein herein can completely block production of human IL-33 stimulated IL-18 with an IC50 value of less than or equal to 700 pM, less than or
- 15 equal to 675 pM, or less than or equal to 650 pM (e.g., 631 pM) at an IL-33 concentration of 10ng/mL. In one embodiment, the IL-33 binding protein herein can completely block production of human IL-33 stimulated IL-18 with a pIC50 value of less than or equal to 9.5 or less than or equal to 9.25 (e.g., 9.20) at an IL-33 concentration of 10ng/mL.

- 20 An IL-33 binding protein described herein may comprise a VH region that is at least 90% identical to SEQ ID NO:20 and/or a VL region that is at least 90% identical to SEQ ID NO:25, wherein SEQ ID NO:20 comprises:

QVQLVQSGAEVKKPGASVKVSCKASGYTFISYGMHWVRQAPGQGLEWMGEINPHGGSTS
YAQKFX₁GRVTMTTRDTSTSTVYMELSSLRSEDTAVYYCARPSAAYSHYLGX₂DX₃WGRGT
LVTVSS; and

- 25 wherein SEQ ID NO:25 comprises:

DIQMTQSPSSVSASVGDRVTITCRX₄SQGIX₅WLX₆WYQQKPGKAPKLLIYAX₇SX₈LQS
GVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQAX₉VX₁₀PLTFGGGTKVEIK; and

wherein:

- 30 X₁ = K or Q; X₂ = I or L; X₃ = I, L, or M; X₄ = A or T; X₅ = P or S; X₆ = A or S; X₇ = A or G; X₈ = R or S; X₉ = H or N; and X₁₀ = F or L.

An IL-33 binding protein described herein may comprise a VH region that is at least 90% identical to SEQ ID NO:21 and/or a VL region that is at least 90% identical to SEQ ID NO:26.

An IL-33 binding protein described herein may comprise a VH region that is at least 90% identical to SEQ ID NO:22 and/or a VL region that is at least 90% identical to SEQ ID NO:26.

5 An IL-33 binding protein described herein may comprise a VH region that is at least 90% identical to SEQ ID NO:23 and/or a VL region that is at least 90% identical to SEQ ID NO:27.

An IL-33 binding protein described herein may comprise a VH region that is at least 90% identical to SEQ ID NO:24 and/or a VL region that is at least 90% identical to SEQ ID NO:28.

10 An IL-33 binding protein described herein may comprise a VH region that is at least 95% identical to SEQ ID NO:20 and/or a VL region that is at least 95% identical to SEQ ID NO:25, wherein SEQ ID NO:20 comprises:

QVQLVQSGAEVKKPGASVKVSCKASGYTFISYGMHWVRQAPGQGLEWMGEINPHGGSTS
YAQKFX₁GRVTMTRDTSTSTVYMELSSLRSED₁TAVYYCARPSAAYSHYLGX₂DX₃WGRGT
LVTVSS; and

15 wherein SEQ ID NO:25 comprises:

DIQMTQSPSSVSASVGDRVITITCRX₄SQGISX₅WLX₆WYQQKPGKAPKLLIYAX₇SX₈LQS
GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQAX₉VX₁₀PLTFGGGTKVEIK; and

wherein:

20 X₁ = K or Q; X₂ = I or L; X₃ = I, L, or M; X₄ = A or T; X₅ = P or S; X₆ = A or S; X₇ = A or G; X₈ = R or S; X₉ = H or N; and X₁₀ = F or L.

An IL-33 binding protein described herein may comprise a VH region that is at least 95% identical to SEQ ID NO:21 and/or a VL region that is at least 95% identical to SEQ ID NO:26.

An IL-33 binding protein described herein may comprise a VH region that is at least 95%

25 identical to SEQ ID NO:22 and/or a VL region that is at least 95% identical to SEQ ID NO:26.

An IL-33 binding protein described herein may comprise a VH region that is at least 95% identical to SEQ ID NO:23 and/or a VL region that is at least 95% identical to SEQ ID NO:27.

An IL-33 binding protein described herein may comprise a VH region that is at least 95% identical to SEQ ID NO:24 and/or a VL region that is at least 95% identical to SEQ ID NO:28.

30 An IL-33 binding protein described herein may comprise a VH region that is 100%

identical to SEQ ID NO:20 and/or a VL region that is 100% identical to SEQ ID NO:25, wherein SEQ ID NO:20 comprises:

QVQLVQSGAEVKKPGASVKVSCKASGYTFISYGMHWVRQAPGQGLEWMGEINPHGGSTS
 YAQKFX₁GRVTMTTRDTSTSTVYMEISSLRSEDTAVYYCARPSAAYSHYLGX₂DX₃WGRGT
 5 LVTVSS; and

wherein SEQ ID NO:25 comprises:

DIQMTQSPSSVSASVGDRVTITCRX₄SQGISX₅WLX₆WYQQKPGKAPKLLIYAX₇SX₈LQS
 GVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQAX₉VX₁₀PLTFGGGTKVEIK; and

wherein:

10 X₁ = K or Q; X₂ = I or L; X₃ = I, L, or M; X₄ = A or T; X₅ = P
 or S; X₆ = A or S; X₇ = A or G; X₈ = R or S; X₉ = H or N; and X₁₀ =
 F or L.

An IL-33 binding protein described herein may comprise a VH region that is 100%
 identical to SEQ ID NO:21 and/or a VL region that is 100% identical to SEQ ID NO:26. An IL-
 15 33 binding protein described herein may comprise a VH region that is 100% identical to SEQ ID
 NO:21 and a VL region that is 100% identical to SEQ ID NO:26. An IL-33 binding protein
 described herein may comprise a VH region that is 100% identical to SEQ ID NO:22 and/or a
 VL region that is 100% identical to SEQ ID NO:26. An IL-33 binding protein described herein
 may comprise a VH region that is 100% identical to SEQ ID NO:22 and a VL region that is
 20 100% identical to SEQ ID NO:26. An IL-33 binding protein described herein may comprise a
 VH region that is 100% identical to SEQ ID NO:23 and/or a VL region that is 100% identical to
 SEQ ID NO:27. An IL-33 binding protein described herein may comprise a VH region that is
 100% identical to SEQ ID NO:23 and a VL region that is 100% identical to SEQ ID NO:27. An
 IL-33 binding protein described herein may comprise a VH region that is 100% identical to SEQ
 25 ID NO:24 and/or a VL region that is 100% identical to SEQ ID NO:28. An IL-33 binding protein
 described herein may comprise a VH region that is 100% identical to SEQ ID NO:24 and a VL
 region that is 100% identical to SEQ ID NO:28.

An IL-33 binding protein described herein may comprise a Heavy Chain (HC) sequence
 that is at least 90% identical to SEQ ID NO:29 and/or a Light Chain (LC) that is at least 90%
 30 identical to SEQ ID NO:34, wherein SEQ ID NO:29 comprises:

QVQLVQSGAEVKKPGASVKVSCKASGYTFISYGMHWVRQAPGQGLEWMGEINPHGGSTS
 YAQKFX₁GRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARPSAAYSHYLGX₂DX₃WGRGT
 LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
 PAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCTPPC
 5 PAPELLGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
 VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFL
 YSKLTVDKSRWQQGNVFCSCVMHEALHNYHTQKSLSLSPGK; and

wherein SEQ ID NO:34 comprises:

10 DIQMTQSPSSVSASVGDRVITITCRX₄SQGISX₅WLX₆WYQQKPGKAPKLLIYAX₇SX₈LQS
 GVPSRFGSGSGTDFTLTISLQPEDFATYYCQQAX₉VX₁₀PLTFGGGTKEIKRTVAAP
 SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST
 YLSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC; and

wherein:

15 X₁ = K or Q; X₂ = I or L; X₃ = I, L, or M; X₄ = A or T; X₅ = P
 or S; X₆ = A or S; X₇ = A or G; X₈ = R or S; X₉ = H or N; and X₁₀ =
 F or L.

An IL-33 binding protein described herein may comprise a Heavy Chain (HC) sequence
 that is at least 90% identical to SEQ ID NO:30 and/or a Light Chain (LC) sequence that is at
 20 least 90% identical to SEQ ID NO:35. An IL-33 binding protein described herein may comprise
 an HC sequence that is at least 90% identical to SEQ ID NO:31 and/or an LC sequence that is at
 least 90% identical to SEQ ID NO:35. An IL-33 binding protein described herein may comprise
 an HC sequence that is at least 90% identical to SEQ ID NO:32 and/or an LC sequence that is at
 least 90% identical to SEQ ID NO:36. An IL-33 binding protein described herein may comprise
 25 an HC sequence that is at least 90% identical to SEQ ID NO:33 and/or an LC sequence that is at
 least 90% identical to SEQ ID NO:37.

An IL-33 binding protein described herein may comprise a Heavy Chain (HC) sequence
 that is at least 95% identical to SEQ ID NO:29 and/or a Light Chain (LC) that is at least 95%
 identical to SEQ ID NO:34, wherein SEQ ID NO:29 comprises:

30 QVQLVQSGAEVKKPGASVKVSCKASGYTFISYGMHWVRQAPGQGLEWMGEINPHGGSTS
 YAQKFX₁GRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARPSAAYSHYLGX₂DX₃WGRGT

LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
 PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC
 PAPELLGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
 TKPREEQYNSTYRVSVLTVHLQDNLGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
 5 VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFL
 YSKLTVDKSRWQQGNVFCVMHEALHNHYTQKSLSLSPGK; and

wherein SEQ ID NO:34 comprises:

DIQMTQSPSSVSASVGDRVTITCRX₄SQGISX₅WLX₆WYQQKPGKAPKLLIYAX₇SX₈LQS
 GVPSRFGSGSGTDFTLTISSLQPEDFATYYCQAX₉VX₁₀PLTFGGGTKVEIKRTVAAP
 10 SVFIFFPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST
 YSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC; and

wherein:

X₁ = K or Q; X₂ = I or L; X₃ = I, L, or M; X₄ = A or T; X₅ = P
 or S; X₆ = A or S; X₇ = A or G; X₈ = R or S; X₉ = H or N; and X₁₀ =
 15 F or L.

An IL-33 binding protein described herein may comprise a Heavy Chain (HC) sequence
 that is at least 95% identical to SEQ ID NO:30 and/or a Light Chain (LC) sequence that is at
 least 95% identical to SEQ ID NO:35. An IL-33 binding protein described herein may comprise
 an HC sequence that is at least 95% identical to SEQ ID NO:31 and/or an LC sequence that is at
 20 least 95% identical to SEQ ID NO:35. An IL-33 binding protein described herein may comprise
 an HC sequence that is at least 95% identical to SEQ ID NO:32 and/or an LC sequence that is at
 least 95% identical to SEQ ID NO:36. An IL-33 binding protein described herein may comprise
 an HC sequence that is at least 95% identical to SEQ ID NO:33 and/or an LC sequence that is at
 least 95% identical to SEQ ID NO:37.

25 An IL-33 binding protein described herein may comprise a Heavy Chain (HC) sequence
 that is 100% identical to SEQ ID NO:29 and/or a Light Chain (LC) that is 100% identical to SEQ
 ID NO:34, wherein SEQ ID NO:29 comprises:

QVQLVQSGAEVKKPGASVKVSCKASGYTFISYGMHWVRQAPGQGLEWMGEINPHGGSTS
 YAQKFX₁GRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARPSAAYSHYLGX₂DX₃WGRGT
 30 LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
 PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC

PAPELLGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFL
YSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK; and

5 wherein SEQ ID NO:34 comprises:

DIQMTQSPSSVSASVGDRVTITCR₄SQGISX₅WLX₆WYQQKPGKAPKLLIYAX₇SX₈LQS
GVPSRFGSGSGTDFTLTISSLQPEDFATYYCQQAX₉VX₁₀PLTFGGGTKVEIKRTVAAP
SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS
T YSLSSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC; and

10 wherein:

X₁ = K or Q; X₂ = I or L; X₃ = I, L, or M; X₄ = A or T; X₅ = P
or S; X₆ = A or S; X₇ = A or G; X₈ = R or S; X₉ = H or N; and X₁₀ =
F or L.

An IL-33 binding protein described herein may comprise a Heavy Chain (HC) sequence
15 that is 100% identical to SEQ ID NO:30 and/or a Light Chain (LC) sequence that is 100%
identical to SEQ ID NO:35. An IL-33 binding protein described herein may comprise an HC
sequence that is 100% identical to SEQ ID NO:31 and/or an LC sequence that is 100% identical
to SEQ ID NO:35. An IL-33 binding protein described herein may comprise an HC sequence
that is 100% identical to SEQ ID NO:32 and/or an LC sequence that is 100% identical to SEQ ID
20 NO:36. An IL-33 binding protein described herein may comprise an HC sequence that is 100%
identical to SEQ ID NO:33 and/or an LC sequence that is 100% identical to SEQ ID NO:37.

An IL-33 binding protein provided herein can comprise a sequence that is a variant amino
acid sequence. A nucleic acid sequence of an IL-33 binding protein provided herein can
comprise a variant nucleic acid sequence. A variant nucleic acid sequence herein can be of an IL-
25 33 binding protein provided herein or of a variant thereof. The variant sequence substantially
retains the biological characteristics of the unmodified protein, such as binding affinity for IL-33,
cross-reactivity with both human and cynomolgus IL-33, and half-life.

A VH or VL (or HC or LC) sequence may be a variant sequence of a VH or VL (or HC
or LC) sequence provided herein with up to 10 amino acid substitutions, additions, or deletions.
30 Such a variant sequence may have 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid substitution(s),
addition(s), or deletion(s).

An HC sequence may be a variant sequence of an HC sequence provided herein with up to 40 amino acid substitutions, additions, or deletions. An HC variant sequence may have up to 35, up to 30, up to 25, up to 20, up to 15, or up to 10 amino acid substitutions, additions, or deletions. An HC variant sequence may have 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid substitutions, additions, or deletions.

An LC sequence may be a variant sequence of an LC sequence provided herein with up to 20 amino acid substitutions, additions, or deletions. An LC variant sequence may have up to 15, up to 10, or up to 5 amino acid substitutions, additions, or deletions. An LC variant sequence may have 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid substitutions, additions, or deletions.

A sequence variation may exclude one or more or all of the CDRs. For example, the CDRs portion of the VH or VL (or HC or LC) sequence can be free of a sequence variation, and the variation can be present in a non-CDR portion of a VH or VL (or HC or LC) sequence, i.e., such that the CDR sequences are intact. A variation can be a substitution, such as a conservative substitution, for example, as provided in **Table 2A** or **Table 2B**.

An antigen binding protein having a variant sequence can substantially retain the biological characteristics of an unmodified antigen binding protein, such as inhibiting binding of IL-33 to the ST2 receptor. A binding property (e.g., KD, Kd, or Ka) of an IL-33 binding protein having a variant sequence can be substantially identical to an unmodified IL-33 binding protein. A binding property (e.g., KD, Kd, or Ka) of a variant sequence can be at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to that of an unmodified IL-33 binding protein.

An antigen binding protein as described herein, may be encoded by one or more isolated nucleic acid sequences. In an embodiment, the nucleic acid encoding the VH region is selected from the group consisting of SEQ ID NOs:38-43, wherein:

h = a, c, or t; m = a or c; r = a or g; y = c or t; and n = a, c, g, or t.

In an embodiment, the nucleic acid encoding the VH region is selected from the group consisting of SEQ ID NOs:44-47.

In an embodiment, the nucleic acid encoding the VL region is selected from the group consisting of SEQ ID NOs:48-55, wherein:

k = g or t; m = a or c; r = a or g; s = c or g; y = c or t;
and n = a, c, g, or t.

In an embodiment, the nucleic acid encoding the VL region is selected from the group consisting of SEQ ID NOs:56-58.

5 In an embodiment, the nucleic acid encodes any one of the following combinations of VH region and VL region nucleic acid sequences: SEQ ID NO:38 and SEQ ID NO:48; SEQ ID NO:38 and SEQ ID NO:49; SEQ ID NO:38 and SEQ ID NO:50; SEQ ID NO:38 and SEQ ID NO:51; SEQ ID NO:38 and SEQ ID NO:52; SEQ ID NO:38 and SEQ ID NO:53; SEQ ID NO:38 and SEQ ID NO:54; SEQ ID NO:38 and SEQ ID NO:55; SEQ ID NO:39 and SEQ ID NO:48; SEQ ID NO:39 and SEQ ID NO:49; SEQ ID NO:39 and SEQ ID NO:50; SEQ ID NO:39 and SEQ ID NO:51; SEQ ID NO:39 and SEQ ID NO:52; SEQ ID NO:39 and SEQ ID NO:53; SEQ ID NO:39 and SEQ ID NO:54; SEQ ID NO:39 and SEQ ID NO:55; SEQ ID NO:43 and SEQ ID NO:48; SEQ ID NO:43 and SEQ ID NO:49; SEQ ID NO:43 and SEQ ID NO:50; SEQ ID NO:43 and SEQ ID NO:51; SEQ ID NO:43 and SEQ ID NO:52; SEQ ID NO:43 and SEQ ID NO:53; SEQ ID NO:43 and SEQ ID NO:54; or SEQ ID NO:43 and SEQ ID NO:55, wherein:

h = a, c, or t; k = g or t; m = a or c; r = a or g; s = c or g; y = c or t; and n = a, c, g, or t.

20 In an embodiment, the nucleic acid encodes any one of the following combinations of VH region and VL region nucleic acid sequences: SEQ ID NO:44 and SEQ ID NO:56; SEQ ID NO:45 and SEQ ID NO:56; SEQ ID NO:46 and SEQ ID NO:57; or SEQ ID NO:47 and SEQ ID NO:58.

In an embodiment, the nucleic acid encoding the heavy chain is selected from the group consisting of SEQ ID NOs:59-64, wherein:

25 h = a, c, or t; m = a or c; r = a or g; y = c or t; and n = a, c, g, or t.

In an embodiment, the nucleic acid encoding the heavy chain is selected from the group consisting of SEQ ID NOs:65-68.

30 In an embodiment, the nucleic acid encoding the light chain is selected from the group consisting of SEQ ID NOs:69-76, wherein:

k = g or t; m = a or c; r = a or g; s = c or g; y = c or t;
and n = a, c, g, or t.

In an embodiment, the nucleic acid encoding the light chain is selected from the group consisting of SEQ ID NOs:77-79.

5 In an embodiment, the nucleic acid encodes any one of the following combinations of heavy chain and light chain nucleic acid sequences: SEQ ID NO:59 and SEQ ID NO:69; SEQ ID NO:59 and SEQ ID NO:70; SEQ ID NO:59 and SEQ ID NO:71; SEQ ID NO:59 and SEQ ID NO:72; SEQ ID NO:59 and SEQ ID NO:73; SEQ ID NO:59 and SEQ ID NO:74; SEQ ID NO:59 and SEQ ID NO:75; SEQ ID NO:60 and SEQ ID NO:69; SEQ ID NO:60 and SEQ ID NO:70; SEQ ID NO:60 and SEQ ID NO:71; SEQ ID NO:60 and SEQ ID NO:72; SEQ ID NO:60 and SEQ ID NO:73; SEQ ID NO:60 and SEQ ID NO:74; SEQ ID NO:60 and SEQ ID NO:75; SEQ ID NO:64 and SEQ ID NO:69; SEQ ID NO:64 and SEQ ID NO:70; SEQ ID NO:64 and SEQ ID NO:71; SEQ ID NO:64 and SEQ ID NO:72; SEQ ID NO:64 and SEQ ID NO:73; SEQ ID NO:64 and SEQ ID NO:74; or SEQ ID NO:64 and SEQ ID NO:75, wherein:
15 h = a, c, or t; k = g or t; m = a or c; r = a or g; s = c or g; y = c or t; and n = a, c, g, or t.

In an embodiment, the nucleic acid encodes any one of the following combinations of heavy chain and light chain nucleic acid sequences: SEQ ID NO:65 and SEQ ID NO:77; SEQ ID NO:66 and SEQ ID NO:77; SEQ ID NO:67 and SEQ ID NO:78; or SEQ ID NO:68 and SEQ ID NO:79.
20

Antigen binding proteins may be prepared by any of a number of conventional techniques. For example, antigen binding proteins may be purified from cells that naturally express them (e.g., an antibody can be purified from a hybridoma that produces it) or produced in recombinant Expression systems. Production of an IL-33 binding protein may be achieved in a cell in vitro or in vivo by delivering exogenous isolated nucleic acids encoding the IL-33 binding protein, for example, a heavy chain and a light chain of an antibody.
25

A number of different expression systems and purification regimes can be used to generate the antigen binding protein of the invention. Generally, host cells are transformed with a recombinant expression vector encoding the desired antigen binding protein. The expression vector may be maintained by the host as a separate genetic element or integrated into the host chromosome depending on the expression system. Expression vectors within the scope of the
30

disclosure may provide necessary elements for eukaryotic or prokaryotic expression and include viral promoter driven vectors, such as CMV promoter driven vectors, e.g., pcDNA3.1, pCEP4, and their derivatives, Baculovirus expression vectors, *Drosophila* expression vectors, and expression vectors that are driven by mammalian gene promoters such as human Ig gene promoters. Other examples include prokaryotic expression vectors, such as T7 promoter driven vectors, e.g., pET41, lactose promoter driven vectors, and arabinose gene promoter driven vectors. Those of ordinary skill in the art will recognise many other suitable expression vectors and expression systems.

Also provided herein are recombinant host cells. The host cell may be an isolated host cell. The host cell is usually not part of a multicellular organism (e.g., plant or animal). For example, a host cell can be a single celled organism, or can be an individual cell of a multicellular organism that is separate from that organism. A host cell can be part of a multicellular organism, for example, a plant or animal. The host cell may be a non-human host cell.

A wide range of host cells can be employed, including Prokaryotes (including Gram-negative or Gram-positive bacteria, for example, *Escherichia coli*, *Bacilli* sp., *Pseudomonas* sp., *Corynebacterium* sp.), Eukaryotes including yeast (for example, *Saccharomyces cerevisiae*, *Pichia pastoris*), fungi (for example, *Aspergillus* sp.), or higher Eukaryotes including insect cells and cell lines of mammalian origin (for example, CHO, NS0, PER.C6, HEK293, HeLa, COS-1, COS-7, BHK21, BSC-1, HepG2, 653, SP2/0, myeloma, lymphoma cells, or any derivative thereof). A recombinant cell according to the disclosure may be generated by transfection, cell fusion, immortalisation, or other procedures well known in the art. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian host cells are known in the art.

The cells can be cultured under conditions that promote expression of the antigen binding protein using a variety of equipment, such as shake flasks, spinner flasks, and bioreactors. The polypeptide is recovered by conventional protein purification procedures. Protein purification procedures typically consist of a series of unit operations comprised of various filtration and chromatographic processes developed to selectively concentrate and isolate the antigen binding protein. The purified antigen binding protein may be formulated in a pharmaceutically acceptable composition.

The skilled person will appreciate that, upon production of an antigen binding protein, such as an antibody in a host cell, post-translational modifications may occur. For example, this may include the cleavage of certain leader sequences, the addition of various sugar moieties in various glycosylation patterns, non-enzymatic glycation, deamidation, oxidation, disulfide bond scrambling, and other cysteine variants, such as free sulfhydryls, racemized disulfides, thioethers and trisulfide bonds, isomerization, C-terminal lysine clipping, and N-terminal glutamine cyclisation. The present invention encompasses the use of antigen binding proteins that have been subjected to, or have undergone, one or more post-translational modifications. Thus an “antigen binding protein” or “antibody” of the invention includes an “antigen binding protein” or “antibody”, respectively, as defined earlier that has undergone a post-translational modification such as described herein.

Glycation is a post-translational non-enzymatic chemical reaction between a reducing sugar, such as glucose, and a free amine group in the protein, and is typically observed at the epsilon amine of lysine side chains or at the N-Terminus of the protein. Glycation can occur during production and storage only in the presence of reducing sugars.

Deamidation, which can occur during production and storage, is an enzymatic reaction primarily converting asparagine (N) to iso-aspartic acid (iso-aspartate) and aspartic acid (aspartate) (D) at approximately 3:1 ratio. This deamidation reaction is therefore related to isomerization of aspartate (D) to iso-aspartate. The deamidation of asparagine and the isomerization of aspartate both involve the intermediate succinimide. To a much lesser degree, deamidation can occur with glutamine residues in a similar manner. Deamidation can occur in a CDR, in a Fab (non-CDR region), or in the Fc region.

Oxidation can occur during production and storage (i.e., in the presence of oxidizing conditions) and results in a covalent modification of a protein, induced either directly by reactive oxygen species or indirectly by reaction with secondary by-products of oxidative stress. Oxidation happens primarily with methionine residues, but may occur at tryptophan and free cysteine residues. Oxidation can occur in a CDR, in a Fab (non-CDR) region, or in the Fc region.

Disulfide bond scrambling can occur during production and basic storage conditions. Under certain circumstances, disulfide bonds can break or form incorrectly, resulting in unpaired cysteine residues (-SH). These free (unpaired) sulfhydryls (-SH) can promote shuffling.

The formation of a thioether and racemization of a disulfide bond can occur under basic

conditions, in production or storage, through a beta elimination of disulfide bridges back to cysteine residues via a dehydroalanine and persulfide intermediate. Subsequent crosslinking of dehydroalanine and cysteine results in the formation of a thioether bond or the free cysteine residues can reform a disulfide bond with a mixture of *D*- and *L*-cysteine.

5 Trisulfides result from insertion of a sulfur atom into a disulfide bond (Cys-S-S-S-Cys) and are formed due to the presence of hydrogen sulfide in production cell culture.

N-terminal glutamine (Q) and glutamate (glutamic acid) (E) in the heavy chain and/or light chain is likely to form pyroglutamate (pGlu) via cyclization. Most pGlu formation happens in the production bioreactor, but it can be formed non-enzymatically, depending on pH and
10 temperature of processing and storage conditions. Cyclization of N-terminal Q or E is commonly observed in natural human antibodies.

C-terminal lysine clipping is an enzymatic reaction catalyzed by carboxypeptidases, and is commonly observed in recombinant and natural human antibodies. Variants of this process include removal of lysine from one or both heavy chains due to cellular enzymes from the
15 recombinant host cell. Administration to the human subject/patient is likely to result in the removal of any remaining C-terminal lysines.

Fc engineering methods can be applied to modify the functional or pharmacokinetics properties of an antibody. Effector function may be altered by making mutations in the Fc region that increase or decrease binding to C1q or Fcγ receptors and modify complement-dependent
20 cytotoxicity (CDC) or antibody-dependent cell-mediated cytotoxicity (ADCC) activity, respectively. Modifications to the glycosylation pattern of an antibody can also be made to change the effector function. The *in vivo* half-life of an antibody can be altered by making mutations that affect binding of the Fc to the FcRn (Neonatal Fc Receptor).

Substitutions that increase the binding affinity of IgG to FcRn at pH 6.0 while
25 maintaining the pH dependence of the interaction with target, by engineering the constant region, have been extensively studied (Ghetie et al., Nature Biotech. 15: 637-640, 1997; Hinton et al., JBC 279: 6213-6216, 2004; Dall'Acqua et al., 10 J Immunol 117: 1129-1138, 2006). The *in-vivo* half-life of antigen binding proteins of the present invention may be altered by modification of a heavy chain constant domain or an FcRn binding domain therein.

30 In adult mammals, FcRn, also known as the neonatal Fc receptor, plays a key role in maintaining serum antibody levels by acting as a protective receptor that binds and salvages

antibodies of the IgG isotype from degradation. IgG molecules are endocytosed by endothelial cells and, if they bind to FcRn, are recycled out of the cells back into circulation. In contrast, IgG molecules that enter the cells and do not bind to FcRn and are targeted to the lysosomal pathway where they are degraded.

5 FcRn is believed to be involved in both antibody clearance and the transcytosis across tissues (see Junghans R.P. (1997) *Immunol. Res.*, 16, 29-57 and Ghetie et al. (2000) *Annu. Rev. Immunol.* 18, 739-766). Human IgG1 residues determined to interact directly with human FcRn include Ile253, Ser254, Lys288, Thr307, Gln311, Asn434 and His435. Mutations at any of these positions may enable increased serum half-life and/or altered effector properties of antigen
10 binding proteins of the invention.

Antigen binding proteins of the present invention may have amino acid modifications that increase the affinity of the constant domain or fragment thereof for FcRn. Increasing the half-life (i.e., serum half-life) of therapeutic and diagnostic IgG antibodies and other bioactive molecules has many benefits including reducing the amount and/or frequency of dosing of these molecules.
15 In one embodiment, an antigen binding protein of the invention comprises all or a portion (an FcRn binding portion) of an IgG constant domain having one or more of the following amino acid modifications.

For example, with reference to IgG1, M252Y/S254T/T256E (commonly referred to as “YTE” mutations) and M428L/N434S (commonly referred to as “LS” mutations) increase FcRn
20 binding at pH 6.0 (Wang et al. 2018).

Half-life can also be enhanced by T250Q/M428L, V259I/V308F/M428L, N434A, and T307A/E380A/N434A mutations (with reference to IgG1 and Kabat numbering) (Monnet et al.).

Half-life and FcRn binding can also be extended by introducing H433K and N434F mutations (commonly referred to as “HN” or “NHance” mutations) (with reference to IgG1)
25 (WO2006/130834).

WO00/42072 discloses a polypeptide comprising a variant Fc region with altered FcRn binding affinity, which polypeptide comprises an amino acid modification at any one or more of amino acid positions 238, 252, 253, 254, 255, 256, 265, 272, 286, 288, 303, 305, 307, 309, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 386, 388, 400, 413, 415, 424, 433, 434, 435, 436,
30 439, and 447 of the Fc region (EU index numbering).

WO02/060919 discloses a modified IgG comprising an IgG constant domain comprising

one or more amino acid modifications relative to a wild-type IgG constant domain, wherein the modified IgG has an increased half-life compared to the half-life of an IgG having the wild-type IgG constant domain, and wherein the one or more amino acid modifications are at one or more of positions 251, 253, 255, 285-290, 308-314, 385-389, and 428-435.

5 Shields et al. (2001, J Biol Chem; 276:6591-604) used alanine scanning mutagenesis to alter residues in the Fc region of a human IgG1 antibody and then assessed the binding to human FcRn. Positions that effectively abrogated binding to FcRn when changed to alanine include I253, S254, H435, and Y436. Other positions showed a less pronounced reduction in binding as follows: E233-G236, R255, K288, L309, S415, and H433. Several amino acid positions
10 exhibited an improvement in FcRn binding when changed to alanine; notable among these are P238, T256, E272, V305, T307, Q311, D312, K317, D376, E380, E382, S424, and N434. Many other amino acid positions exhibited a slight improvement (D265, N286, V303, K360, Q362, and A378) or no change (S239, K246, K248, D249, M252, E258, T260, S267, H268, S269, D270, K274, N276, Y278, D280, V282, E283, H285, T289, K290, R292, E293, E294, Q295, Y296,
15 N297, S298, R301, N315, E318, K320, K322, S324, K326, A327, P329, P331, E333, K334, T335, S337, K338, K340, Q342, R344, E345, Q345, Q347, R356, M358, T359, K360, N361, Y373, S375, S383, N384, Q386, E388, N389, N390, K392, L398, S400, D401, K414, R416, Q418, Q419, N421, V422, E430, T437, K439, S440, S442, S444, and K447) in FcRn binding.

 The most pronounced effect with respect to improved FcRn binding was found for
20 combination variants. At pH 6.0, the E380A/N434A variant showed over 8-fold better binding to FcRn, relative to native IgG1, compared with 2-fold for E380A and 3.5-fold for N434A. Adding T307A to this resulted in a 12-fold improvement in binding relative to native IgG1. In one embodiment, the antigen binding protein of the invention comprises the E380A/N434A mutations and has increased binding to FcRn.

25 Dall'Acqua et al. (2002, J Immunol.;169:5171-80) describes random mutagenesis and screening of human IgG1 hinge-Fc fragment phage display libraries against mouse FcRn. They disclosed random mutagenesis of positions 251, 252, 254-256, 308, 309, 311, 312, 314, 385-387, 389, 428, 433, 434, and 436. The major improvements in IgG1-human FcRn complex stability occur when substituting residues located in a band across the Fc-FcRn interface (M252, S254,
30 T256, H433, N434, and Y436) and to lesser extent substitutions of residues at the periphery, such as V308, L309, Q311, G385, Q386, P387, and N389. The variant with the highest affinity

to human FcRn was obtained by combining the M252Y/S254T/T256E (“YTE”) and H433K/N434F/Y436H mutations and exhibited a 57-fold increase in affinity relative to the wild-type IgG1. The in vivo behaviour of such a mutated human IgG1 exhibited a nearly 4-fold increase in serum half-life in cynomolgus monkey as compared to wild-type IgG1.

5 The present invention therefore provides an antigen binding protein with optimized binding to FcRn. In a preferred embodiment, the antigen binding protein comprises at least one amino acid modification in the Fc region of said antigen binding protein, wherein said modification is at an amino acid position selected from the group consisting of 226, 227, 228, 230, 231, 233, 234, 239, 241, 243, 246, 250, 252, 256, 259, 264, 265, 267, 269, 270, 276, 284,
10 285, 288, 289, 290, 291, 292, 294, 297, 298, 299, 301, 302, 303, 305, 307, 308, 309, 311, 315, 317, 320, 322, 325, 327, 330, 332, 334, 335, 338, 340, 342, 343, 345, 347, 350, 352, 354, 355, 356, 359, 360, 361, 362, 369, 370, 371, 375, 378, 380, 382, 384, 385, 386, 387, 389, 390, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 403, 404, 408, 411, 412, 414, 415, 416, 418, 419, 420, 421, 422, 424, 426, 428, 433, 434, 438, 439, 440, 443, 444, 445, 446 and 447 of the Fc
15 region.

Additionally, various publications describe methods for obtaining physiologically active molecules with modified half-lives, either by introducing an FcRn-binding polypeptide into the molecules (WO97/43316, US5869046, US5747035, WO96/32478 and WO91/14438) or by fusing the molecules with antibodies whose FcRn-binding affinities are preserved, but affinities
20 for other Fc receptors have been greatly reduced (WO99/43713), or fusing with FcRn binding domains of antibodies (WO00/09560, US4703039).

Further provided herein are IL-33 binding proteins that bind to an epitope of IL-33.

In one embodiment, the epitope comprises SEQ ID NO:80. In one embodiment, the epitope comprises SEQ ID NO:81. In one embodiment, the epitope comprises SEQ ID NO:80
25 and SEQ ID NO:81. In one embodiment, the epitope is a linear epitope. In one embodiment, the epitope is determined from deuterium exchange in HDX-MS analysis.

In one embodiment, the epitope comprises SEQ ID NO:82. In one embodiment, the epitope comprises SEQ ID NO:83. In one embodiment, the epitope comprises SEQ ID NO:82
30 and SEQ ID NO:83. In one embodiment, the epitope comprises SEQ ID NO:82, SEQ ID NO:83, and one or more of the following sequences: SEQ ID NO:84, SEQ ID NO:85, and LSE (residues 267-269). For example, and not limitation, in one embodiment, the epitope comprises SEQ ID

NO:82, SEQ ID NO:83, and SEQ ID NO:84. In another example, and not for limitation, in one embodiment, the epitope comprises SEQ ID NO:82, SEQ ID NO:83, and SEQ ID NO:85. In another example, and not for limitation, in one embodiment, the epitope comprises SEQ ID NO:82, SEQ ID NO:83, and LSE (residues 267-269). In one embodiment the epitope is a
5 conformational epitope. In one embodiment, the epitope is determined using Cryo-EM.

In one embodiment, the epitope comprises SEQ ID NO:86. In one embodiment, the epitope comprises SEQ ID NO:87. In one embodiment, the epitope comprises SEQ ID NO:86 and SEQ ID NO:87. In one embodiment, the epitope comprises SEQ ID NO:86, SEQ ID NO:87, and one or more of the following sequences: SEQ ID NO:84, SEQ ID NO:85, and LSE (residues
10 267-269). In one embodiment, the epitope is determined from deuterium exchange in HDX-MS analysis and/or using Cryo-EM.

In one embodiment, the IL-33 binding protein comprises a means for binding to the epitope described herein.

In one embodiment, the epitope comprises any embodiment listed above and the IL-33 binding protein comprises any IL-33 binding protein described herein. In one embodiment, the affinity of IL-33 for the ST2 receptor is not affected when the IL-33 is complexed with the IL-33
15 binding protein. In one embodiment, the IL-33 is human IL-33. In one embodiment, the IL-33 binding protein further comprises one or more of the following:

- (a) the IL-33 binding protein does not bind to human IL-1 α ;
- 20 (b) the IL-33 binding protein does not bind to human IL-1 β ;
- (c) the IL-33 binding protein does not bind to human oxidized IL-33;
- (d) the IL-33 binding protein does not bind to cynomolgus oxidized IL-33;
- (e) the IL-33 binding protein inhibits IL-33 induced superoxide generation from isolated eosinophils;
- 25 (f) the IL-33 binding protein inhibits IL-33 induced IFN- γ secretion from CD4⁺ T cells;
- (g) the IL-33 binding protein inhibits IL-33 induced IL-8 secretion from human umbilical vein endothelial cells (HUVECs);
- (h) the IL-33 binding protein inhibits IL-33 induced IL-6 secretion from human umbilical vein endothelial cells (HUVECs);

- (i) the IL-33 binding protein inhibits IL-33 induced β -hexosaminidase release from basophils;
- (j) the IL-33 binding protein can completely block production of human IL-33 stimulated IL-6;
- 5 (k) the IL-33 binding protein can completely block production of human IL-33 stimulated TNF- α ;
- (l) the IL-33 binding protein can completely block production of human IL-33 stimulated IL-13;
- (m) the IL-33 binding protein can completely block production of human IL-33 stimulated IL-18; and
- 10 (n) the IL-33 binding protein can completely block production of human IL-33 stimulated IL-6.

Further provided herein are IL-33 binding proteins that bind to IL-33 and compete for binding to the IL-33 with a reference IL-33 binding protein that binds to any epitope described above. In one embodiment, the IL-33 binding protein comprises a means for binding IL-33. In one embodiment, the reference IL-33 binding protein that binds to any epitope described above comprises:

15

- (i) CDRH1, CDRH2, and CDRH3 from SEQ ID NO:20 and CDRL1, CDRL2, and CDRL3 from SEQ ID NO:25, wherein SEQ ID NO:20 comprises:
- 20 QVQLVQSGAEVKKPGASVKVSCKASGYTFISYGMHWVRQAPGQGLEWMGEIN
PHGGSTSYAQKFX₁GRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARPSAAY
SHYLGX₂DX₃WGRGTLTVSS; and

wherein SEQ ID NO:25 comprises:

25 DIQMTQSPSSVSASVGDRVTITCRX₄SQGISX₅WLX₆WYQQKPGKAPKLLIYA
X₇SX₈LQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQAX₉VX₁₀PLTFG
GGTKVEIK; and

wherein:

X₁ = K or Q; X₂ = I or L; X₃ = I, L, or M; X₄ = A or T;

X₅ = P or S; X₆ = A or S; X₇ = A or G; X₈ = R or S; X₉ =

30 H or N; and X₁₀ = F or L; or

- (ii) CDRH1, CDRH2, and CDRH3 from any one of SEQ ID NOs:21-24 and CDRL1, CDRL2, and CDRL3 from any one of SEQ ID NOs:26-28; or
- (iii) one or more CDR variants of (i) or (ii), wherein the variant has 1, 2, or 3 amino acid modifications.

5 In one embodiment, the IL-33 reference binding protein comprises:

- (i) CDRH1, CDRH2, and CDRH3 from SEQ ID NO:21 and CDRL1, CDRL2, and CDRL3 from SEQ ID NO:26;
- (ii) CDRH1, CDRH2, and CDRH3 from SEQ ID NO:22 and CDRL1, CDRL2, and CDRL3 from SEQ ID NO:26;
- 10 (iii) CDRH1, CDRH2, and CDRH3 from SEQ ID NO:23 and CDRL1, CDRL2, and CDRL3 from SEQ ID NO:27;
- (iv) CDRH1, CDRH2, and CDRH3 from SEQ ID NO:24 and CDRL1, CDRL2, and CDRL3 from SEQ ID NO:28;
- (v) CDRH1 of SEQ ID NO:1, CDRH2 of SEQ ID NO:3, CDRH3 of SEQ ID NO:6, CDRL1 of SEQ ID NO:10, CDRL2 of SEQ ID NO:14, and CDRL3 of SEQ ID NO:18;
- 15 (vi) CDRH1 of SEQ ID NO:1, CDRH2 of SEQ ID NO:4, CDRH3 of SEQ ID NO:7, CDRL1 of SEQ ID NO:10, CDRL2 of SEQ ID NO:14, and CDRL3 of SEQ ID NO:18;
- 20 (vii) CDRH1 of SEQ ID NO:1, CDRH2 of SEQ ID NO:3, CDRH3 of SEQ ID NO:8, CDRL1 of SEQ ID NO:11, CDRL2 of SEQ ID NO:15, and CDRL3 of SEQ ID NO:18; or
- (viii) CDRH1 of SEQ ID NO:1, CDRH2 of SEQ ID NO:3, CDRH3 of SEQ ID NO:7, CDRL1 of SEQ ID NO:12, CDRL2 of SEQ ID NO:16, and CDRL3 of SEQ ID NO:19.
- 25

In one embodiment, the IL-33 is human IL-33.

Further provided herein are IL-33 binding proteins that bind to IL-33 and compete for binding to the IL-33 with a reference IL-33 binding protein, wherein the reference IL-33 binding protein comprises:

- 30 (i) CDRH1, CDRH2, and CDRH3 from SEQ ID NO:20 and CDRL1, CDRL2, and CDRL3 from SEQ ID NO:25, wherein SEQ ID NO:20 comprises:

QVQLVQSGAEVKKPGASVKVSCKASGYTFISYGMHWVRQAPGQGLEWMGEIN
PHGGSTSYAQKFX₁GRVTMTRDTSTSTVYMESSLRSED₁AVYYCARPSAAY
SHYLGX₂DX₃WGRGTLTVSS; and

wherein SEQ ID NO:25 comprises:

5 DIQMTQSPSSVSASVGDRVTITCRX₄SQGISX₅WLX₆WYQQKPGKAPKLLIYA
X₇SX₈LQSGVPSRFS₁SGSGSGTDFTLT₁ISS₁LQPEDFATYYCQQA₉VX₁₀PLT₁FG
GGTKVEIK; and

wherein:

10 X₁ = K or Q; X₂ = I or L; X₃ = I, L, or M; X₄ = A or T;
X₅ = P or S; X₆ = A or S; X₇ = A or G; X₈ = R or S; X₉ =
H or N; and X₁₀ = F or L; or

(ii) CDRH1, CDRH2, and CDRH3 from any one of SEQ ID NOs:21-24 and CDRL1,
CDRL2, and CDRL3 from any one of SEQ ID NOs:26-28; or

15 (iii) one or more CDR variants of (i) or (ii), wherein the variant has 1, 2, or 3 amino
acid modifications.

In one embodiment, the IL-33 reference binding protein comprises:

- (i) CDRH1, CDRH2, and CDRH3 from SEQ ID NO:21 and CDRL1, CDRL2, and
CDRL3 from SEQ ID NO:26;
- 20 (ii) CDRH1, CDRH2, and CDRH3 from SEQ ID NO:22 and CDRL1, CDRL2, and
CDRL3 from SEQ ID NO:26;
- (iii) CDRH1, CDRH2, and CDRH3 from SEQ ID NO:23 and CDRL1, CDRL2, and
CDRL3 from SEQ ID NO:27;
- (iv) CDRH1, CDRH2, and CDRH3 from SEQ ID NO:24 and CDRL1, CDRL2, and
CDRL3 from SEQ ID NO:28;
- 25 (v) CDRH1 of SEQ ID NO:1, CDRH2 of SEQ ID NO:3, CDRH3 of SEQ ID NO:6,
CDRL1 of SEQ ID NO:10, CDRL2 of SEQ ID NO:14, and CDRL3 of SEQ ID
NO:18;
- 30 (vi) CDRH1 of SEQ ID NO:1, CDRH2 of SEQ ID NO:4, CDRH3 of SEQ ID NO:7,
CDRL1 of SEQ ID NO:10, CDRL2 of SEQ ID NO:14, and CDRL3 of SEQ ID
NO:18;

- (vii) CDRH1 of SEQ ID NO:1, CDRH2 of SEQ ID NO:3, CDRH3 of SEQ ID NO:8, CDRL1 of SEQ ID NO:11, CDRL2 of SEQ ID NO:15, and CDRL3 of SEQ ID NO:18; or
- (viii) CDRH1 of SEQ ID NO:1, CDRH2 of SEQ ID NO:3, CDRH3 of SEQ ID NO:7, CDRL1 of SEQ ID NO:12, CDRL2 of SEQ ID NO:16, and CDRL3 of SEQ ID NO:19.

In one embodiment, the IL-33 is human IL-33.

An antigen binding protein as described herein may be incorporated into pharmaceutical compositions for use in the treatment of the human diseases described herein. In one embodiment, the pharmaceutical composition comprises an antigen binding protein in combination with one or more pharmaceutically acceptable carriers and/or excipients. Such compositions comprise a pharmaceutically acceptable carrier as known and called for by acceptable pharmaceutical practice.

Pharmaceutical compositions may be administered by injection or continuous infusion (examples include, but are not limited to, intravenous, intraperitoneal, intradermal, subcutaneous, intramuscular, intraocular, and intraportal administration). In one embodiment, the composition is suitable for intravenous administration. In one embodiment, the composition is suitable for subcutaneous administration. Pharmaceutical compositions may be suitable for topical administration (which includes, but is not limited to, epicutaneous, inhaled, intranasal, or ocular administration) or enteral administration (which includes, but is not limited to, oral, vaginal, or rectal administration).

A subject in need may be delivered one or more nucleic acids encoding an antigen binding protein provided herein, such as a heavy chain and a light chain of an antibody. The heavy chain and the light chain of the antibody may be delivered by the same or separate nucleic acids. The nucleic acids may be DNA or RNA. The nucleic acids encoding the IL-33 binding protein may be delivered without a delivery vehicle (i.e., "naked") or delivered with a viral or non-viral delivery vehicle (i.e., as a viral vector, adsorbed to or encapsulated in liposomes or polymer-based vehicles, and the like). The nucleic acid may include elements such as a poly A tail, a 5' untranslated region (UTR), and/or a 3' UTR. The nucleic acids may be mRNA. The mRNA may include a cap structure. The mRNA may be self-replicating RNA.

The nucleic acid coding for the IL-33 binding protein may be modified or unmodified.

The nucleic acids coding for the IL-33 binding protein may comprise at least one chemical modification. Nucleic acids (e.g., mRNAs) can be modified to enhance stability by including one or more chemical modifications. Such chemical modifications include, but are not limited to, a modified nucleotide, a modified sugar backbone, and the like. Also provided herein is a method of producing an IL-33 binding protein in a cell, tissue, or organism comprising contacting said cell, tissue, or organism with a composition comprising an isolated nucleic acid comprising at least one chemical modification and which encodes the IL-33 binding protein. Also provided herein is a method of producing an IL-33 binding protein in a cell in vitro or in vivo comprising contacting said cell with a composition comprising a nucleic acid comprising at least one chemical modification and which encodes an IL-33 binding protein.

Pharmaceutical compositions provided herein can comprise an effective amount of an antigen binding protein, such as an IL-33 binding protein. A pharmaceutical composition may comprise between 0.5 mg and 10 g of an IL-33 binding protein, and in some cases, can comprise between 5 mg and 1 g of an IL-33 binding protein. In an embodiment, the IL-33 binding protein is an antibody. In one embodiment, the IL-33 binding protein is an IgG1 antibody (e.g., human IgG1 antibody). In one embodiment, the IL-33 binding protein is a human IgG1k antibody.

A pharmaceutical composition may comprise between 0.5 mg and 10 g or between 5 mg and 1 g of an IL-33 binding protein comprising a VH region that is at least 90% identical to SEQ ID NO:20 and a VL region that is at least 90% identical to SEQ ID NO:25, wherein SEQ ID NO:20 comprises:

QVQLVQSGAEVKKPGASVKVSCKASGYTFISYGMHWVRQAPGQGLEWMGEINPHGGSTS
YAQKFX₁GRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARPSAAYSHYLGX₂DX₃WGRGT
LTVVSS; and

wherein SEQ ID NO:25 comprises:

DIQMTQSPSSVSASVGDRVTITCRX₄SQGISX₅WLX₆WYQQKPGKAPKLLIYAX₇SX₈LQS
GVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQAX₉VX₁₀PLTFGGGTKVEIK; and

wherein:

X₁ = K or Q; X₂ = I or L; X₃ = I, L, or M; X₄ = A or T; X₅ = P or S; X₆ = A or S; X₇ = A or G; X₈ = R or S; X₉ = H or N; and X₁₀ = F or L. In one embodiment, the VH region is at least 95% identical to SEQ ID NO:20 and

the VL region is at least 95% identical to SEQ ID NO:25. In one embodiment, the VH region is 100% identical to SEQ ID NO:20 and the VL region is 100% identical to SEQ ID NO:25.

A pharmaceutical composition may comprise between 0.5 mg and 10 g or between 5 mg and 1 g of an IL-33 binding protein comprising a VH region that is at least 90% identical to SEQ ID NO:21 and a VL region that is at least 90% identical to SEQ ID NO:26. In one embodiment, the VH region is at least 95% identical to SEQ ID NO:21 and the VL region is at least 95% identical to SEQ ID NO:26. In one embodiment, the VH region is 100% identical to SEQ ID NO:21 and the VL region is 100% identical to SEQ ID NO:26.

A pharmaceutical composition may comprise between 0.5 mg and 10 g or between 5 mg and 1 g of an IL-33 binding protein comprising a VH region that is at least 90% identical to SEQ ID NO:22 and a VL region that is at least 90% identical to SEQ ID NO:26. In one embodiment, the VH region is at least 95% identical to SEQ ID NO:22 and the VL region is at least 95% identical to SEQ ID NO:26. In one embodiment, the VH region is 100% identical to SEQ ID NO:22 and the VL region is 100% identical to SEQ ID NO:26.

A pharmaceutical composition may comprise between 0.5 mg and 10 g or between 5 mg and 1 g of an IL-33 binding protein comprising a VH region that is at least 90% identical to SEQ ID NO:23 and a VL region that is at least 90% identical to SEQ ID NO:27. In one embodiment, the VH region is at least 95% identical to SEQ ID NO:23 and the VL region is at least 95% identical to SEQ ID NO:27. In one embodiment, the VH region is 100% identical to SEQ ID NO:23 and the VL region is 100% identical to SEQ ID NO:27.

A pharmaceutical composition may comprise between 0.5 mg and 10 g or between 5 mg and 1 g of an IL-33 binding protein comprising a VH region that is at least 90% identical to SEQ ID NO:24 and a VL region that is at least 90% identical to SEQ ID NO:28. In one embodiment, the VH region is at least 95% identical to SEQ ID NO:24 and the VL region is at least 95% identical to SEQ ID NO:28. In one embodiment, the VH region is 100% identical to SEQ ID NO:24 and the VL region is 100% identical to SEQ ID NO:28.

A pharmaceutical composition may comprise between 0.5 mg and 10 g or between 5 mg and 1 g of an IL-33 binding protein comprising an HC that is at least 90% identical to SEQ ID NO:29 and an LC that is at least 90% identical to SEQ ID NO:34, wherein SEQ ID NO:29 comprises:

QVQLVQSGAEVKKPGASVKVSCKASGYTFISYGMHWVRQAPGQGLEWMGEINPHGGSTS
 YAQKFX₁GRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARPSAAYSHYLGX₂DX₃WGRGT
 LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
 PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCTPPC
 5 PAPELLGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
 VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFL
 YSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK; and

wherein SEQ ID NO:34 comprises:

10 DIQMTQSPSSVSASVGDRVITITCRX₄SQGISX₅WLX₆WYQQKPGKAPKLLIYAX₇SX₈LQS
 GVPSRFGSGSGTDFTLTISLQPEDFATYYCQQAX₉VX₁₀PLTFGGGTKEIKRTVAAP
 SVFIFPPSDEQLKSGTASVVCLLNNFYFPREAKVQWKVDNALQSGNSQESVTEQDSKDST
 YLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC; and

wherein:

15 X₁ = K or Q; X₂ = I or L; X₃ = I, L, or M; X₄ = A or T; X₅ = P
 or S; X₆ = A or S; X₇ = A or G; X₈ = R or S; X₉ = H or N; and X₁₀
 = F or L. In one embodiment, the HC is at least 95% identical to SEQ ID NO:29 and the
 LC is at least 95% identical to SEQ ID NO:34. In one embodiment, the HC is 100% identical
 to SEQ ID NO:29 and the LC is 100% identical to SEQ ID NO:34.

20 A pharmaceutical composition may comprise between 0.5 mg and 10 g or between 5
 mg and 1 g of an IL-33 binding protein comprising an HC that is at least 90% identical to
 SEQ ID NO:30 and an LC that is at least 90% identical to SEQ ID NO:35. In one
 embodiment, the HC is at least 95% identical to SEQ ID NO:30 and the LC is at least 95%
 identical to SEQ ID NO:35. In one embodiment, the HC is 100% identical to SEQ ID NO:30
 25 and the LC is 100% identical to SEQ ID NO:35.

A pharmaceutical composition may comprise between 0.5 mg and 10 g or between 5
 mg and 1 g of an IL-33 binding protein comprising an HC that is at least 90% identical to
 SEQ ID NO:31 and an LC that is at least 90% identical to SEQ ID NO:35. In one
 embodiment, the HC is at least 95% identical to SEQ ID NO:31 and the LC is at least 95%
 30 identical to SEQ ID NO:35. In one embodiment, the HC is 100% identical to SEQ ID NO:31
 and the LC is 100% identical to SEQ ID NO:35.

A pharmaceutical composition may comprise between 0.5 mg and 10 g or between 5 mg and 1 g of an IL-33 binding protein comprising an HC that is at least 90% identical to SEQ ID NO:32 and an LC that is at least 90% identical to SEQ ID NO:36. In one embodiment, the HC is at least 95% identical to SEQ ID NO:32 and the LC is at least 95% identical to SEQ ID NO:36. In one embodiment, the HC is 100% identical to SEQ ID NO:32 and the LC is 100% identical to SEQ ID NO:36.

A pharmaceutical composition may comprise between 0.5 mg and 10 g or between 5 mg and 1 g of an IL-33 binding protein comprising an HC that is at least 90% identical to SEQ ID NO:33 and an LC that is at least 90% identical to SEQ ID NO:37. In one embodiment, the HC is at least 95% identical to SEQ ID NO:33 and the LC is at least 95% identical to SEQ ID NO:37. In one embodiment, the HC is 100% identical to SEQ ID NO:33 and the LC is 100% identical to SEQ ID NO:37.

The pharmaceutical composition may be included in a kit containing the antigen binding protein together with other medicaments and/or with instructions for use. For convenience, the kit may comprise the reagents in predetermined amounts with instructions for use. The kit may also include devices used for administration of the pharmaceutical composition.

The antigen binding protein described herein may also be used in methods of treatment (e.g., for IL-33 mediated diseases). It will be appreciated by those skilled in the art that references herein to treatment refer to the treatment of established conditions. However, compounds of the invention may, depending on the condition, also be useful in the prevention of certain diseases. The antigen binding protein described herein is used in an effective amount for therapeutic, prophylactic, or preventative treatment. A therapeutically effective amount of the antigen binding protein described herein is an amount effective to ameliorate or reduce one or more symptoms of, or to prevent or cure, the disease.

Provided herein are methods of treating a disease or condition in a subject in need thereof comprising administering to said subject a therapeutically effective amount of the IL-33 binding protein or pharmaceutical composition as defined herein. The subject may be an animal or a human. In an embodiment, the subject is a human.

The IL-33 binding proteins described herein are provided for use in therapy. In one embodiment, IL-33 binding proteins are provided for use in the treatment of a disease or

condition. In one embodiment, the IL-33 binding protein comprises a VH region comprising SEQ ID NO:20 and a VL region comprising SEQ ID NO:25, wherein SEQ ID NO:20 comprises:

QVQLVQSGAEVKKPGASVKVSCKASGYTFISYGMHWVRQAPGQGLEWMGEINPHGGSTS
YAQKFX₁GRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARPSAAYSHYLGX₂DX₃WGRGT
5 LVTVSS; and

wherein SEQ ID NO:25 comprises:

DIQMTQSPSSVSASVGDRVTITCRX₄SQGIX₅WLX₆WYQQKPGKAPKLLIYAX₇SX₈LQS
GVPSRFGSGSGTDFTLTISLQPEDFATYYCQQAX₉VX₁₀PLTFGGGTKVEIK; and

wherein:

10 X₁ = K or Q; X₂ = I or L; X₃ = I, L, or M; X₄ = A or T; X₅ = P
or S; X₆ = A or S; X₇ = A or G; X₈ = R or S; X₉ = H or N; and X₁₀ =
F or L.

In one embodiment, an IL-33 binding protein is provided for use in the treatment of a
disease or condition, wherein the IL-33 binding protein comprises a VH region comprising SEQ
15 ID NO:21 and a VL region comprising SEQ ID NO:26. In one embodiment, an IL-33 binding
protein is provided for use in the treatment of a disease or condition, wherein the IL-33 binding
protein comprises a VH region comprising SEQ ID NO:22 and a VL region comprising SEQ ID
NO:26. In one embodiment, an IL-33 binding protein is provided for use in the treatment of a
disease or condition, wherein the IL-33 binding protein comprises a VH region comprising SEQ
20 ID NO:23 and a VL region comprising SEQ ID NO:27. In one embodiment, an IL-33 binding
protein is provided for use in the treatment of a disease or condition, wherein the IL-33 binding
protein comprises a VH region comprising SEQ ID NO:24 and a VL region comprising SEQ ID
NO:28.

In one embodiment, an IL-33 binding protein is provided for use in the treatment of a
25 disease or condition, wherein the IL-33 binding protein comprises an HC sequence comprising
SEQ ID NO:29 and an LC sequence comprising SEQ ID NO:34, wherein SEQ ID NO:29
comprises:

QVQLVQSGAEVKKPGASVKVSCKASGYTFISYGMHWVRQAPGQGLEWMGEINPHGGSTS
YAQKFX₁GRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARPSAAYSHYLGX₂DX₃WGRGT
30 LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCTPPC

PAPELLGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGGSFFL
YSKLTVDKSRWQQGNVFCSCVMHEALHNYHTQKSLSLSPGK; and

5 wherein SEQ ID NO:34 comprises:

DIQMTQSPSSVSASVGDRVITITCR₄SQGISX₅WLX₆WYQQKPGKAPKLLIYAX₇SX₈LQS
GVPSRFGSGSGTDFTLTISLQPEDFATYYCQQAX₉VX₁₀PLTFGGGTKVEIKRTVAAP
SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST
YLSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC; and

10 wherein:

X₁ = K or Q; X₂ = I or L; X₃ = I, L, or M; X₄ = A or T; X₅ = P
or S; X₆ = A or S; X₇ = A or G; X₈ = R or S; X₉ = H or N; and
X₁₀ = F or L.

In one embodiment, an IL-33 binding protein is provided for use in the treatment of a
15 disease or condition, wherein the IL-33 binding protein comprises an HC sequence comprising
SEQ ID NO:30 and an LC sequence comprising SEQ ID NO:35. In one embodiment, an IL-33
binding protein is provided for use in the treatment of a disease or condition, wherein the IL-33
binding protein comprises an HC sequence comprising SEQ ID NO:31 and an LC sequence
comprising SEQ ID NO:35. In one embodiment, an IL-33 binding protein is provided for use in
20 the treatment of a disease or condition, wherein the IL-33 binding protein comprises an HC
sequence comprising SEQ ID NO:32 and an LC sequence comprising SEQ ID NO:36. In one
embodiment, an IL-33 binding protein is provided for use in the treatment of a disease or
condition, wherein the IL-33 binding protein comprises an HC sequence comprising SEQ ID
NO:33 and an LC sequence comprising SEQ ID NO:37.

25 Also provided is a method for treatment of a disease or condition in a subject in need
thereof comprising administering to said subject a therapeutically effective amount of an IL-33
binding protein or a pharmaceutical composition described herein. Also provided is a method for
treatment of a disease or condition in a subject in need thereof comprising administering to said
subject a therapeutically effective amount of an IL-33 binding protein, wherein the IL-33 binding
30 protein comprises a VH region comprising SEQ ID NO:20 and a VL region comprising SEQ ID
NO:25, wherein SEQ ID NO:20 comprises:

QVQLVQSGAEVKKPGASVKVSCKASGYTFISYGMHWVRQAPGQGLEWMGEINPHGGSTS
 YAQKFX₁GRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARPSAAYSHYLGX₂DX₃WGRGT
 LVTVSS; and

wherein SEQ ID NO:25 comprises:

5 DIQMTQSPSSVSASVGDRVTITCRX₄SQGISX₅WLX₆WYQQKPGKAPKLLIYAX₇SX₈LQS
 GVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQAX₉VX₁₀PLTFGGGTKVEIK; and

wherein:

X₁ = K or Q; X₂ = I or L; X₃ = I, L, or M; X₄ = A or T; X₅ = P
 or S; X₆ = A or S; X₇ = A or G; X₈ = R or S; X₉ = H or N; and X₁₀ =
 10 F or L.

Also provided is a method for treatment of a disease or condition in a subject in need
 thereof comprising administering to said subject a therapeutically effective amount of an IL-33
 binding protein, wherein the IL-33 binding protein comprises a VH region comprising SEQ ID
 NO:21 and a VL region comprising SEQ ID NO:26. Also provided is a method for treatment of a
 15 disease or condition in a subject in need thereof comprising administering to said subject a
 therapeutically effective amount of an IL-33 binding protein, wherein the IL-33 binding protein
 comprises a VH region comprising SEQ ID NO:22 and a VL region comprising SEQ ID NO:26.
 Also provided is a method for treatment of a disease or condition in a subject in need thereof
 comprising administering to said subject a therapeutically effective amount of an IL-33 binding
 20 protein, wherein the IL-33 binding protein comprises a VH region comprising SEQ ID NO:23
 and a VL region comprising SEQ ID NO:27. Also provided is a method for treatment of a
 disease or condition in a subject in need thereof comprising administering to said subject a
 therapeutically effective amount of an IL-33 binding protein, wherein the IL-33 binding protein
 comprises a VH region comprising SEQ ID NO:24 and a VL region comprising SEQ ID NO:28.

25 In an embodiment, a method for treatment of a disease or condition in a subject in need
 thereof is provided comprising administering to said subject a therapeutically effective amount of
 an IL-33 binding protein, wherein the IL-33 binding protein comprises an HC sequence
 comprising SEQ ID NO:29 and an LC sequence comprising SEQ ID NO:34, wherein SEQ ID
 NO:29 comprises:

30 QVQLVQSGAEVKKPGASVKVSCKASGYTFISYGMHWVRQAPGQGLEWMGEINPHGGSTS
 YAQKFX₁GRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARPSAAYSHYLGX₂DX₃WGRGT

LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
 PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCTPPC
 PAPELLGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
 5 VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFL
 YSKLTVDKSRWQQGNVFCVMHEALHNHYTQKSLSLSPGK; and

wherein SEQ ID NO:34 comprises:

DIQMTQSPSSVSASVGDRVTITCR₄SQGISX₅WLX₆WYQQKPGKAPKLLIYAX₇SX₈LQS
 GVPSRFGSGSGTDFTLTISSLQPEDFATYYCQQAX₉VX₁₀PLTFGGGTKVEIKRTVAAP
 10 SVFIFFPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKST
 YSLSSITLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC; and

wherein:

X₁ = K or Q; X₂ = I or L; X₃ = I, L, or M; X₄ = A or T; X₅ = P
 or S; X₆ = A or S; X₇ = A or G; X₈ = R or S; X₉ = H or N; and
 15 X₁₀ = F or L.

In an embodiment, a method for treatment of a disease or condition in a subject in need thereof is provided comprising administering to said subject a therapeutically effective amount of an IL-33 binding protein, wherein the IL-33 binding protein comprises an HC sequence comprising SEQ ID NO:30 and an LC sequence comprising SEQ ID NO:35. In an embodiment,
 20 a method for treatment of a disease or condition in a subject in need thereof is provided comprising administering to said subject a therapeutically effective amount of an IL-33 binding protein, wherein the IL-33 binding protein comprises an HC sequence comprising SEQ ID NO:31 and an LC sequence comprising SEQ ID NO:35. In an embodiment, a method for treatment of a disease or condition in a subject in need thereof is provided comprising
 25 administering to said subject a therapeutically effective amount of an IL-33 binding protein, wherein the IL-33 binding protein comprises an HC sequence comprising SEQ ID NO:32 and an LC sequence comprising SEQ ID NO:36. In an embodiment, a method for treatment of a disease or condition in a subject in need thereof is provided comprising administering to said subject a therapeutically effective amount of an IL-33 binding protein, wherein the IL-33 binding protein
 30 comprises an HC sequence comprising SEQ ID NO:33 and an LC sequence comprising SEQ ID NO:37.

As previously described, in one embodiment, the IL-33 binding protein is provided for use in the treatment of a disease or condition. In one embodiment, the IL-33 binding protein is provided for use in the treatment of an IL-33 mediated disorder. In some embodiments, the IL-33 mediated disorder is a respiratory disorder, an inflammatory condition, an immune disorder, a fibrotic disorder, an eosinophilic disorder, an infection, pain, a central nervous system disorder, a solid tumor, or an ophthalmologic disorder.

Exemplary IL-33 mediated disorders that may be treated with an IL-33 binding protein include, for example, and not limitation, respiratory disorders (e.g., chronic obstructive pulmonary disease (COPD), asthma, bronchitis, bronchiolitis, acute respiratory failure, inflammatory lung disorders), inflammatory conditions (e.g., chronic obstructive pulmonary disease (COPD), asthma, bronchitis, bronchiolitis, acute respiratory failure, inflammatory lung diseases, diabetic kidney disease, endometriosis, chronic urticaria, atopic dermatitis, allergic rhinitis, rheumatoid arthritis, sepsis, septic shock), immune disorders (e.g., asthma, allergy, anaphylaxis, anaphylactic shock, allergic rhinitis, rheumatoid arthritis, psoriasis, inflammatory bowel disease (IBD), Crohn's disease, diabetes, liver disease), fibrotic disorders (e.g., pulmonary fibrosis (e.g., idiopathic pulmonary fibrosis)), eosinophilic disorders (e.g., eosinophil-associated gastrointestinal disorders (EGIDs) (e.g., eosinophilic esophagitis)), infections (e.g., viral infections, helminth infections, protozoan infections), pain (e.g., inflammatory pain), central nervous system disorders (e.g., Alzheimer's disease), solid tumors (e.g., lung, breast, colon, prostate, kidney, liver, pancreas, stomach, intestinal, brain, bone, or skin tumors), and ophthalmologic disorders (e.g., age-related macular degeneration (AMD), retinopathy). It will be understood by one of ordinary skill in the art that a particular disease may fall into more than one of the above-listed categories. For example, asthma is a respiratory disorder and may also be classified as an inflammatory condition and an immune disorder.

For example, and not limitation, in some embodiments, the IL-33 mediated disorder is chronic obstructive pulmonary disease (COPD), asthma, bronchitis, bronchiolitis, acute respiratory failure, inflammatory lung diseases, diabetic kidney disease, endometriosis, chronic rhinosinusitis with nasal polyps, food hypersensitivity, food allergy (e.g., peanut allergy), allergic rhinitis, eosinophilic esophagitis, atopic dermatitis, cystic fibrosis, or chronic urticaria.

For example, and not limitation, in other embodiments, the IL-33 mediated disorder is chronic obstructive pulmonary disease (COPD), asthma, COPD overlap syndrome (ACOS),

chronic bronchitis, emphysema, chronic rhinosinusitis with or without nasal polyps, allergic rhinitis, sepsis, septic shock, atopic dermatitis, diabetic kidney disease, rheumatoid arthritis, vasculitis, graft-versus-host disease (GvHD), uveitis, chronic idiopathic urticaria, sinusitis, or pancreatitis.

- 5 In one embodiment, the IL-33 binding protein is used to treat a respiratory disorder. Examples of respiratory disorders include, but are not limited to, chronic obstructive pulmonary disease (COPD), asthma, asthma and COPD overlap syndrome (ACOS), bronchitis, bronchiolitis, acute respiratory failure, inflammatory lung diseases, bronchiectasis, and emphysema.
- 10 Examples of inflammatory lung diseases include, but are not limited to, chronic obstructive pulmonary disease (COPD), asthma (e.g., allergic asthma), emphysema, sarcoidosis, acute respiratory distress syndrome (ARDS), eosinophilic pulmonary inflammation, pulmonary inflammation (e.g., cytokine storm syndrome (CSS), cytokine release syndrome (CRS)), infection-induced pulmonary conditions (e.g., related to viral infection (e.g., influenza,
- 15 parainfluenza, respiratory syncytial virus (RSV), rotavirus, human metapneumovirus), bacterial infection, fungal infection (e.g., *Aspergillus*), parasitic infection, or prion infection), pulmonary conditions related to gastric aspiration, pulmonary conditions related to environmental or occupational exposure (e.g., asbestosis, silicosis, berylliosis), immune dysregulation, pulmonary conditions related to physical trauma (e.g., ventilator injury), pneumonia (e.g., community-
- 20 acquired pneumonia, hospital-acquired pneumonia, bacterial pneumonia, viral pneumonia, fungal pneumonia, aspiration pneumonia, chemical pneumonia), pneumonitis (e.g., checkpoint inhibitor pneumonitis), acute lung injury, chronic lung disease, eosinophilic bronchitis, bronchopulmonary dysplasia, airway exacerbations, allergen-induced pulmonary conditions, histiocytosis, unified airway disease, and lymphangiomyomatosis. Examples of viral infections include, but are not
- 25 limited to, respiratory tract viral infections related to an influenza virus (e.g., Influenza virus A, Influenza virus B), respiratory syncytial virus (RSV), parainfluenza virus (e.g., hPIV-1, hPIV-2, hPIV-3, hPIV-4), rhinovirus, adenovirus, coxsackie virus, coronaviruses (e.g., SARS-CoV-1, SARS-CoV-2, MERS-CoV), adenovirus, metapneumovirus, cytomegalovirus, echo virus, herpes simplex virus, or smallpox. Examples of bacterial infections include, but are not limited to,
- 30 Chlamydia pneumoniae or Mycoplasma pneumoniae.

In one embodiment, the IL-33 binding protein is used to reduce or prevent a respiratory infection in the lung, airways, or small airways.

For example, and not limitation, in one embodiment, the respiratory disorder is chronic obstructive pulmonary disease (COPD), asthma, asthma and COPD overlap syndrome (ACOS),
5 bronchitis, bronchiolitis, acute respiratory failure, or an inflammatory lung disease.

Chronic Obstructive Pulmonary Disease (COPD) is a lung disease characterized by persistent respiratory symptoms caused by airway or alveoli abnormalities. See, e.g., Global Initiative for Chronic Obstructive Lung Disease (GOLD): Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease (2024 Report),
10 available at goldcopd.org/wp-content/uploads/2024/02/GOLD-2024_v1.2-11Jan24_WMV.pdf (last accessed November 8, 2024), which is incorporated herein by reference in its entirety. COPD symptoms include dyspnea, cough, sputum production, and/or activity limitation, and the disease often progresses in stages. Diagnostic criteria include a ratio of a forced expiratory volume in 1 second (FEV1) to the forced vital capacity (FVC) being below a threshold (e.g.,
15 $FEV1/FVC < 0.7$) as measured by spirometry. In one embodiment, the subject has COPD and an elevated level of eosinophils. Alternatively, the subject has COPD and does not have an elevated level of eosinophils.

Asthma is an inflammatory disease of the airways characterized by reversible airflow obstruction and bronchospasm. Common symptoms include wheezing, coughing, chest tightness,
20 and shortness of breath. Examples of asthma include, but are not limited to, allergic asthma, severe asthma, moderate to severe asthma, mild asthma, chronic asthma, asthma due to smoking, exercise-induced asthma, drug-induced asthma (e.g., aspirin-induced asthma, nonsteroidal anti-inflammatory drug (NSAID)-induced asthma), atopic asthma, non-atopic asthma, and occupational asthma. In one embodiment, the asthma is eosinophilic asthma. Alternatively, the
25 asthma is non-eosinophilic asthma. In one embodiment, the asthma is allergic asthma. Alternatively, the asthma is non-allergic asthma. In one embodiment, the asthma is not controlled with treatment or resistant to treatment (e.g., with corticosteroids). In one embodiment, the asthma is steroid resistant asthma. In one embodiment, the asthma is steroid sensitive asthma. In one embodiment, the asthma is steroid refractory asthma. In one embodiment, the asthma is
30 severe refractory asthma. In one embodiment, the asthma is an asthma exacerbation. In one embodiment, the asthma is due to smoking.

- In one embodiment, the IL-33 binding protein is used to treat an inflammatory condition. Examples of inflammatory conditions include, but are not limited to, chronic obstructive pulmonary disease (COPD), asthma, asthma and COPD overlap syndrome (ACOS), bronchitis, bronchiolitis, acute respiratory failure, inflammatory lung diseases, endometriosis, diabetic
- 5 kidney disease, **chronic rhinosinusitis with nasal polyps**, allergic rhinitis, eosinophilic esophagitis, atopic dermatitis, cystic fibrosis, chronic urticaria, allergy, anaphylaxis (e.g., due to peanuts or bee stings), anaphylactic shock, eosinophilic inflammation, rhinosinusitis, nasal polyps, arthritis (e.g., rheumatoid arthritis, osteoarthritis, psoriatic arthritis, enteropathic arthritis), ankylosing spondylitis, osteoporosis, bone erosion, airway inflammation, airway
- 10 hyperreactivity, airway hyperresponsiveness, pneumonitis, vasculitis, arteritis, angiogenesis, cardiovascular disease (e.g., heart failure), dermatitis, psoriasis, scleroderma, fibrosis, multiple sclerosis, lupus, dermatomyositis, myolitis, polymyolitis, dermatomyolitis, gastrointestinal inflammatory disorders, hepatitis, sepsis, septic shock, Behcet's disease, Sjögren's syndrome, giant cell arteritis, Churg-Strauss syndrome, Henoch-Schönlein purpura, Reiter's syndrome
- 15 (reactive arthritis), Still's disease, graft versus host disease (GVHD), allograft rejection, seronegative enthesopathy and arthropathy (SEA) syndrome, interstitial cystitis, polymyalgia rheumatica, bullous pemphigoid (BP), polyarteritis nodosa (PAN), granulomatosis with polyangiitis (GPA), cartilage inflammation, inflammatory pain, or mast cell-mediated inflammatory diseases.
- 20 Examples of inflammatory lung diseases include, but are not limited to, chronic obstructive pulmonary disease (COPD), asthma (e.g., allergic asthma), emphysema, sarcoidosis, acute respiratory distress syndrome (ARDS), eosinophilic pulmonary inflammation, infection-induced pulmonary conditions (e.g., related to viral infection (e.g., influenza, parainfluenza, respiratory syncytial virus (RSV), rotavirus, human metapneumovirus), bacterial infection,
- 25 fungal infection (e.g., Aspergillus), parasitic infection, or prion infection), pulmonary conditions related to gastric aspiration, pulmonary conditions related to environmental or occupational exposure (e.g., asbestosis, silicosis, berylliosis), immune dysregulation, pulmonary conditions related to physical trauma (e.g., ventilator injury), pneumonia (e.g., community-acquired pneumonia, hospital-acquired pneumonia, bacterial pneumonia, viral pneumonia, fungal
- 30 pneumonia, aspiration pneumonia, chemical pneumonia), acute lung injury, chronic lung disease,

bronchopulmonary dysplasia, airway exacerbations, allergen-induced pulmonary conditions, histiocytosis, unified airway disease, and lymphangiomyomatosis.

Examples of gastrointestinal inflammatory conditions include, but are not limited to, inflammatory bowel disease (IBD), ulcerative colitis (UC), Crohn's disease (CD), or colitis (e.g., related to environmental exposure (e.g., chemotherapy, radiation therapy), infectious colitis, ischemic colitis, necrotizing enterocolitis, collagenous or lymphocytic colitis, colitis related to conditions (e.g., chronic granulomatous disease, celiac disease), food allergy, food hypersensitivity, gastritis, infectious gastritis or enterocolitis (e.g., *Helicobacter pylori*-infected chronic active gastritis, and gastrointestinal inflammation related to infection.

In some embodiments, the inflammatory condition is a type 2 inflammatory disease. Examples of type 2 inflammatory diseases include, but are not limited to, asthma, viral exacerbations of allergic asthma, allergic rhinitis, chronic rhinosinusitis with nasal polyps, atopic dermatitis, chronic spontaneous urticaria, allergic bronchopulmonary aspergillosis, eosinophilic esophagitis, eosinophilic gastritis, eosinophilic gastroenteritis, eosinophilic colitis, allergic conjunctivitis, eosinophilia, fibrosis, allergy, anaphylaxis, anaphylactic shock, and food allergies.

For example, and not limitation, in some embodiments, the inflammatory condition is chronic obstructive pulmonary disease (COPD), asthma, bronchitis, airway inflammation, allergic rhinitis, atopic dermatitis, endometriosis, rheumatoid arthritis, sepsis, or septic shock.

In one embodiment, the IL-33 binding protein is used to treat an immune disorder.

Examples of immune disorders include, but are not limited to, asthma (e.g., allergic asthma), atopic dermatitis, allergic rhinitis, allergic fungal rhinosinusitis, allergy, anaphylaxis, anaphylactic shock, allergic bronchopulmonary aspergillosis, allergic conjunctivitis, arthritis (e.g., rheumatoid arthritis, psoriatic arthritis, juvenile rheumatoid arthritis), psoriasis, plaque psoriasis, guttate psoriasis, inverse psoriasis, pustular psoriasis, erythrodermic psoriasis, inflammatory bowel disease (IBD), Crohn's disease, ulcerative colitis, celiac disease, Sjögren's syndrome, Guillain-Barré syndrome, Raynaud's syndrome, Addison's disease, myasthenia gravis, thyroiditis (e.g., Graves' disease), bullous pemphigoid, paraneoplastic syndrome, infection with human immunodeficiency viruses (HIV), autoimmune hepatitis, pancreatitis, diabetes (e.g., type I diabetes), a compromised immune system (e.g., due to infection (e.g., HIV) or chemotherapy), and liver diseases (e.g., fatty liver disease (e.g., metabolic dysfunction-associated steatotic liver disease), primary biliary cirrhosis, primary sclerosing cholangitis, non-

alcoholic steatohepatitis (NASH)). In one embodiment, the immune disorder is mediated at least in part by mast cells.

For example, and not limitation, in some embodiments, the immune disorder is asthma, allergy, anaphylaxis, anaphylactic shock, allergic rhinitis, atopic dermatitis, psoriasis, inflammatory bowel disease (IBD), Crohn's disease, rheumatoid arthritis, psoriatic arthritis, diabetes, or liver disease.

In one embodiment, the IL-33 binding protein is used to treat fibrosis. As used herein, "fibrotic disorder" or "fibrosis" refer to conditions characterized by formation of excess of fibrous connective tissue in an organ or tissue. Fibrotic disorders include, but are not limited to, fibrosis due to pathological conditions or diseases, fibrosis due to physical trauma, fibrosis due to radiation damage, and fibrosis due to exposure to chemotherapeutics.

Examples of fibrotic disorders include, but are not limited to, pulmonary fibrosis (e.g., idiopathic pulmonary fibrosis, drug-induced pulmonary fibrosis (e.g., bleomycin-induced pulmonary fibrosis), asbestosis-related pulmonary fibrosis, bronchiolitis obliterans syndrome); fibrosis related to acute lung injury or acute respiratory distress (e.g., bacterial pneumonia induced fibrosis, trauma induced fibrosis, viral pneumonia induced fibrosis, ventilator induced fibrosis, non-pulmonary sepsis induced fibrosis, aspiration induced fibrosis); hepatic fibrosis (e.g., fibrosis related to cirrhosis (e.g., alcohol-induced cirrhosis, viral-induced cirrhosis, post-hepatitis C cirrhosis, primary biliary cirrhosis), alcohol-induced liver fibrosis, non-alcoholic steatohepatitis (NASH), biliary duct injury, schistosomiasis, cholangitis (e.g., sclerosing cholangitis), infection-induced liver fibrosis, viral-induced liver fibrosis, and autoimmune-induced hepatitis); renal fibrosis (e.g., tubulointerstitial fibrosis, scleroderma, diabetic nephritis, glomerular nephritis); dermal fibrosis (e.g., scleroderma, hypertrophic or keloid scarring, nephrogenic fibrosing dermatopathy, burns); myelofibrosis; neurofibromatosis; fibroma; fibrotic adhesions resulting from surgical procedures; cardiac fibrosis (e.g., fibrosis related to myocardial infarction); vascular fibrosis (e.g., fibrosis related to postangioplasty arterial restenosis, stent restenosis, atherosclerosis); ocular fibrosis (e.g., fibrosis related to post cataract surgery, proliferative vitreoretinopathy, retro-orbital fibrosis); bone marrow fibrosis (e.g., idiopathic myelofibrosis, drug-induced myelofibrosis); gastrointestinal fibrosis; colon fibrosis; intestinal fibrosis; pancreatic fibrosis; silicosis; radiation-induced fibrosis; scleroderma; sclerosis; cystic fibrosis; stent restenosis; and atherosclerosis.

In one embodiment, the fibrotic disorder is pulmonary fibrosis related to idiopathic pulmonary fibrosis (IDF), nonspecific interstitial pneumonia (NSIP) (e.g., cellular, fibrotic), cryptogenic organizing pneumonia (COP), sarcoidosis, adult respiratory distress syndrome, respiratory bronchiolitis, bronchiolitis obliterans, fibrosis with collagen vascular disease, Hermansky-Pudlak syndrome, or histiocytosis X. In one embodiment, the pulmonary fibrosis is idiopathic pulmonary fibrosis.

The fibrotic disorder may be organ-specific or systemic. The fibrotic disorder may be a result of a chronic disease, immune dysregulation, an infection, a toxin, medical intervention, and/or physical trauma. For example, and not limitation, the fibrotic disorder may be a result of interstitial lung disease; inhalation of environmental or occupational debris, dusts, fibers, fumes, smoke, or vapors; inhalation of chemicals or molds; alcohol abuse; cigarette smoking; hypertension; inflammation (e.g., glomerulonephritis, pancreatitis); viral infection (e.g., viral hepatitis); autoimmune disease (e.g., metabolic disorders (e.g., diabetes), Crohn's disease, inflammatory bowel disease (IBD), scleroderma); allergy; sepsis; adverse reaction to medications; aspirin overdose; hypersensitivity to environmental antigens; exposure to chlorine or fluorocarbons; exposure to herbicides; exposure to radiation; chemotherapy; treatment with an immune checkpoint inhibitor; immune dysregulation; or cancer.

In one embodiment, the IL-33 binding protein is used to treat an eosinophilic disorder. As used herein, "eosinophilic disorders" refer to conditions characterized by excess eosinophil levels either locally or systemically.

Examples of eosinophilic disorders include, but are not limited to, asthma (e.g., atopic asthma, severe asthma, drug-induced asthma (e.g., aspirin-induced asthma), COPD, atopic dermatitis, allergic rhinitis, fibrosis (e.g., pulmonary fibrosis (e.g., IPF, pulmonary fibrosis related to sclerosis, hepatic fibrosis), eosinophilic esophagitis, eosinophilic inflammation, non-allergic rhinitis, nasal polyps, allergic bronchopulmonary aspergillosis, chronic eosinophilic pneumonia, eosinophilic bronchitis, celiac disease, Churg-Strauss syndrome, eosinophilic-myalgia syndrome, hypereosinophilic syndrome, eosinophilic gastritis, eosinophilic gastroenteritis, eosinophilic enteritis, eosinophilic colitis, Crohn's disease, inflammatory bowel disease (IBD), scleroderma, endomyocardial fibrosis, aspirin intolerance, obstructive sleep apnea, cancer (e.g., glioblastoma (e.g., glioblastoma multiforme), non-Hodgkin's lymphoma), edema (e.g., angioedema), infection (e.g., helminth infection), or onchocercal dermatitis.

In one embodiment, the eosinophilic disorder is an eosinophil-associated gastrointestinal disorder (EGID), for example, and not limitation, eosinophilic esophagitis, eosinophilic gastritis, eosinophilic gastroenteritis, eosinophilic enteritis, or eosinophilic colitis.

5 In one embodiment, the IL-33 binding protein is used to treat an infection. The infection may be due to a cause including, but not limited to, a viral infection (e.g., influenza, respiratory syncytial virus (RSV)), a helminth infection (e.g., nematodiasis (e.g., trichuriasis)), or a protozoan infection (e.g., *Leishmania major* infection).

10 In one embodiment, the IL-33 binding protein is used to treat pain. The pain may be related to inflammatory pain, hyperalgesia (e.g., mechanical hyperalgesia), allodynia, or hypernociception (e.g., cutaneous hypernociception, articular hypernociception). The hypernociception may or may not be induced by an antigen.

15 In one embodiment, the IL-33 binding protein is used to treat a central nervous system disorder. Examples of central nervous system disorders include, but are not limited to, Alzheimer's disease, subarachnoid hemorrhage, infection of the central nervous system (e.g., viral infection), bipolar disorder, and neurodegenerative diseases. Examples of neurodegenerative diseases include, but are not limited to Alzheimer's disease, multiple sclerosis, Parkinson's disease, Huntington's disease, and experimental autoimmune encephalomyelitis.

20 In one embodiment, the IL-33 binding protein is used to treat a cancer or tumorigenic disorder. Examples of cancer or tumorigenic disorders include lung cancer, ovarian cancer, breast cancer, prostate cancer, endometrial cancer, renal cancer, esophageal cancer, pancreatic cancer, squamous cell carcinoma, uveal melanoma, cervical cancer, colorectal cancer, bladder cancer, brain cancer, pancreatic cancer, head and neck cancer, liver cancer, leukemia, lymphoma, Hodgkin's disease, multiple myeloma, melanoma, gastric cancer, astrocytoma, stomach cancer, 25 and pulmonary adenocarcinoma.

30 In one embodiment, the IL-33 binding protein is used to treat a solid tumor. Examples of solid tumors include, but are not limited to, tumors of the lung, breast, ovary, uterus, prostate, male genital organ, kidney, liver, pancreas, brain, head and neck, oral cavity, stomach, duodenum, small intestine, large intestine, gastrointestinal tract, colon, anus, gall bladder, labium, nasopharynx, urinary organs, bladder, skin, connective tissue (e.g., sarcoma), or bone.

For example, and not limitation, in one embodiment, the solid tumor is a breast tumor, a colon tumor, a prostate tumor, a lung tumor, a kidney tumor, a liver tumor, a pancreas tumor, a stomach tumor, an intestinal tumor, a brain tumor, a bone tumor, or a skin tumor.

In one embodiment, the IL-33 binding protein is used to inhibit tumor growth,
5 progression, and/or metastasis.

In one embodiment, the IL-33 binding protein is used to treat an ophthalmological disorder. In one embodiment, the ophthalmological disorder is related to angiogenesis and/or atrophy. Examples of ophthalmological disorders include, but are not limited to, age-related macular degeneration (AMD) (e.g., wet AMD, dry AMD, intermediate AMD, advanced AMD,
10 geographic atrophy (GA)), macular degeneration, macular edema, diabetic macular edema (DME) (e.g., non-center involved DME, center involved DME), retinopathy (e.g., high-altitude retinopathy), diabetic retinopathy (DR) (e.g., proliferative DR (PDR), non-proliferative DR (NPDR), high-altitude DR), hypertensive retinopathy, ischemia-related retinopathies, retinopathy of prematurity (ROP), conjunctivitis (e.g., infectious conjunctivitis, non-infectious conjunctivitis
15 (e.g., allergic conjunctivitis)), choroidal neovascularization (CNV) (e.g., myopic CNV), corneal neovascularization, diseases related to corneal neovascularization, retinal neovascularization, diseases related to retinal/choroidal neovascularization, intraocular neovascularization, iris neovascularization, central serous retinopathy (CSR), pathologic myopia, von Hippel-Lindau disease, histoplasmosis of the eye, uveitis (e.g., infectious uveitis, non-infectious uveitis),
20 vasculitis, retinitis (e.g., CMV retinitis), blepharitis, dry eye, traumatic eye injury, Sjögren's syndrome, Norrie disease, Stargardt disease, Leber congenital amaurosis, familial exudative vitreoretinopathy (FEVR), retinal abnormalities related to osteoporosis-pseudoglioma syndrome (OPPG), subconjunctival hemorrhage, rubeosis, ocular neovascular disease, neovascular glaucoma, retinitis pigmentosa (RP), retinal angiomatous proliferation, macular telangiectasia,
25 retinal degeneration, cystoid macular edema (CME), papilledema, ocular melanoma, retinal blastoma, retinoschisis, rubeosis (e.g., rubeosis iridis), fibrotic disorders of the eye (e.g., proliferative vitreoretinopathy), choroiditis (e.g., multifocal choroiditis), ocular histoplasmosis, and ophthalmological disorders related to ocular neovascularization, vascular leakage, retinal edema, and/or retinal atrophy.

30 Corneal neovascular may be related to a plurality of diseases and disorders including, but not limited to, Sjögren's syndrome, Terrien marginal degeneration, infections (e.g., Herpes

- simplex infections, Herpes zoster infections, Mycobacteria infections, protozoan infections), systemic lupus erythematosus (SLE), rheumatoid arthritis, ulcers (e.g., Mooren's ulcer, bacterial ulcers, fungal ulcers), vitamin A deficiency, syphilis, traumatic eye injury, chemical burns, polyarteritis nodosa, Stevens-Johnson syndrome, granulomatous diseases (e.g., sarcoidosis,
- 5 Wegener's granulomatosis), acne, rosacea, epidemic keratoconjunctivitis, atopic keratoconjunctivitis, superior limbic keratoconjunctivitis, keratoconjunctivitis sicca, phlyctenular keratoconjunctivitis, marginal keratolysis, surgical procedures (e.g., radial keratotomy), scleritis, Kaposi's sarcoma, lipid degeneration (e.g., primary lipid keratopathy), corneal graft rejection, and contact lens overuse.
- 10 Choroidal neovascularization and retinal vascular defects may be related to a plurality of diseases and disorders including, but not limited to, diabetic retinopathy, macular degeneration, systemic lupus erythematosus, retinopathy of prematurity, retina edema (e.g., macular edema), traumatic eye injury, surgical procedures (e.g., laser eye surgery), sickle cell anemia, sarcoidosis, syphilis, Lyme disease, Behcet's disease, Eales disease, Paget's disease, presumed ocular
- 15 histoplasmosis syndrome, Best disease (Best vitelliform macular dystrophy (BVMD)), myopia, vein occlusion, artery occlusion, carotid obstructive disease, pseudoxanthoma elasticum, retinal detachment, toxoplasmosis, mycobacterial infections, infections resulting in retinitis or choroiditis (e.g., multifocal choroiditis), uveitis, vitritis, pars planitis, optic disc pits, and hyperviscosity syndrome.
- 20 Retinal atrophy may be related to a plurality of diseases and disorders including, but not limited to, age-related macular degeneration (AMD), macular atrophy, diabetic retinopathy, Stargardt disease, Sorsby's fundus dystrophy (SFD), retinoschisis, and retinitis pigmentosa. In one embodiment, the AMD is geographic atrophy or advanced AMD (e.g., advanced dry AMD). In one embodiment, the AMD is dry AMD. In one embodiment, the macular atrophy is related to
- 25 neovascularization and/or geographic atrophy.
- In one embodiment, the ophthalmological disorder is an intraocular neovascular disease. Examples of intraocular neovascular diseases include, but are not limited to, age-related macular degeneration (AMD), diabetic retinopathy, ischemia-related retinopathies, proliferative retinopathies, diabetic macular edema, pathological myopia, von Hippel-Lindau disease,
- 30 presumed ocular histoplasmosis syndrome, retinal vein occlusion (RVO) (e.g., CRVO, BRVO),

choroidal neovascularization (CNV), corneal neovascularization, retinal neovascularization, and retinopathy of prematurity (ROP).

For example, and not limitation, in one embodiment, the ophthalmological disorder is age-related macular degeneration (AMD), retinopathy of the eye, polypoidal choroidal
5 vasculopathy (PCV), diabetic macular edema, dry eye disease, Behcet's disease, retina detachment, glaucoma, uveitis (e.g., infectious and non-infectious uveitis), retinitis pigmentosa, Leber congenital amaurosis, Stargardt disease, traumatic eye injury, or conjunctivitis (e.g., infectious conjunctivitis, non-infectious conjunctivitis, allergic conjunctivitis). In one embodiment, the AMD is geographic atrophy (GA), wet AMD, or dry AMD. In one
10 embodiment, the GA. In one embodiment, the retinopathy of the eye is diabetic retinopathy (DR) or retinopathy of prematurity (ROP). In one embodiment, the retinopathy of the eye is high-altitude retinopathy. In one embodiment, the conjunctivitis is infection conjunctivitis or non-infections conjunctivitis. In one embodiment, the conjunctivitis is allergic conjunctivitis.

In one embodiment, the IL-33 binding protein or the pharmaceutical composition
15 described herein is administered with other medicaments. For example, and not limitation, the IL-33 binding protein or the pharmaceutical composition described herein may be administered with a beta2-agonist (e.g., a short-acting beta2-agonist (SABA) or a long-acting beta2-agonist (LABA),) an anticholinergic (e.g., a short-acting anticholinergic (SAMA) or a long-acting anticholinergic (LAMA)), a methylxanthine, a corticosteroid (e.g., an inhaled corticosteroid
20 (ICS), an oral corticosteroid (OCS), an intravenous corticosteroid, a topical corticosteroid) or steroid, a phosphodiesterase-4 inhibitor (e.g., roflumilast), a leukotriene receptor antagonist (LTA) (e.g., montelukast, zafirlukast), a mucolytic agent (e.g., erdosteine, carbocysteine, N-acetylcysteine), an analgesic, an antiviral, an antibiotic, an antifungal agent, aspirin, a
25 antihypertensive agent (e.g., calcium channel blocker, thiazide diuretic, beta-blocker, alpha-blocker, angiotensin-converting enzyme (ACE) inhibitor, angiotensin II receptor antagonist), a mast cell stabilizer (e.g., nedocromil sodium, cromolyn sodium), an antioxidant, oxygen, or a combination thereof. In one embodiment, one or more of the other medicaments is included in a kit with the IL-33 binding protein or the pharmaceutical composition described herein.

30 Examples of SABAs include, but are not limited to, fenoterol, levalbuterol, salbutamol (albuterol), pirbuterol, metaproterenol, and terbutaline. Examples of LABAs include, but are not

limited to, arformoterol, formoterol, indacaterol, olodaterol, and salmeterol. Examples of SAMAs include, but are not limited to, ipratropium bromide and oxitropium bromide. Examples of LAMAs include, but are not limited to, aclidinium bromide, glycopyrronium bromide, tiotropium, umeclidinium, glycopyrrolate, and revefenacin. Examples of corticosteroids include, but are not limited to, prednisone, prednisolone (e.g., methylprednisolone), dexamethasone, dexamethasone triamcinolone, hydrocortisone, betamethasone, beclomethasone, budesonide, mometasone, flunisolide, dexamethasone acetate/phenobarbital/theophylline, fluticasone propionate, and fluticasone furoate. Examples of methylxanthines include, but are not limited to, aminophylline and theophylline.

In one embodiment, the IL-33 binding protein or the pharmaceutical composition described herein may be administered with a combination treatment including, but not limited to, a SABA and a SAMA in one device (e.g., fenoterol and ipratropium, salbutamol and ipratropium); a LABA and a LAMA in one device (e.g., formoterol and aclidinium, formoterol and glycopyrronium, indacaterol and glycopyrronium, vilanterol and umeclidinium, olodaterol and tiotropium); a LABA and an ICS in one device (e.g., salmeterol and fluticasone propionate, formoterol and beclomethasone, formoterol and budesonide, formoterol and mometasone, vilanterol and fluticasone furoate); or a LABA, LAMA, and ICS in one device (e.g., fluticasone, umeclidinium, and vilanterol; beclomethasone, formoterol, and glycopyrronium; budesonide, formoterol, and glycopyrrolate).

For example, and not limitation, a subject with COPD or asthma is provided with the IL-33 binding protein or the pharmaceutical composition described herein administered with an additional treatment including a SABA, a SAMA, a LABA, a LAMA, and/or a corticosteroid or steroid (e.g., an ICS). In one embodiment, a subject with COPD is provided with the “standard of care”, which refers to treatments commonly used to treat COPD (e.g., maintenance therapy), including an ICS and a LABA, a LAMA and a LABA, or an ICS, a LAMA, and a LABA.

In a first aspect of the invention, the present disclosure provides an IL-33 binding protein comprising:

- (i) CDRH1, CDRH2, and CDRH3 from SEQ ID NO:20 and CDRL1, CDRL2, and CDRL3 from SEQ ID NO:25, wherein SEQ ID NO:20 comprises:

QVQLVQSGAEVKKPGASVKVSCASGYTFISYGMHWVRQAPGQGLEWMGEINPH
GGSTSYAQKFX₁GRVTMTRDTSTSTVYMESSLRSEDTAVYYCARPSAAYSHYLG
X₂DX₃WGRGTLVTVSS; and

wherein SEQ ID NO:25 comprises:

5 DIQMTQSPSSVSASVGDRVTITCRX₄SQGIX₅WLX₆WYQQKPGKAPKLLIYAX₇S
X₈LQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQAX₉VX₁₀PLTFGGGTKV
EIK; and

wherein:

10 X₁ = K or Q; X₂ = I or L; X₃ = I, L, or M; X₄ = A or T; X₅ =
P or S; X₆ = A or S; X₇ = A or G; X₈ = R or S; X₉ = H or N;
and X₁₀ = F or L; or

(ii) one or more CDR variants of (i), wherein the variant has 1, 2, or 3 amino acid
modifications.

15 In a second aspect of the invention, the present disclosure provides an IL-33 binding
protein, wherein the IL-33 binding protein comprises:

(i) CDRH1, CDRH2, and CDRH3 from any one of SEQ ID NOs:21-24 and CDRL1,
CDRL2, and CDRL3 from any one of SEQ ID NOs:26-28; or

(ii) one or more CDR variants of (i), wherein the variant has 1, 2, or 3 amino acid
modifications.

20 In an embodiment of the first aspect or the second aspect of the invention, the IL-33
binding protein comprises CDRH1, CDRH2, and CDRH3 from SEQ ID NO:21 and CDRL1,
CDRL2, and CDRL3 from SEQ ID NO:26.

25 In an embodiment of the first aspect or the second aspect of the invention, the IL-33
binding protein comprises CDRH1, CDRH2, and CDRH3 from SEQ ID NO:22 and CDRL1,
CDRL2, and CDRL3 from SEQ ID NO:26.

In an embodiment of the first aspect or the second aspect of the invention, the IL-33
binding protein comprises CDRH1, CDRH2, and CDRH3 from SEQ ID NO:23 and CDRL1,
CDRL2, and CDRL3 from SEQ ID NO:27.

30 In an embodiment of the first aspect or the second aspect of the invention, the IL-33
binding protein comprises CDRH1, CDRH2, and CDRH3 from SEQ ID NO:24 and CDRL1,
CDRL2, and CDRL3 from SEQ ID NO:28.

In an embodiment of the first aspect or the second aspect of the invention, the IL-33 binding protein comprises CDRH1 of SEQ ID NO:1, CDRH2 of SEQ ID NO:3, CDRH3 of SEQ ID NO:6, CDRL1 of SEQ ID NO:10, CDRL2 of SEQ ID NO:14, and CDRL3 of SEQ ID NO:18.

5 In an embodiment of the first aspect or the second aspect of the invention, the IL-33 binding protein comprises CDRH1 of SEQ ID NO:1, CDRH2 of SEQ ID NO:4, CDRH3 of SEQ ID NO:7, CDRL1 of SEQ ID NO:10, CDRL2 of SEQ ID NO:14, and CDRL3 of SEQ ID NO:18.

In an embodiment of the first aspect or the second aspect of the invention, the IL-33 binding protein comprises CDRH1 of SEQ ID NO:1, CDRH2 of SEQ ID NO:3, CDRH3 of SEQ ID NO:8, CDRL1 of SEQ ID NO:11, CDRL2 of SEQ ID NO:15, and CDRL3 of SEQ ID NO:18.

10 In an embodiment of the first aspect or the second aspect of the invention, the IL-33 binding protein comprises CDRH1 of SEQ ID NO:1, CDRH2 of SEQ ID NO:3, CDRH3 of SEQ ID NO:7, CDRL1 of SEQ ID NO:12, CDRL2 of SEQ ID NO:16, and CDRL3 of SEQ ID NO:19.

In an embodiment of any one of the preceding aspects or corresponding embodiments, the IL-33 binding protein comprises a heavy chain variable (VH) domain having at least 90% identity to any one of SEQ ID NOs:20-24 and a light chain variable (VL) domain having at least 90% identity to any one of SEQ ID NOs:25-28.

In an embodiment of any one of the preceding aspects or corresponding embodiments, the IL-33 binding protein comprises a VH domain having at least 90% identity to SEQ ID NO:22 and a VL domain having at least 90% identity SEQ ID NO:26.

20 In an embodiment of any one of the preceding aspects or corresponding embodiments, the IL-33 binding protein is an antibody or binding fragment thereof.

In an embodiment of any one of the preceding aspects or corresponding embodiments, the IL-33 binding protein is a monoclonal antibody or binding fragment thereof.

25 In an embodiment of any one of the preceding aspects or corresponding embodiments, the antibody or binding fragment thereof is a human IgG antibody or binding fragment thereof.

In an embodiment of any one of the preceding aspects or corresponding embodiments, the human IgG antibody or binding fragment thereof is a human IgG1 antibody or binding fragment thereof.

30 In an embodiment of the first aspect or the second aspect of the invention, the human IgG1 antibody or binding fragment thereof is a human IgG1 κ antibody or binding fragment thereof.

In an embodiment of the first aspect or the second aspect of the invention, the antibody comprises a modified Fc region.

In an embodiment of the first aspect or the second aspect of the invention, the modified Fc region comprises Fc mutations to extend half-life. In one embodiment, the modified Fc region comprises Fc mutations to extend half-life as compared to an IL-33 antibody without any Fc mutations to extend half-life. In one embodiment, the modified Fc region comprises Fc mutations to extend half-life as compared to an IL-33 antibody without said Fc mutations to extend half-life.

In an embodiment of the first aspect or the second aspect of the invention, the Fc mutation is YTE.

In a third aspect of the invention, the present disclosure provides an IL-33 binding protein comprising a heavy chain (HC) having at least 90% identity to any one of SEQ ID NOs:29-33 and a light chain (LC) having at least 90% identity to any one of SEQ ID NOs:34-37, wherein SEQ ID NO:29 comprises:

QVQLVQSGAEVKKPGASVKVSCKASGYTFISYGMHWVRQAPGQGLEWMGEIN
PHGGSTSYAQKFX₁GRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARPSAAY
SHYLGX₂DX₃WGRGTLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY
FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN
VNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLYI
TREPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE
LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSK
LTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK; and

wherein SEQ ID NO:34 comprises:

DIQMTQSPSSVSASVGDRVTITCRX₄SQGISX₅WLX₆WYQQKPGKAPKLLIYA
X₇SX₈LQSGVPSRFSGSGSGTDFTLTITSSLPEDFATYYCQQAX₉VX₁₀PLTFG
GGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD
NALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSS
PVTKSFNRGEC; and

wherein:

$X_1 = K \text{ or } Q; X_2 = I \text{ or } L; X_3 = I, L, \text{ or } M; X_4 = A \text{ or } T;$
 $X_5 = P \text{ or } S; X_6 = A \text{ or } S; X_7 = A \text{ or } G; X_8 = R \text{ or } S; X_9 =$
 $H \text{ or } N; \text{ and } X_{10} = F \text{ or } L.$

In an embodiment of the third aspect of the invention, the IL-33 binding protein is an
5 antibody comprising an HC having at least 90% identity to SEQ ID NO:31 and an LC having at
least 90% identity to SEQ ID NO:35.

In an embodiment of the third aspect of the invention, the IL-33 binding protein is an
antibody comprising an HC of SEQ ID NO:31 and an LC of SEQ ID NO:35.

In a fourth aspect of the invention, the present disclosure provides a pharmaceutical
10 composition comprising the IL-33 binding protein as defined in any one of the above aspects or
embodiments of the invention and a pharmaceutically acceptable excipient.

In a fifth aspect of the invention, the present disclosure provides a method of treating or
preventing a disease or condition in a human in need thereof comprising administering to the
human a therapeutically effective amount of the IL-33 binding protein of any one of the first
15 three aspects of the invention and corresponding embodiments, or the pharmaceutical
composition of the fourth aspect of the invention.

In a sixth aspect of the invention, the present disclosure provides an IL-33 binding
protein of any one of the first three aspects of the invention and corresponding embodiments, or
a pharmaceutical composition of the fourth aspect of the invention, for use in treating or
20 preventing a disease or condition.

In a seventh aspect of the invention, the present disclosure provides use of the IL-33
binding protein of any one of the first three aspects of the invention and corresponding
embodiments, or the pharmaceutical composition of the fourth aspect of the invention, in the
manufacture of a medicament for treating or preventing a disease or condition.

25 In an embodiment of the method of the fifth aspect of the invention, the IL-33 binding
protein or pharmaceutical composition for use of the sixth aspect of the invention, or the use of
the seventh aspect of the invention, the disease or condition is chronic obstructive pulmonary
disease (COPD), asthma, bronchitis, bronchiolitis, acute respiratory failure, inflammatory lung
diseases, diabetic kidney disease, endometriosis, chronic rhinosinusitis with nasal polyps, food
30 hypersensitivity, food allergy, peanut allergy, allergic rhinitis, eosinophilic oesophagitis, atopic
dermatitis, cystic fibrosis, or chronic urticaria.

In an embodiment of the method of the fifth aspect of the invention, the IL-33 binding protein or pharmaceutical composition for use of the sixth aspect of the invention, or the use of the seventh aspect of the invention, the disease or condition is COPD.

5 In an eighth aspect of the invention, the present disclosure provides a nucleic acid sequence or plurality of nucleic acid sequences encoding an IL-33 binding protein according to any one of the first three aspects of the invention and corresponding embodiments.

In a ninth aspect of the invention, the present disclosure provides a nucleic acid sequence or plurality of nucleic acid sequences comprising any one of SEQ ID NOs:59-64 and/or any one of SEQ ID NOs:69-76.

10 In a tenth aspect of the invention, the present disclosure provides a nucleic acid sequence or plurality of nucleic acid sequences comprising any one of SEQ ID NOs:65-68 and/or any one of SEQ ID NOs:77-79.

In an eleventh aspect of the invention, the present disclosure provides a nucleic acid sequence or plurality of nucleic acid sequences comprising SEQ ID NO:66 and/or SEQ ID
15 NO:77.

In a twelfth aspect of the invention, the present disclosure provides an expression vector comprising the nucleic acid sequence or plurality of nucleic acid sequences of the eighth, ninth, tenth, or eleventh aspects of the invention.

20 In a thirteenth aspect of the invention, the present disclosure provides a host cell that comprises the nucleic acid sequence or plurality of nucleic acids of any one of the eighth, ninth, tenth, or eleventh aspects of the invention, or the expression vector of the twelfth aspect of the invention.

In a fourteenth aspect of the invention, the present invention provides a method of producing an IL-33 binding protein, comprising culturing the host cell as defined in the
25 thirteenth aspect of the invention under conditions suitable for expression of said nucleic acid sequence, plurality of nucleic acid sequences, or vector, whereby a polypeptide comprising the IL-33 binding protein is produced.

In a fifteenth aspect of the invention, the present disclosure provides an IL-33 binding protein produced by the method of the fourteenth aspect of the invention.

30 In a sixteenth aspect of the invention, the present disclosure provides an IL-33 binding protein that binds to human IL-33 at amino acid residues 219-227 (SEQ ID NO:87).

In one embodiment of the sixteenth aspect, the IL-33 binding protein also binds to human IL-33 at amino acid residues 164-182 (SEQ ID NO:86).

In one embodiment, the present disclosure provides the IL-33 binding protein of the sixteenth aspect of the invention and corresponding embodiments, wherein the IL-33 binding protein further binds one or more of the following sequences: SEQ ID NO:84, SEQ ID NO:85, and LSE (residues 267-269).

In one embodiment, the present disclosure provides the IL-33 binding protein of the sixteenth aspect of the invention and corresponding embodiments, wherein the IL-33 binding protein is further defined in any of the first, second, third, and fifteenth aspects of the invention and corresponding embodiments.

In a seventeenth aspect of the invention, the present disclosure provides an IL-33 binding protein that binds to human IL-33 and competes for binding to the human IL-33 with a reference IL-33 binding protein that binds to the IL-33 at amino acid residues 219-227 (SEQ ID NO:87), and optionally at amino acid residues 164-182 (SEQ ID NO:86), wherein the reference IL-33 binding protein comprises:

- (i) CDRH1, CDRH2, and CDRH3 from SEQ ID NO:20 and CDRL1, CDRL2, and CDRL3 from SEQ ID NO:25, wherein SEQ ID NO:20 comprises:

QVQLVQSGAEVKKPGASVKVSCKASGYTFISYGMHWVRQAPGQGLEWMGEIN
PHGGSTSYAQKFX₁GRVTMTTRDTSTSTVYMELSSLRSEDTAVYYCARPSAAY
SHYLGX₂DX₃WGRGTLTVSS; and

wherein SEQ ID NO:25 comprises:

DIQMTQSPSSVSASVGDRVTITCRX₄SQGISX₅WLX₆WYQQKPGKAPKLLIYA
X₇SX₈LQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQAX₉VX₁₀PLTFG
GGTKVEIK; and

wherein:

X₁ = K or Q; X₂ = I or L; X₃ = I, L, or M; X₄ = A or T;
X₅ = P or S; X₆ = A or S; X₇ = A or G; X₈ = R or S; X₉ =
H or N; and X₁₀ = F or L;

- (ii) CDRH1, CDRH2, and CDRH3 from any one of SEQ ID NOs:21-24 and CDRL1, CDRL2, and CDRL3 from any one of SEQ ID NOs:26-28; or

(iii) one or more CDR variants of (i) or (ii), wherein the variant has 1, 2, or 3 amino acid modifications.

In one embodiment of the seventeenth aspect, the reference IL-33 binding protein also binds to human IL-33 at amino acid residues 164-182 (SEQ ID NO:86).

5 In one embodiment, the present disclosure provides the IL-33 binding protein of the seventeenth aspect of the invention and corresponding embodiments, wherein the reference IL-33 binding protein further binds one or more of the following sequences: SEQ ID NO:84, SEQ ID NO:85, and LSE (residues 267-269).

10 In an eighteenth aspect, the present invention provides an IL-33 binding protein that binds to human IL-33 and competes for binding to the human IL-33 with a reference IL-33 binding protein, wherein the reference IL-33 binding protein comprises:

(i) CDRH1, CDRH2, and CDRH3 from SEQ ID NO:20 and CDRL1, CDRL2, and CDRL3 from SEQ ID NO:25, wherein SEQ ID NO:20 comprises:

15 QVQLVQSGAEVKKPGASVKVSCKASGYTFISYGMHWVRQAPGQGLEWMGEIN
PHGGSTSYAQKFX₁GRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARPSAAY
SHYLGX₂DX₃WGRGTLTVTVSS; and

wherein SEQ ID NO:25 comprises:

20 DIQMTQSPSSVSASVGDRVTITCRX₄SQGISX₅WLX₆WYQQKPGKAPKLLIYA
X₇SX₈LQSGVPSRFSGSGSGTDFTLTITSSLPEDFATYYCQQA₉VX₁₀PLTFG
GGTKVEIK; and

wherein:

X₁ = K or Q; X₂ = I or L; X₃ = I, L, or M; X₄ = A or T;
X₅ = P or S; X₆ = A or S; X₇ = A or G; X₈ = R or S; X₉ =
H or N; and X₁₀ = F or L;

25 (ii) CDRH1, CDRH2, and CDRH3 from any one of SEQ ID NOs:21-24 and CDRL1, CDRL2, and CDRL3 from any one of SEQ ID NOs:26-28; or

(iii) one or more CDR variants of (i) or (ii), wherein the variant has 1, 2, or 3 amino acid modifications.

EXAMPLES

Example 1 – Generation and isolation of IL-33 binding proteins

Fully human antibodies specific for human IL-33 were generated using a yeast-based platform and isolated using biotinylated recombinant human and cynomolgus IL-33 combined with magnetic cell sorting and FACS-based selection techniques. Antibody 22A06-1 was identified from the naïve yeast libraries as a hit and its heavy and light chain variable regions were sequenced.

22A06-1 underwent two further successive cycles of affinity maturation, yielding four high-performing clones: 22A06-429, 22A06-458, 22A06-481 and 22A06-502. Anti-IL-33 antibody 22A06-458 was reformatted with the 'YTE' Fc mutations for half-life extension.

Example 2 - Affinity in solution of an IL-33 binding protein to human and cynomolgus IL-33

The affinity of IL-33 binding protein 22A06-458 for human and cynomolgus monkey IL-33 at room temperature (25°C) and 37°C was determined using an MSD-SET (MESOSCALE DISCOVERY Solution Equilibrium Titration) assay. Two anti-IL-33 antibodies (in-house) were included in the assay as positive controls and a human IgG1 isotype control (in-house) was included as a negative control for non-specific binding.

A. Methods

Two identical 96 well polypropylene plates were prepared with biotinylated human IL-33 (in-house) at a constant concentration of 30pM and the antibodies to be tested titrated 1 in 3 from 1nM to 5×10^{-5} nM across the plate, with a final 1 in 10 dilution to 5×10^{-6} nM. All antibodies were tested in duplicate within the plate. Both plates were incubated for 24 hours, one at room temperature, the other at 37°C.

After 24 hours, the same antibodies (20nM in PBS) were coated on to two identical standard bind MSD plates (Mesoscale Discovery #L15XA) for 30 minutes at room temperature. Plates were then blocked with starting block blocking buffer for 30 minutes with shaking at 700 rpm, followed by three washes with wash buffer. The incubated solutions were added to the MSD plates (each incubation temperature on its own MSD plate) for 150 seconds with shaking at 700 rpm followed by one wash. Antigen captured on the plate was detected with SULFO-TAG -labeled streptavidin (Mesoscale Discovery #R32AD-1). The plates were washed three times with wash buffer and then read on the MSD Sector Imager instrument using 1 × Read

Buffer T (Mesoscale Discovery #R92TC-1) with surfactant. The percent free antigen was plotted as a function of titrated antibody concentration in GraphPad Prism and fit to a quadratic equation.

To determine the affinity to cynomolgus monkey IL-33 (in-house), the assay was repeated with cynomolgus IL-33 at a constant concentration of 62.5pM and the antibodies titrated 1 in 3 from 12nM to 6×10^{-4} nM with a final 1 in 10 dilution to 6×10^{-5} nM.

B. Results

The affinity of 22A06-458 for human IL-33 is 3.3pM (range 1.8-6pM) at 25°C and 13.5pM (range 0-42pM) at 37°C. The affinity of 22A06-458 for cynomolgus IL-33 at 25°C is 27.5pM (range 4.6-43pM) and at 37°C is 56.5pM (range 30-98pM). See **Table 3** for results.

The positive control antibodies (mAb1 and mAb2) bound both human and cynomolgus IL-33, while the negative isotype control antibody showed no binding to IL-33. All the assay results met the acceptance criteria of $R^2 > 0.96$.

All the samples were run in duplicate within the plates and the reported results are therefore an average of n=2. One of the 22A06-458 replicates in the cynomolgus IL-33 25°C assay failed acceptance criteria due to an automation pipetting error. This plate was repeated and hence the result for this sample is an average of n=3.

Table 3. Affinity in solution of IL-33 antibodies to human and cynomolgus IL-33 by MSD-SET

Antibody	Antigen	Temp (°C)	Average K_D (pM)	Lower 95% confidence interval (pM)	Upper 95% confidence interval (pM)
22A06-458	human IL-33	25	3.3	1.8	6
		37	13.5	0	42
	cyno IL-33	25	27.5*	4.6	43
		37	56.5	30	98
Anti IL-33 control mAb1	human IL-33	25	<3.0	0	2.6
		37	9.65	0	17
	cyno IL-33	25	125	95	320
		37	565	130	990
Anti IL-33 control mAb2	human IL-33	25	<3.0	0	1.6
		37	<3.0	0	9
	cyno IL-33	25	61.5	20	100
		37	140	48	240

Affinity values are an average of n=2 replicates, apart from (*) which is n=3

Affinity values of 0 are not possible, but these lower confidence interval values of zero are due to the limits set in the prism macro used to analyze the results.

Following a similar procedure, affinities were determined for 22A06-429, 22A06-481 and 22A06-502 for human IL-33 to be between 1 pM and 5 pM at 25°C. Affinities were
5 determined for 22A06-429, 22A06-481 and 22A06-502 for cynomolgus IL-33 to be between 10 pM and 70 pM at 25°C.

Example 3 - Specificity of IL-33 binding protein for IL-33 over IL-1 α and IL-1 β

The specificity of IL-33 binding protein 22A06-458 for IL-33 over the most closely related proteins, IL-1 α and IL-1 β , was determined using a single shot binding assay on a
10 FORTEBIO OCTET RED384 biolayer interferometry (BLI) instrument.

A. Methods

22A06-458 at 20 μ g/mL PBSF (PBS + 0.1% IgG free BSA, in-house) was captured on protein A dip and read biosensors (Fortebio #18-5013) to a level of 1nm. The loaded sensors were dipped into either human IL-33 (in-house), IL-1 α (R&D Systems #200-LA), or IL-1 β
15 (R&D Systems #201-LB) all diluted to 100nM in PBSF for 300 seconds. The sensors were then dipped back into buffer for the dissociation phase for 600 seconds. Blank sensors were included to check for non-specific binding of the proteins to the sensors. Regeneration of the biosensor tips was carried out using 10mM glycine pH 1.5 (in-house). The analysis was run at 25°C, with a plate shaker speed of 1000rpm. Data were aligned to the baseline, but no kinetics model was
20 applied to the data.

B. Results

There was no non-specific binding of the proteins to the protein A sensors and there was visible binding of 22A06-458 to human IL-33, confirming that the assay was working. There was no visible binding of 22A06-458 to human IL-1 α or human IL-1 β .

25 **Example 4 - Competition between IL-33 binding protein and ST2 receptor for binding to IL-33**

A FORTEBIO OCTET RED384 BLI instrument was used to determine whether HEK expressed IL-33 binding protein 22A06-458 competes with the ST2 receptor for binding to IL-33. An anti-IL-33 positive control antibody (in-house) was included for comparison.

A. Methods

30 1. Competition with ST2-Fc receptor for binding to IL-33

22A06-458 at 20µg/mL in PBSF (phosphate buffered saline + 0.1% IgG free BSA, in-house) was captured on protein A dip and read biosensors (Fortebio #18-5013). The loaded sensors were blocked with a human IgG1 isotype control (in-house) at 100µg/mL for 15 minutes. The loaded and blocked sensors were dipped into ST2-Fc (R&D Systems #523-ST) at 20µg/mL
5 (100nM) to check that blocking was complete and that no binding of the Fc tag to the protein A sensors was observed.

The loaded and blocked sensors were dipped into human IL-33 (in-house) at 256nM in PBSF for 300 seconds and then into ST2-Fc at 100nM for 300 seconds. The assay was repeated in the reverse orientation with ST2-Fc loaded onto the protein A sensors, which were then dipped
10 into human IL-33 followed by the antibody.

A self-binning control was included where both the loading step and second binding step were carried out in ST2-Fc to check that the receptor competed with itself for binding to human IL-33 and that the assay format was therefore working.

Regeneration of the biosensor tips was carried out using 10mM glycine pH 1.5 (in-house). The analysis was run at 25°C, with a plate shaker speed of 1000rpm. Data were aligned
15 to the baseline, but no kinetics model was applied to the data.

2. Competition with monomeric ST2 for binding to IL-33

The method described in this example above was repeated with the secondary binding step being performed in either ST2-Fc (dimeric) or ST2-his (monomeric). The ST2 was at a
20 concentration of 75nM, and the association and dissociation phases were both 180 seconds long. The assay format was not reversed in this experiment, but all other conditions remain the same as described above.

B. Results

1. Competition with ST2-Fc (dimeric) receptor for binding to IL-33

25 No significant non-specific binding of IL-33 or ST2 to the protein A sensors was observed; therefore, all binding signals seen are specific. No second binding event was observed in the self-binning control step (ST2 competing against itself); therefore, the assay format was working.

When the 22A06-458:IL-33 complex captured on to the sensor was dipped into ST2-Fc, then a clear second binding event was visible, demonstrating that ST2 can bind to IL-33 that is bound to the antibody.

When the ST2:IL-33 complex was dipped into the solution of antibody, then only a very small binding signal (just above the blank buffer signal) was observed. This signal was smaller than would be expected for the binding of a full-size antibody and was much smaller than the ST2 signal in the reverse orientation.

The competition between 22A06-458 and ST2 appears to be dependent on binding order/orientation. When ST2 is bound to IL-33 first, the antibody is unable to bind properly.

When the antibody is bound first, ST2 is still able to bind IL-33. Results are shown in **Table 4**.
Table 4. *Competition with ST2-Fc receptor for binding to IL-33*

	Second Binding Event	
Loaded to sensors	22A06-458	ST2-Fc
22A06-458	ND	Non-competitor
ST2-Fc	Competitor	Competitor

ND = not determined

2. Competition with monomeric ST2-his (monomeric) receptor for binding to IL-33

No significant non-specific binding of IL-33 or ST2 to the protein A sensors or the blocking antibody was observed; therefore, all binding signals seen are specific.

When ST2 was binned against itself (i.e., loaded onto the sensor and as the second binding component in solution) as either the his-tagged monomeric version or the dimeric Fc tagged version, no second binding event was observed. Therefore, ST2 is competing with itself for binding to IL-33 which demonstrates that the assay format was working.

For 22A06-458 and the anti-IL-33 control antibody, a binding signal was observed as the antibody binds to IL-33 and then a second binding signal was observed for ST2 binding to IL-33 in both its his-tagged and Fc tagged forms. The binding signal was larger for the dimeric version because the molecular weight is larger.

Therefore, in this assay format 22A06-458 is non-competitive with the ST2 receptor and both the monomeric and dimeric ST2 receptor can form a ternary complex with the IL-33:antibody complexes. Results are shown in **Table 5**.

Table 5. *Competition with monomeric ST2 receptor for binding to IL-33*

		Second Binding Step	
		ST2-his	ST2-Fc
Loaded onto Sensors	22A06-458	Non-competitor	Non-competitor
	Anti IL-33 control	Non-competitor	Non-competitor
	ST2-Fc	Competitor	Competitor

Example 5 - Affinity of IL-33 binding protein:IL-33 complex to ST2

A FORTEBIO OCTET RED384 was used to determine the difference in the binding affinity of IL-33 for ST2 receptor with or without IL-33 binding protein 22A06-458 present. An anti-IL-33 positive control antibody (in-house) was included for comparison.

A. Methods

Human ST2-his (R&D Systems #523-ST) at 20µg/mL in PBSF (PBS + 0.1% IgG free BSA, in-house) was captured onto anti-his dip and read biosensors (ForteBio #18-5120); blank sensors were also included for referencing. All the sensors were then blocked with an irrelevant his tagged protein (OX40-his, in-house) to prevent the his tag on human IL-33 binding to the sensors.

The loaded and blocked sensors were dipped into human IL-33 (in-house) at 100nM for 900 seconds and the dissociation step was carried out in PBSF buffer for 600 seconds. The assay was then repeated with the IL-33 pre-mixed with a ten-fold excess of 22A06-458 (1µM) and the assay repeated with all other steps remaining the same.

Regeneration of the biosensor tips was carried out using 10mM glycine pH 1.5 (in-house). The analysis was run at 25°C, with a plate shaker speed of 1000rpm. The data were double referenced (blank reference sensors and 0nM data subtracted) and aligned to the baseline. A steady state equilibrium model with a global fit was applied to the data using the FORTEBIO data analysis software v8.0.

The experiment was repeated on a separate day with fresh solutions to give n=2 data, which were averaged to give the reported result.

B. Results

No binding of IL-33-his to the blocked anti-his sensors was observed (demonstrating that the blocking was complete). No non-specific binding of 22A06-458 to the sensors was observed, though some was seen for the anti-IL-33 control antibody; however, this was removed in the double referencing process.

The affinity values determined in this experiment are for comparison purposes only, as after a 900 second association phase the binding curves had not reached equilibrium and were therefore not likely to do so within a reasonable time frame.

Binding of IL-33 to ST2 receptor was observed with an affinity of 7.2nM. Binding of the 22A06-458:IL-33 complex to ST2 was also observed and the binding signal was larger, which would be expected as the combined molecular weight is larger than IL-33 alone. The affinity of the 22A06-458:IL-33 complex for ST2 was 11.5nM.

The positive control antibody:IL-33 complex bound to ST2 with an affinity of 2.4nM.

These affinities are within normal assay variation of one another and are therefore comparable. Therefore, the affinity of IL-33 for ST2 receptor is not affected when IL-33 is complexed with 22A06-458. Results are shown in **Table 6**.

Table 6. *Affinity of IL-33 binding protein:IL-33 complex to ST2 vs affinity of IL-33 to ST2*

Sample	Average KD (nM)
Human IL-33	7.2
IL-33 + 22A06-458	11.5
IL-33 + anti IL-33 control	2.4

Example 6 - Binding of IL-33 binding protein to oxidized human and cynomolgus IL-33

A FORTEBIO OCTET RED384 biolayer interferometry (BLI) instrument was used to determine whether 22A06-458 binds to oxidized human and cynomolgus IL-33 (the physiologically inactive form). An anti-oxidized IL-33 positive control antibody (in-house) was included for reference.

A. Methods

Oxidized and reduced human and cynomolgus IL-33 (in-house) were captured via their his-tags to anti-his dip and read biosensors (Fortebio #18-5120); blank sensors were also

included for referencing. The sensors were then dipped into 22A06-458 at 1 μ M for 300 seconds (the positive control antibody was at 150nM). The dissociation step was carried out in PBSF buffer (in-house) for 300 seconds.

Regeneration of the biosensor tips was carried out using 10mM glycine pH 1.5 (in-house). The analysis was run at 25°C, with a plate shaker speed of 1000rpm. The data were aligned to the baseline, but no kinetics model was applied.

B. Results

No non-specific binding of the antibodies to the anti-his sensors was observed; therefore, the binding signals observed were specific. The positive control antibody had a large, clear binding signal to oxidized human IL-33 and a small but visible binding signal to cynomolgus IL-33, demonstrating the suitability of the oxidized material. The positive control antibody does not bind to the reduced form of IL-33.

22A06-458 had a very large binding signal to reduced human and cynomolgus IL-33, showing that the assay was working as expected. There was no significant binding signal observed for 22A06-458 to either human or cynomolgus oxidized IL-33. For the cynomolgus protein, the signal was completely flat. For human IL-33, the signal was lower than the normal binding threshold cut-offs (0.07nm vs threshold of 0.1nm) and much lower than the signal for reduced IL-33 (0.07nm vs 2.5nm). At the high concentrations used (1 μ M), this would be considered a non-binder.

These results were also replicated using different techniques (BIAcore and MSD-SET), which found the same results.

Example 7 – HDX-MS of IL-33 binding protein

Hydrogen-deuterium exchange coupled to mass spectrometry (HDX-MS, WATERS SYNAPT G2-SI) data for IL-33 binding protein 22A06-458 demonstrated protection at the peptides: YESQHPSNESGDGVDGKMLM [residues 66-85 for 6H-IL33(113-270), in-house] (SEQ ID NO:80) and within the peptide FVLHNMHSNC (residues 120-129) (SEQ ID NO:81). Accordingly, these protected areas are good candidates for the epitope of mAb 22A06-458.

Structural modelling was carried out using publicly available complex structures of human IL-33 and ST2 (4KC3, Liu et al., 2013, PNAS, 110(37), 14918-14923), and human IL1 β , human IL1R1 and human IL1RAcP (4DEP, Thomas et al., 2012, 19, 455-457) to determine the

potential binding mechanism for 22A06-458. The ST2 and IL1R1 protein chains from the two structures were superimposed using CCG (Chemical Computing Group) MOE (Molecular Operating Environment) 2015.1001 to orientate IL1RAcP in relation to the IL33/ST2 complex. Through this structural modeling, the aforementioned two protected peptides were identified as peptide loops within IL-33. If the 22A06-458 mAb were to interact with these loops, it could potentially inhibit binding of both ST2 and IL-1RAcP to IL-33. However, residues within the potential epitope of the mAb interact mainly with residues within domain 3 of ST2 and domains 2 & 3 of IL-1RAcP, and the prominent flexible loop within IL-33, especially, is distant to domains 1 & 2 of ST2. Counting the number of ST2 residues located within 4.5Å of IL-33, 18 are within domain 3 (of which 14 are those interacting with the HDX/MS peptides), 14 within domain 2, and 8 within domain 1. Over half of the contacts between IL-33 and ST2 are thus within domains 1 & 2 (22 residues). The linker between domains 2 and 3 of ST2 is 9 residues in length, and one could thus possibly conceive that domain 3 could potentially swing out of the way of 22A06-458, which may allow the antibody to bind IL-33, while at the same time domains 1 & 2 of ST2 are also free to bind IL-33.

Example 8 - Binding of IL-33 binding protein to FcγR receptors

The binding of IL-33 binding protein 22A06-458 to recombinant soluble human and cynomolgus Fc gamma receptors (FcγR) was assessed using a PROTEON XPR36 (BIORAD) surface plasmon resonance (SPR) instrument. Human IgG1 wildtype and human IgG1 Fc disabled isotype antibodies were included in the analysis as an assay control.

A. Methods

A murine anti-poly-histidine IgG was immobilized on a GLM biosensor chip (BioRad #176-5012) by primary amine coupling (GE Healthcare #BR100050). Antibodies to be tested were used as the analyte and passed over at 1024nM, 256nM, 64nM, 16nM, and 4nM with an injection of 0nM (i.e., buffer alone) used to double reference the binding curves. The murine anti-poly-histidine IgG surface was regenerated with 100mM phosphoric acid between interactions. The run was carried out at 25°C using HBS-EP as running buffer (Teknova #H8022). Data were fitted to the equilibrium model inherent to the PROTEON XPR36 (BIORAD) analysis software using a global R-max value.

B. Results

The anti-RSV wildtype and Fc disabled control antibodies behaved as expected. The wildtype antibody bound all human and cyno Fc gamma receptors and the Fc disabled control did not.

22A06-458 binds to all the human and cynomolgus monkey Fc gamma receptors tested with affinities which are comparable to the human IgG1 wildtype control antibody (see **Table 7** and **Table 8**).

Table 7. Binding of 22A06-458 to recombinant soluble human Fc gamma receptors

Antibody	K _D (nM)					
	hFcγRI	hFcγRIIa (H131)	hFcγRIIa (R131)	hFcγRIIb	hFcγRIIIa (V158)	hFcγRIIIa (F158)
22A06-458	24.3	574.0	502.0	5220.0	215.0	987.0
Anti-RSV hIgG1 WT control	28.3	884.0	528.0	Weak*	232.0	735.0
Anti-RSV hIgG1 Fc disabled control	NB	NB	NSB	NSB	NB	NB

NB = no binding

NSB = non-specific binding

10 Weak* = Binding observed, but too weak to generate an accurate value

Table 8. Binding to recombinant soluble cynomolgus monkey Fc gamma receptors

Antibody	cFcγRIIa	cFcγRIIb	cFcγRIIIa
22A06-458	2110.0	1100.0	119.0
Anti-RSV hIgG1 WT control	2210.0	1450.0	141.0
Anti-RSV hIgG1 Fc disabled control	NB	NB	NB

NB= no binding

Example 9 - Binding of IL-33 binding protein to recombinant human and cynomolgus monkey neonatal receptor (FcRn)

Binding of IL-33 binding protein 22A06-458 to human and cynomolgus recombinant neonatal receptor (FcRn) at pH 6.0 and pH 7.4 was assessed using a PROTEON XPR36 (BIORAD) surface plasmon resonance (SPR) instrument. A human IgG1 isotype control was included in the experiment as a control for the assay and a human IgG1 control containing the YTE mutation was included for comparison purposes.

A. Methods

Protein L (Thermo Scientific #21189) was immobilized on a CM5 chip (GE Healthcare #BR-1005-30) by primary amine coupling (GE Healthcare #BR100050). This surface was then used to capture the antibodies via the light chain and human and cynomolgus recombinant soluble FcRn were then passed over as the analyte at 512nM, 128nM, 32nM, 8nM, and 2nM. An injection of buffer alone (i.e., 0nM) was used to double reference the binding curves.

Regeneration of the protein L surface was carried out using 10mM Glycine-HCl pH 1.5.

The assay was run at 25°C in HBS-EP pH 7.4 buffer (Teknova #H8022) and repeated in HBS-EP pH 6.0 buffer. Data was analyzed using the equilibrium model inherent to the PROTEON's analysis software.

B. Results

The human IgG1 wildtype control performed as expected (i.e., binding was observed at pH 6.0 and no binding was observed at pH 7.4).

22A06-458 has binding affinities to human and cynomolgus FcRn at pH 6.0 comparable to those of the human IgG1 YTE control (**Table 9**). 22A06-458 shows a 5-to-6-fold improvement in its binding affinities to human and cynomolgus monkey FcRn at pH 6.0 compared to the hIgG1 wildtype control. There is also some weak low-level binding visible at pH 7.4 which is not visible for the wildtype IgG1 molecule. The binding affinities of 22A06-458 for human and cynomolgus monkey FcRn are comparable to the YTE control and improved over those seen for the wildtype control.

Table 9. Binding to recombinant human and cynomolgus monkey neonatal receptor (FcRn)

Antibody	K _D (nM)	
	Human	Cyno

	pH 6.0	pH 7.4	pH 6.0	pH 7.4
22A06-458	25.0	1230	24.7	2560
Anti-RSV hIg1 wildtype control	129	No binding	146	No binding
Human IgG1 YTE control	30.4	4330	39.3	2760

Example 10 - Binding of IL-33 binding protein to human complement component C1q

Binding of IL-33 binding protein 22A06-458 to human recombinant soluble complement C1q was assessed using a PROTEON XPR36 (BIORAD) surface plasmon resonance (SPR) instrument. Human IgG1 wildtype and human IgG1 Fc disabled isotype antibodies were included in the analysis as an assay control.

A. Methods

The antibodies to be tested were immobilized on a GLC chip (BioRad #176-5011) by primary amine coupling (GE Healthcare #BR100050). C1q (Sigma #C1740-5mg) was passed over the immobilized antibodies at 512nM, 128nM, 32nM, 8nM, 2nM, and 0nM (i.e., buffer alone). The blank interspot region of the chip was used to double reference binding curves. The analysis was carried out at 25°C and the running buffer for was HBS-EP (pH 7.4) with 10mM CaCl₂ (Teknova #H8022). Data were fitted to the equilibrium model, inherent to the PROTEON XPR36 (BIORAD) analysis software using a global R-max value.

B. Results

The anti-RSV wildtype and Fc disabled control antibodies behaved as expected (i.e., the wildtype antibody bound C1q and the Fc disabled control did not).

The binding affinities of 22A06-458 and the human IgG1 wildtype control antibody to human C1q were 213nM and 680nM, respectively (see **Table 10**). The affinity of 22A06-458 in this analysis is stronger than that of the wildtype control but is within the range of normal assay variation and is therefore comparable.

Table 10. Binding of 22A06-458 to human complement component C1q

Antibody	K _D (nM)
22A06-458	213

Anti-RSV hIgG1 wildtype control	680
Anti-RSV hIgG1 Fc disabled control	No binding

Example 11 - Characterization of human FcγRIIIa engagement with the complex of IL-33 binding protein and IL-33

Studies were carried out to assess the risk that cells treated with IL-33 binding protein 22A06-458 could become targets for antibody-dependent cellular cytotoxicity (ADCC) via engagement of FcγRIIIa.

A. Methods

To determine a positive control antibody for the target/effector cell engagement assay, anti-her1, anti-her2, anti-CD20, or anti-CD52 antibodies were mixed with 1.25×10^4 target HEK-BLUE IL-33 cells (InvivoGen #HKB-HIL-33) for 45 minutes at 37°C/5%CO₂. After the addition of 7.5×10^4 Jurkat-NFAT-FcγRIIIa-Luc cells (Promega #G7102) to each test well, all four antibodies were at assay concentrations from 1.7×10^{-4} to 66.7nM. After a further 6 hours of incubation at 37°C/5%CO₂, assay plates were allowed to equilibrate to room temperature for 15 minutes before the addition of ONE-GLO luciferase reagent (Promega #E6120). Luminescence intensity was determined using a Perkin Elmer multilabel VICTOR plate reader.

To determine the engagement of target HEK-BLUE IL-33 and effector Jurkat-NFAT-FcγRIIIa-Luc cells with complexed IL-33/22A06-458, IL-33 (in-house) was initially incubated with 22A06-458 for 30 minutes at room temperature. Separately, IL-33 was pre-incubated with either anti-RSV or anti-CD52 assay control antibodies.

Antibody/IL-33 mixtures were incubated with 1.25×10^6 target HEK-BLUE IL-33 cells per well of a 96-well plate (Costar #3197) for 45 minutes at 37°C/5%CO₂. After the addition of 7.5×10^6 Jurkat-NFAT-FcγRIIIa-Luc cells, IL-33 was at 1nM while test antibodies were present over a concentration range from 1.7×10^{-4} to 66.7nM. After a further six hours of incubation at 37°C/5%CO₂, assay plates were equilibrated to room temperature for 15 minutes before the addition of ONE-GLO luciferase reagent. Luminescence intensity was determined as above.

Each antibody was screened over an increasing concentration range. Each concentration of antibody was assayed in duplicate. Mean raw luminescence values were calculated and the standard deviations determined.

Raw data values were imported into Microsoft Excel and Target/Effector cell engagement at each antibody concentration was expressed as fold change using **Equation 1**:

$$\frac{\text{Mean luminescence value at test antibody concentration}}{\text{Mean luminescence value in absence of test antibody}} \quad \text{Equation 1}$$

Data were plotted in Grafit (Erithacus Software).

5 B. Results

Four antibodies (anti-her1, anti-her2, anti-CD20, and anti-CD52) were screened in a target HEK-BLUE IL-33, Jurkat NFAT-FcγRIIIa-Luc effector cell engagement assay. There was an absence of enhanced luminescent signal with an anti-her1, anti-her2, or anti-CD20 antibody. However, when compared to the absence of antibody, an anti-CD52 antibody showed enhanced
10 luminescence at concentrations in excess of 2.7nM. There was a 2.4-fold increase in cell engagement at 66.7nM. Based on this observation, the anti-CD52 antibody was selected as the assay positive control.

While an enhanced luminescence signal was seen when HEK-BLUE IL-33 and Jurkat-NFAT-FcγRIIIa-Luc cells were assayed with an anti-CD52 antibody (concentrations >2.7nM)
15 that had been pre-incubated with IL-33, there was no evidence of any change in luminescent signal when these two cell types were pre-incubated with anti-IL-33/anti-RSV, anti-IL-33/mAb1, or anti-IL-33/22A06-458 in two independent experiments.

Example 12 – Inhibition of multiple IL-33 isoforms by IL-33 binding protein

Several isoforms of IL-33 have been reported that could arise in vivo from alternative
20 mRNA splicing or by the action of different proteases present in the lung, dependent on the nature of the airway inflammation. For example, Lefrancais et al. (PNAS, 2012; 109:1673-8) reported that the activity of neutrophil proteases (Cathepsin G and neutrophil elastase) can lead to the generation of a number of mature biologically active forms of IL-33 (IL-33₉₅₋₂₇₀, IL-33₉₉₋₂₇₀, and IL-33₁₀₉₋₂₇₀). These forms were shown to be produced by activated human neutrophils ex vivo, to
25 be biologically active in vivo, and to have a ~10-fold higher activity than full-length IL-33 in cellular assays. Another novel form of IL-33 was reported as a short splice variant that lacks exon 3 containing a proposed caspase-1 cleavage site (Hong et al., J Biol Chem 2011; 286: 20078-86) termed spIL-33. Mature IL-33 (IL-33₁₁₃₋₂₇₀), the spIL-33, and the cleaved forms of IL-33 were recombinantly expressed and the ability of 22A06-458 to inhibit their activity was assessed in a

HEK-BLUE reporter assay. 22A06-458 was able to completely inhibit all isoforms of IL-33 but showed a decrease in activity against IL-33 isoforms compared to mature IL-33₁₁₃₋₂₇₀ (**Table 11**). spIL-33 showed the smallest variation compared with IL-33₁₁₃₋₂₇₀, with IL-33₉₉₋₂₇₀ showing the largest difference. It should be noted that this is not likely to reflect biological activity differences as only a single concentration of each IL-33 isoform was tested and the assay was not optimised to the activity for each individual cytokine.

Table 11. *Inhibition by 22A06-458 of 25pM of different isoforms of IL-33 in HEK-Blue reporter assay*

	IC50 (pM) (with 95% confidence intervals included)				
	95-270 IL-33	99-270 IL-33	109-270 IL-33	spIL-33	113-270 IL-33
22A06-458	162.2 (147.8 to 178.3)	436.2 (298.3 to 676.9)	254 (230.2 to 280.7)	90.26 (72.32 to 113.6)	18.18 (12.79 to 25.25)

Example 13 – Inhibition of eosinophil superoxide generation by IL-33 binding protein

Studies were carried out to measure the transient generation of superoxide anions in response to IL-33 stimulation of isolated eosinophils.

A. Methods

Whole human blood was collected from high eosinophil donors. Typically, 60mL was collected by venipuncture per donor into a sterile container with anti-coagulant, sodium heparin solution (10IU/mL).

Within 1 hour of collection, and using a microbiological safety cabinet, 20mL of anti-coagulated blood samples were transferred into 50mL falcon tubes and diluted by addition of 10mL PBS (Thermo Fisher #14190144) and 10mL 4% w/v dextran (Sigma Aldrich #31392-50G) solution. After gentle inversion to mix the blood, the tubes were allowed to stand on ice for up to 30 min allowing the red blood cells (RBC) to sediment.

The granulocytes were separated from the peripheral blood mononuclear cells (PBMCs) by layering the RBC depleted cell suspension (~30mL) onto 15mL FICOLL-PAQUE (Sigma

Aldrich #GE17-1440-02) pre-loaded into 50mL falcon tubes followed by centrifugation in a swing bucket rotor (300xg; 25 min; 18°C, no break).

The plasma and PBMCs were aspirated off leaving the RBC contaminated granulocyte pellets. Each separate cell pellet was re-suspended in 300µL PBS and pooled into a fresh falcon tube.

Contaminating RBCs were lysed through hypotonic shock by the addition of 20mL ice cold water (20-30s) followed by neutralization with the addition of 20mL 2x concentrated PBS.

The granulocytes were pelleted by centrifugation (300xg/ 5 min) and re-suspended in 1mL PBS and counted on a hemocytometer.

Untouched eosinophils were purified using an eosinophil isolation kit (Miltenyi Biotec #130-092-101) as per manufacturer's instructions. Non-eosinophils were indirectly magnetically labeled using a cocktail of biotin-conjugated antibodies as well as anti-Biotin MicroBeads. Highly pure eosinophils were obtained by depletion of the magnetically labeled cells. Isolated eosinophils were diluted to a cell density of 1×10^6 per mL in superoxide assay buffer (1x phosphate buffered saline (with $\text{Ca}^{2+}/\text{Mg}^{2+}$) + 0.1% BSA).

22A06-458 antibody was diluted to 8x final top assay concentration of 0.5µg/mL (3.33nM). Subsequent 1 in 3 serial dilutions were then carried out in superoxide assay buffer to generate an 8-point concentrations response curve. The diluted antibody was then mixed 1:1 (e.g., 30µL + 30µL in a V bottom polypropylene 96 well plate) with a single concentration of recombinant human (rhu) IL-33 (also made up to 8x final assay concentration (FAC) of 0.3ng/mL (15pM)). These pre-complex mixtures (now at 4x FAC) were incubated for 30 min at 37°C. The final concentration range of the antibody tested was 0.00152nM – 3.3nM.

For the control no antibody treatment (Positive control), IL-33 was mixed 1:1 with assay buffer and the Negative control consisted of assay buffer alone.

Sterile 384 well, white clear bottom plates (Corning #3707) were pre-coated with 1% BSA/PBS solution (25µL /well; 1hr incubation at 37°C). After aspiration the wells were washed once with 25 µL PBS.

30 µL of eosinophil cell suspension (at 1×10^6 cells/mL) was added per well (30×10^3 cells/well) followed by addition of 15 µL of pre-mixed luminol + enhancer solution (each kit component diluted 1:10 into PBS assay buffer) and allowed to pre-incubate with eosinophils for 15 min at 37°C.

Next, 15 µL of pre-complexed 22A06-458 + IL-33 was then added to the eosinophils in triplicate per concentration.

Non-complexed IL-33 and buffer alone were included as positive and negative controls, respectively.

5 The assay plate was then immediately placed into the SPECTRAMAX plate reader set at 37°C and luminescence read at 5 min intervals for a total time period of 60 min.

The peak/max response achieved during the transient superoxide response over the 60 min time period was calculated by the SPECTRAMAX instrument software (SoftMax Pro-6.4). All data was then calculated as % inhibition of the control response, i.e., relative to the mean of 8
10 high (positive control) and 8 low (negative control) wells on each plate as described in **Equation 2** below:

$$\frac{(\text{Pos Ctrl} - \text{Test Sample})}{(\text{Pos Ctrl} - \text{Neg Ctrl})} \times 100\% \quad \text{Equation 2}$$

A non-linear regression four-parameter curve fit was applied using the Graph Prism software. Data was presented as the mean IC₅₀ value and pIC₅₀ with the standard error of the
15 mean of the mean of n experiments.

B. Results

22A06-458 concentration dependently blocked the production of IL-33 stimulated superoxide in isolated human eosinophils. Inhibition by 22A06-458 was expressed as percentage inhibition of the positive control (15pM IL-33) and the non-stimulated negative control response
20 as shown in **Figure 1**. Inhibition by 22A06-458 was tested in 6 separate donor experiments. The antibody was tested twice in one donor, giving a total of 7 tests. The results have been summarized in **Table 12**. An IC₅₀ value of 19.65 ± 2.18 pM (n=7) was observed. The pIC₅₀ was calculated as 10.73 ± 0.05 (n=7).

Table 12. *Determination of Potency: Inhibition of IL-33 induced superoxide generation from eosinophils by pre-complexed 22A06-458.*
25

	Donor #33778	Donor #91933	Donor #30953	Donor #91885	Donor #91945	Donor #90980		Mean	SEM
	N=1	N=2	N=3	N=4	N=5	N=6	N=7		

pIC₅₀	10.66	10.63	10.78	10.59	10.83	10.98	10.61	10.73	0.05
IC₅₀ (pM)	21.85	23.5	16.5	25.6	14.8	10.5	24.8	19.65	2.18

Example 14 – Inhibition of IFN- γ production from CD4⁺ T cells by IL-33 binding protein

Studies were carried out to measure IL-33 and IL-12 co-stimulated IFN- γ secretion from isolated CD4⁺ T cells.

5 A. Methods

Whole human blood was collected from normal healthy donors. Typically, 80mL was collected by venipuncture per donor into a sterile container with anti-coagulant, sodium heparin solution (10IU/mL).

10 Within 1 hour of collection red blood cells were sedimented as described for the eosinophil preparation above. Following centrifugation, the layer of PBMCs at the Ficoll/plasma interface was gently removed from each tube using a sterile pastette and dispensed (~10-15mL) into a fresh 50mL falcon tube. The volume was made up to 50mL with PBS and the tubes centrifuged for 10 mins at 300g. After removal of the buffer by aspiration the cell pellets were pooled into a volume of 10mL PBS. Cell number was determined using a hemocytometer cell
15 counter.

CD4⁺ T cells were isolated using a CD4⁺ T Cell Isolation kit (Miltenyi Biotec # 130-096-533) according to the manufacturer's instructions. Non-CD4⁺ cells were labeled using a cocktail of biotin-conjugated antibodies. Non-target cells were then magnetically labeled with the CD4⁺ T Cell MicroBead Cocktail. Isolation of highly pure T cells was achieved by depletion of the
20 magnetically labeled non-CD4⁺ T cells. Isolated CD4⁺ T cells were suspended to 1.11×10^6 /mL in RPMI1640 tissue culture media (Thermo Fisher #31870).

A 20x cytokine mix of IL-33 +IL-12 +IL-2 was prepared in RPMI1640 T- cell assay media based on the dilutions shown in **Table 13**. 22A06-458 antibody was diluted to 20x final top assay concentration of 1.5 μ g/mL (10nM).

25 **Table 13. Dilutions for 20x cytokine mix of IL-33 +IL-12 +IL-2.**

Cytokine	Stock conc	Final assay concentrations required	Working stock conc (x20 final conc)	Dilution required
IL-33	2.1mg/mL	2.5ng/mL IL-33	50ng/mL IL33	1 in 42,000

IL-2	1mg/mL	2.5ng/mL IL-2	50ng/mL IL-2	1 in 20,000
IL-12	1mg/mL	12.5ng/mL IL-12	250ng/mL IL12	1 in 4000

Subsequent 1 in 4 serial dilutions were then carried out in culture media to generate a 6-point concentration response curve. The diluted antibody was then mixed 1:1 (e.g., 50 μ L + 50 μ L in a V bottom polypropylene sterile 96 well plate) with the cytokine mix of IL-33/IL-12/IL-2 (made up to 20x final assay concentration of 2.5ng/mL (125pM), 12.5ng/mL and 2.5ng/mL, respectively). The 1:1 diluted mixtures (now at 10x FAC) were incubated for 30 min at 37°C to allow the antibody to complex with the IL-33. The final concentration range of the antibody tested was between 0.0098nM - 10nM.

For the control no antibody treatment (positive control), IL-33 + cytokine mix (IL-12+IL-2) was combined 1:1 with assay media and the negative control consisted of assay media containing cytokine mix without IL-33.

Following co-incubation of 22A06-458 with the IL-33 cytokine mix, 20 μ L was added to a 96 well tissue culture plate in triplicate wells per concentration. Non antibody complexed IL-33 cytokine mix and media containing IL-12+IL-2 alone were included as positive and negative controls respectively. Next, 180 μ L of CD4⁺ T cell suspension was added to the test wells (2×10^5 cells/well) and the plate incubated (5% O₂/95% air) for a period of 18h at 37°C.

After 18h of incubation, the T-cells were centrifuged (300xg/ 5 min) and 150 μ L of the cell free supernatants transferred to a new 96 well plate. MSD plates pre-coated with anti- IFN- γ capture antibody were first pre-blocked with 1%w/v of Blocker B. Standards and samples were then added to the MSD plates and incubated for 1.5h at RT while shaking. IFN- γ specific detection antibody labeled with the MSD SULFO-TAG reagent was then added to all the samples and incubated for a further 1.5h at RT while shaking. After washing plates with PBS + 0.05% Tween-20 three times and addition of 2x Read Buffer to all the samples, the plates were read on the Sector Imager plate reader.

Levels of IFN- γ (pg/mL) were back calculated from the standard curve using the MSD analysis software (Discovery Workbench 4). All data was then calculated as % inhibition of the control response, i.e., relative to the mean of 6 high (positive control) and 6 low (negative control) wells on each plate as described in **Equation 2**. A non-linear regression four-parameter

curve fit was applied using the Graph Prism software. Data was presented as the mean IC₅₀ value and pIC₅₀ with the standard error of the mean.

B. Results

22A06-458 concentration dependently blocked IL-33+IL-12 stimulated IFN- γ secretion from isolated human CD4⁺ T cells. Inhibition by 22A06-458 was expressed as percentage inhibition of the positive control (125pM IL-33) and the non-stimulated negative control response as shown in **Figure 2**. Inhibition by 22A06-458 was tested in 6 separate donor experiments. The antibody was tested twice in one donor, giving a total of 7 tests. The results have been summarized in **Table 14**. An IC₅₀ value of 675.14 $\pm$ 101.39 pM (n=7) was observed and a pIC₅₀ was calculated as 9.2 $\pm$ 0.07 (n=7).

Table 14. *Determination of Potency: Inhibition of IL-33 induced IFN- γ secretion from CD4⁺ T cells by pre-complexed 22A06-458.*

	Donor # 91738	Donor # 91806	Donor # 91936	Donor # 90290	Donor # 33778	Donor # 90980		Mean	SEM
	N=1	N=2	N=3	N=4	N=5	N=6	N=7		
pIC ₅₀	8.97	9.02	9.54	9.22	9.1	9.3	9.24	9.2	0.073
IC ₅₀ (pM)	1056	936	282	591	790	497	574	675.14	101.39

Example 15 – Inhibition of inflammatory cytokine production from HUVEC cells by IL-33 binding protein

Studies were carried out to measure release of IL-8 and IL-6 in response to direct IL-33 stimulation of human umbilical vein endothelial cells (HUVECs).

A. Methods

HUVECs (pooled donor; Promocell #C12203) were cultured in a T75cm² collagen coated tissue culture flask (Greiner bio-one #658950) until approximately 80-90% confluent. After removal of medium and a wash of the cells with PBS (12mL), the adherent cells were detached by the addition of 2mL Tryple Express cell detachment solution (5 min; 37°C) (Thermo Fisher #12604-13). 10mL fresh endothelial culture media was added to the flask to collect the detached cells then transferred to a 50mL flacon tube and centrifuged (300xg; 5min). Pelleted cells were resuspended in 1mL fresh endothelial media and counted using a haemocytometer.

HUVEC cells (passage 7/8/9) were seeded into 96 well tissue culture plates (collagen coated; Corning Biocoat collagen I #354649) at 1×10^4 cells per well (100 μ L) in endothelial growth media. Plates were incubated for 24h at 37°C to allow the cells to settle and adhere.

22A06-458 antibody was diluted to 6x final top assay concentration of 1.5 μ g/mL (10nM). Subsequent 1 in 4 serial dilutions were then carried out in culture media to generate a 6-point concentration response curve. The diluted antibody was then mixed 1:1 (e.g., 100 μ L + 100 μ L in a V bottom polypropylene sterile 96 well plate) with IL-33 (made up to 6x final assay concentration of 10ng/mL (500pM). The 1:1 diluted mixtures (now at 3x FAC) were incubated for 30 min at 37°C to allow the antibody to complex with the IL-33. The final concentration range of the antibody tested was between 0.0098nM - 10nM.

For the control no antibody treatment (Positive control), IL-33 was combined 1:1 with endothelial culture media and the Negative control consisted of endothelial media alone.

24h after HUVEC seeding into 96 well plates, the media was aspirated off and the cells washed once with 100 μ L fresh media by aspiration and 100 μ L was added back to each well. Following co-incubation of 22A06-458 with the IL-33, 50 μ L was added to the 96 well HUVEC culture plate in triplicate wells per concentration. Non antibody complexed IL-33 and media alone were included as positive and negative controls, respectively. The plate was then placed into 37°C incubator (5% O₂/95% air) for a period of 18h. After 18h of incubation, cell free supernatants were transferred to a new 96 well plate.

Concentrations of IL-8 in the cell supernatants were determined using a human IL-8 ELISA kit (R&D Systems DY208-05) according to the manufacture's protocol. Briefly, samples and standards were added to each well of a microplate, which was pre-coated with anti-human IL-8 monoclonal antibody and incubated for 2 hours. Each well was washed and incubated with the enzyme-linked polyclonal antibody specific for human IL-8 for 2h. The wells were washed to remove unbound antibody-enzyme reagent and substrate solution was added to each well. After incubation for 20 min at RT, the enzyme reaction was stopped. IL-8 concentrations were determined by comparison of the optical density values with the standard curve.

For determination of IL-6, standards and samples were added to MSD plates already pre-coated with anti-IL-6 capture antibody from a kit (Mesoscale Discovery #K151AKB-4). The MSD plates were incubated for 1.5h at RT while shaking. IL-6 specific detection antibody labeled with the MSD SULFO-TAG reagent was then added to all the samples and incubated for

a further 1.5h at RT while shaking. Plates were then washed with PBS + 0.05% Tween-20 three times and 2x Read Buffer added to all the samples. The plates were read on the Sector Imager plate reader.

Levels of IL-8 and IL-6 (pg/mL) were back calculated from the standard curve using the SPECTRAMAX analysis software (SoftMax Pro-6.4) and the MSD analysis software (Discovery Workbench 4), respectively. All data was then calculated as % inhibition of the control response, i.e., relative to the mean of 6 high (positive control) and 6 low (negative control) wells on each plate as described in **Equation 2**.

A non-linear regression four-parameter curve fit was applied using the Graph Prism software. Data was presented as the mean IC₅₀ value and pIC₅₀ with the standard error of the mean of the mean of n experiments.

B. Results

22A06-458 concentration dependently blocked IL-33 stimulated IL-8 (**Figure 3A**) and IL-6 (**Figure 3B**) secretion from HUVECs. Inhibition by 22A06-458 was calculated as percentage inhibition of the positive control (500pM IL-33) and the non-stimulated negative control response. Inhibition by 22A06-458 was tested in 3 separate HUVEC passage experiments. The results have been summarized in **Table 15**. IC₅₀ values of 389.90 ± 61.22 pM and 217.30 ± 8.61 pM were observed for IL-8 and IL-6 secretion, respectively. The respective pIC₅₀ values of 9.42 ± 0.07 and 9.66 ± 0.02 for IL-8 and IL-6 secretion were calculated.

Table 15. *Determination of Potency: Inhibition of IL-33 induced IL-8 and IL-6 secretion from HUVECs by pre-complexed 22A06-458.*

Inhibition of IL-8	HUVEC;P7	HUVEC;P8	HUVEC;P9	Mean	SEM
pIC ₅₀	9.31	9.56	9.40	9.42	0.07
IC ₅₀ (pM)	489.00	278.30	402.50	389.90	61.22
Inhibition of IL-6	HUVEC;P7	HUVEC;P8	HUVEC;P9	Mean	SEM
pIC ₅₀	9.67	9.69	9.63	9.66	0.02
IC ₅₀ (pM)	213.90	204.40	233.60	217.30	8.61

Example 16 – Inhibition of basophil degranulation by IL-33 binding protein

Studies were carried out to measure release of the degranulation product, β -hexosaminidase, following co-stimulation with IL-33 and anti-IgE.

A. Methods

Whole human blood was collected from allergic donors. Typically, 60mL was collected by venipuncture per donor into a sterile container with anti-coagulant, sodium heparin solution (10IU/mL).

Within 1 hour of collection, and using a microbiological safety cabinet, basophils were isolated according to the basophil isolation kit (Stem Cell #19069) instructions. 10mL of anti-coagulated blood samples were transferred into 15mL conical tubes and 2mL HETASEP (Stem Cell #07906) added and mixed with the blood by inversion. The tubes were centrifuged at 110g (6 min) at room temperature (RT) with the brake off. The samples were then removed and allowed to sit undisturbed for 5-10 minutes until the red blood cell:plasma interface was approximately 40% of the total volume. The plasma fraction containing the nucleated cells was harvested into a 50mL falcon tubes and 4 parts of cold basophil buffer added to 1 part harvested plasma. Following centrifugation (400g/10 minutes/RT), the supernatant was discarded, and the cell pellet washed to remove excess platelets by further centrifugation at 120g (10 min/RT). After aspiration of the supernatants the cells were counted and resuspended at 5×10^7 /mL in basophil buffer.

Cells at 5×10^7 cells/mL were transferred to 5mL polystyrene round bottom tubes to which 50 μ L of enrichment antibody cocktail (kit reagent) was added and allowed to incubate with the cells for 10 min at RT. 100 μ L of pre-mixed magnetic particles (kit reagent) were then added and mixed with the cells by pipetting and allowed to incubate for 10 min at RT. The volume in the tube was topped up to 2.5mL with basophil buffer and then placed inside the EasySep magnet (Stem Cell #18000) for 5 min/RT. The magnet was picked up and in one continuous motion the enriched cell suspension was poured into a new 5mL tube. The tube inside the magnet was discarded and the tube containing the enriched cell suspension placed into the magnet for a further 5 min. This step was repeated once more and the final contents containing the untouched basophils poured into a 15mL conical tube.

The enriched basophils were centrifuged (300g/ 5 min/ RT) and the cell pellet resuspended in RPMI1640 basophil medium (Thermo Fisher #31870) at a cell density of 1×10^6 /mL.

20 μ L of cell suspension (2×10^4 cells/well) was added per well of a 384 well sterile cell culture plate and the cells allowed to rest for 60 min in a 37°C incubator.

22A06-458 antibody was diluted to 8x final top assay concentration of 10µg/mL (66nM). Subsequent 1 in 10 serial dilutions were then carried out in basophil medium to generate a 5-point concentration response curve. The diluted antibody was then mixed 1:1 (e.g., 50µL + 50µL in a V-bottom polypropylene 96 well plate) with a single concentration of rhu IL-33 (in-house, also made up to 8x FAC) 100ng/mL (5.0nM)). These pre-complex mixtures (now at 4x FAC) were incubated for 40 min at RT. The final concentration range of 22A06-458 tested was 6.6pM - 66nM.

The anti-IgE cross linking antibody was diluted into basophil medium to 4x FAC of 1µg/mL.

10 For the control no 22A06-458 treatment, IL-33 was diluted to 4x FAC (100ng/mL) in basophil media and the negative non-treated control consisted of basophil medium alone.

To duplicate/triplicate wells of basophils in a 384 well plate (Corning #3701), 20µL of basophil medium only was added to represent the non-treated control. To the IL-33 only stimulated control, 10µL basophil medium (RPMI1640 (Thermo Fisher #31870) without phenol red + 10% FBS + 2mM L-glutamine (Thermo Fisher #25030)) plus 10µL of IL-33 was added. The anti-IgE negative control had 10µL basophil medium plus 10µL anti-IgE stimulus added per well. The positive control consisted of addition of 10µL IL-33 plus 10µL anti-IgE. For the test treatments, 10µL of the pre-complexed IL-33+22A06-458 was added per well followed by addition of 10µL anti-IgE. Final assay volume per well consisted of 40µL. The treated plate was incubated for a period of 40 min in a 37°C incubator.

After the incubation period the cell plate was centrifuged at 300g/ 3 min and the cell free supernatants transferred to a new 384 well plate (~30µL).

10µL of the cell free supernatant was then transferred into a black opaque 384 well plate (Greiner #781076) into which 10µL of the β-hexosaminidase substrate (diluted 1 in 100 to 500µM in citrate buffer (0.2M, pH=4.5) was added. After 60min incubation at 37°C, 36µL of Trizma buffer (Sigma Aldrich #T2819) was added to stop the reaction. β-hexosaminidase released from the basophils was quantitated by measuring the fluorescence intensity at Ex:356nm, Em:450nm on the SPECTRAMAX iQ plate reader (SoftMax Pro-6.4).

The fluorescence signal data generated by the β-hexosaminidase interacting with its substrate was first normalized by subtracting the background fluorescence signal from substrate + media only treated wells. The % inhibition of the control response was then calculated, i.e.,

relative to the mean of IL-33 + cross linked IgE (positive control) and cross linked IgE alone (negative control) wells as described in **Equation 2**.

A non-linear regression four-parameter curve fit was applied using the Graph Prism software. Data was presented as the mean IC₅₀ value and pIC₅₀ with the standard error of the mean of the mean of n experiments.

B. Results

IL-33 alone failed to induce direct basophil degranulation by way of β -hexosaminidase release. Co-stimulation of basophils with IL-33 and cross linking of cell bound IgE with an anti-IgE antibody caused a synergistic increase in β -hexosaminidase release. 22A06-458 concentration dependently blocked the synergistic effect of IL-33 on cross linked IgE stimulated β -hexosaminidase release. Inhibition by 22A06-458 (**Figure 4**) was expressed as percentage inhibition of the IL-33 (5.0nM) + cross linked IgE response relative to the negative control response (anti-IgE alone). The inhibitory effect of 22A06-458 was tested in 4 separate donor experiments. The results have been summarized in **Table 16**. An IC₅₀ value of 2.03 ± 0.63 nM (n=4) was observed. The pIC₅₀ was calculated as 8.78 ± 0.11 (n=4).

Table 16. *Determination of Potency: Inhibition of IL-33 induced β -hexosaminidase release from basophils by pre-complexed 22A06-458*

	Donor #90356	Donor #90541	Donor #90290	Donor #33235	Mean	SD	SEM
	N=1	N=2	N=3	N=4			
pIC ₅₀	8.91	9.01	8.34	8.86	8.78	0.30	0.11
IC ₅₀ (nM)	1.23	0.97	4.53	1.39	2.03	1.68	0.63

Example 17 - Inhibition of IL-33/IL-12 stimulated blood IFN- γ release by an IL-33 binding protein

Inhibition of IL-33/IL-12 induced IFN- γ release from human whole blood with an IL-33 binding protein is described below.

A. Methods

Whole human blood was collected from normal healthy donors. Typically, 20mL was collected by venipuncture per donor into a sterile container with anti-coagulant, sodium heparin solution (10IU/mL, Leo Laboratories).

5 A 20x cytokine mix of IL-33 (in-house) +IL-12 (Thermo Fisher #PHC1123) was prepared in PBS assay buffer (Thermo Fisher #14190144) based on the following dilutions:

A volume of rhu IL-12 was made up to 20x Final Assay Concentration (FAC) of 12.5ng/mL. Stock IL-12 (1mg/mL) was diluted 1 in 40 followed by a 1 in 100 dilution into PBS (IL-12 at 20x 12.5ng/mL = 250ng/mL). Subsequent dilutions of IL-33 were made into this PBS/IL-12 buffer.

10 rhu IL-33 was diluted to 3 separate 20x FACs of 100ng/mL, 30ng/mL, and 10ng/mL. First, the highest rhu IL-33 concentration (i.e., 20x 100ng/mL = 2000ng/mL) was prepared by diluting the stock IL-33 (2.1mg/mL) 1 in 100 into PBS/IL-12 then 1 in 10.5 into PBS/IL-12. To make up the second concentration of IL-33 (i.e., 20x 30ng/mL = 600ng/mL), the 2000ng/mL stock was diluted 1 in 3.3 into PBS/IL-12. To make the third concentration of IL-33 (i.e., 20x
15 10ng/mL = 200ng/mL), the 2000ng/mL preparation stock was diluted 1 in 10 into PBS/IL-12.

22A06-458 anti-IL-33 mAb was produced at a stock concentration of 9.95mg/mL in 20mM Histidine, 180mM Trehalose, 40mM Arginine, 8mM Methionine, 0.05mM EDTA, pH 6. 20μL aliquots were stored at -80°C and were thawed and diluted to the appropriate concentration on day of use.

20 22A06-458 mAb was diluted to 20x final top assay concentration of 10μg/mL (67nM) by diluting the stock 1 in 49.5 to 200μg/mL. Subsequent 1 in 4 serial dilutions were then carried out in PBS in a sterile polypropylene 96 well U-bottom plate to generate a 7-point (0.164nM – 67nM) and 6-point (0.654nM –67nM) concentration response curve for pre-complexed and non-complexed 22A06-458, respectively.

25 To pre-complex the mAb with IL-33, the diluted antibody was then mixed 1:1 (e.g., 50μL + 50μL) with each of the prepared concentrations of IL-33/IL-12. The 1:1 diluted mixtures (now at 10x FAC) were incubated for 30 min at room temperature (RT) to allow the antibody to complex with the IL-33.

30 For the non-complexing of 22A06-458 with IL-33, the diluted mAb was not mixed with the IL-33 and was left at the 20x FAC.

For the no antibody treatment control (positive control), each test IL-33/IL-12 concentration was either diluted 1:1 with PBS buffer to maintain the same concentration as with the pre-complexed preparation or left at the 20x FAC.

The negative control for the assay was represented by PBS/IL-12 (without IL-33). Other assay controls included, consisted of PBS alone and PBS/IL-33 (without IL-12). These controls demonstrate that stimulation of blood with either IL-12 or IL-33 alone induce little or no IFN- γ release.

Following complex formation of 22A06-458 with each of the test concentrations of IL-33/IL-12, 20 μ L was added in duplicate wells per sample concentration to a 96 well tissue culture plate containing 180 μ L of pre-dispensed blood and mixed by repeated pipetting. Next, 20 μ L of the positive controls (IL-33 at 100, 30, and 10ng/mL) and negative controls (PBS alone, PBS/IL-12 (without IL-33), and PBS/IL-33 (without IL-12)) were also added to the assay plate and mixed. The assay plates were incubated (5% O₂/95% air) for a period of 20h at 37°C.

For the non-complexed 22A06-458, 10 μ L (at 20x FAC) was added in duplicate per concentration to 180 μ L blood in a 96 well plate and mixed by repeated pipetting. Next, 10 μ L of IL-33/IL-12 (at 20x FAC) was added to the blood and mixed. Positive and negative controls were included as above. The assay plates were incubated (5% O₂/95% air) for a period of 20h at 37°C.

After the 20h incubation period, the blood assay plates were centrifuged (2000rpm for 10 minutes at RT) and 35 μ L of the blood plasma was withdrawn from each well using the 96 well Biomek NXp robot and transferred to a new 96 well polypropylene plate.

Single plex IFN- γ assay kits (Mesoscale Discovery, #K151AEB-4) were used to assay for IFN- γ release in the blood plasma. Standards and plasma samples were added to the MSD plates and incubated for 1.5h at RT while shaking. IFN- γ specific detection antibody labeled with the MSD SULFO-TAG reagent was then added to all the sample wells and incubated for a further 1.5h at RT while shaking. After washing plates with PBS + 0.05% Tween-20 three times and addition of 2x Read Buffer T, the plates were read on the Sector Imager plate reader.

Levels of IFN- γ (pg/mL) were back calculated from the standard curve using the MSD analysis software (Discovery Workbench 4) and plotted against the log₁₀ [M] concentration of 22A06-458. A non-linear regression three-parameter curve fit was applied using the Graph Prism

software v5.0.4. Data was presented as the mean IC₅₀ value and pIC₅₀ with the standard error of the mean (SEM) of number (n) of separate experiments.

B. Results

1. Pre-Complexed

22A06-458 pre-complexed with IL-33 concentration dependently blocked IL-33/IL-12 stimulated IFN- γ release in whole blood. Inhibition by 22A06-458 was tested against IL-33 concentrations of 100ng/mL, 30ng/mL, and 10ng/mL. Respective IC₅₀ values of 3.90 ± 0.76 nM (n=8), 3.24 ± 0.63 nM (n=6), and 1.95 ± 0.58 nM (n=5) were observed for IL-33 concentrations of 100ng/mL (**Table 17**), 30ng/mL (**Table 18**), and 10ng/mL (**Table 19**). Respective pIC₅₀ values of 8.48 ± 0.10 (n=8), 8.53 ± 0.09 (n=6), and 8.80 ± 0.14 nM (n=5) were observed for IL-33 concentrations of 100ng/mL (**Table 17**), 30ng/mL (**Table 18**), and 10ng/mL (**Table 19**).

Table 17. *Determination of Potency: Inhibition of 100ng/mL IL-33/IL-12 induced IFN- γ secretion from whole blood by pre-complexed 22A06-458 (n=8 donors)*

Donor #	#91232	#92187	#92257	#91865	#92186	#91845	#92023	#92258	Mean	SEM
pIC ₅₀	8.55	8.32	8.58	9.05	8.52	8.24	8.46	8.10	8.48	0.10
IC ₅₀ (nM)	2.79	4.75	2.66	0.88	3.01	5.75	3.43	7.9	3.90	0.76

Table 18. *Determination of Potency: Inhibition of 30ng/mL IL-33/IL-12 induced IFN- γ secretion from whole blood by pre-complexed 22A06-458 (n=6 donors)*

Donor #	#91232	#92187	#92257	#91845	#92023	#92258	Mean	SEM
pIC ₅₀	8.83	8.30	8.75	8.41	8.60	8.30	8.53	0.09
IC ₅₀ (nM)	1.47	4.92	1.75	3.88	2.48	4.93	3.24	0.63

Table 19. *Determination of Potency: Inhibition of 10ng/mL IL-33/IL-12 induced IFN- γ secretion from whole blood by pre-complexed 22A06-458 (n=5 donors)*

Donor #	#91232	#92257	#91845	#92023	#92258	Mean	SEM
pIC ₅₀	9.19	9.08	8.56	8.72	8.42	8.80	0.14
IC ₅₀ (nM)	0.63	0.81	2.72	1.88	3.72	1.95	0.58

2. Not Pre-Complexed

22A06-458 that had not been pre-complexed with IL-33 also demonstrated concentration dependent inhibition of IL-33/IL-12 stimulated IFN- γ release in whole blood. Inhibition by 22A06-458 was tested in 6 separate donor experiments against IL-33 concentrations of 30ng/mL and 10ng/mL. Respective IC₅₀ values of 1.57 ± 0.43 nM (n=6) and 0.37 ± 0.09 nM (n=6) were observed for IL-33 concentrations of 30ng/mL (**Table 20**) and 10ng/mL (**Table 21**). Respective pIC₅₀ values of 8.89 ± 0.13 (n=6) and 9.51 ± 0.13 (n=6) were observed for IL-33 concentrations of 30ng/mL (**Table 20**) and 10ng/mL (**Table 21**).

Table 20. *Determination of Potency: Inhibition of 30 ng/mL IL-33 induced IFN- γ secretion from whole blood by non-pre-complexed 22A06-458 (n=6 donors)*

Donor #	#90237	#91986	#32871	#91680	#92251	#91955	Mean	SEM
pIC ₅₀	8.58	8.51	9.01	8.79	9.13	9.31	8.89	0.13
IC ₅₀ (nM)	2.6	3.04	0.97	1.60	0.74	0.48	1.57	0.43

Table 21. *Determination of Potency: Inhibition of 10 ng/mL IL-33 induced IFN- γ secretion from whole blood by non-pre-complexed 22A06-458 (n=6 donors)*

Donor #	#90237	#91986	#32871	#91680	#92251	#91955	Mean	SEM
pIC ₅₀	9.13	9.44	9.67	9.37	9.37	10.06	9.51	0.13
IC ₅₀ (nM)	0.73	0.36	0.21	0.43	0.42	0.08	0.37	0.09

IL-33 alone did not stimulate IFN- γ release from whole blood, whereas IL-12 alone stimulation did cause some increase in basal IFN- γ release in some donors. However, with co-stimulation, IL-33/IL-12 synergistically caused release of large quantities of this cytokine. The concentrations of IFN- γ secreted was highly variable between donors poststimulation with IL-33/IL-12.

The anti-IL-33 monoclonal antibody (22A06-458) concentration dependently inhibited the synergistic effect of IL-33/IL-12 on whole blood IFN- γ release both when pre-complexed with IL-33 as well as when added directly to blood without pre-complexing.

The assay demonstrated that the potency of inhibition varied depending on the concentration of IL-33 used in the assay. Pre-complexing the anti-IL-33 mAb resulted in little difference in potency compared to non-pre-complexing. These in vitro investigations

demonstrate that the anti-IL-33 mAb (22A06-458) can neutralize IL-33 and potentially block pro-inflammatory effects, as measured by IFN- γ secretion in human whole blood.

Example 18 - IL-33 binding protein inhibition of IL-33-induced cytokine production in mouse bone marrow-derived mast cells

5 A. Methods

22A06-458 was provided at a stock concentration of 9.95mg/mL in 20mM Histidine, 180mM Trehalose, 40mM Arginine, 8mM Methionine, 0.05mM EDTA, pH 6. 20 μ L aliquots were stored at -80°C and were thawed and diluted to the appropriate concentration on day of use. Molar concentrations of the antibody were calculated based on a molecular weight of 150kDa.

10 Recombinant human IL-33 (in-house) was provided at a stock concentration of 2.1mg/mL (Mwt of 19.7Kda; therefore [M] = 106.5 μ M) in PBS+0.1% BSA. 20 μ L aliquots were stored at -80°C and were thawed and diluted to the appropriate concentration on day of use.

Recombinant mouse IL-33 (R&D Systems #3626-ML-010) was supplied in lyophilised form from a solution in PBS, EDTA and DTT with a BSA carrier. The protein was reconstituted at 10 μ g/mL in sterile PBS containing 0.1% BSA. Aliquots were stored at -80°C and were thawed and diluted to the appropriate concentration on day of use.

Recombinant rat IL-33 (Biolegend #766404) was supplied at 200 μ g/mL in sterile PBS. Aliquots were stored at -80°C and were thawed and diluted to the appropriate concentration on day of use.

20 Aqua Zombie dead cell dye (Biolegend #423102) was diluted 1:500 in PBS. FcR block (Miltenyi #130-092-575) was diluted 1:10 in PBS. The Fc ϵ R1 (Biolegend #134325), CD117 (Biolegend #105811), and isotype control antibodies (Biolegend #400611) were diluted 1:25 in diluted FcR block.

Mast cells were counted and 1 $\times$ 10⁵ cells added per U bottom 5mL tube (Falcon 352235) with 3mL PBS. The tubes were centrifuged (300g, 5mins), the supernatant discarded, and the cell pellet resuspended in 100 μ L Aqua Zombie. After incubating for 20mins at room temperature 100 μ L of the diluted antibodies were added. The tubes were incubated in the dark at 4°C for 20mins. 3mL of FACS wash buffer was added per tube and centrifuged (300g, 5mins). The supernatant was discarded and 200 μ L PBS+0.1% FCS added. The cells were analyzed on an Attune NxT Flow Cytometer.

Mast cells were collected into a 50mL Falcon tube and centrifuged (1300rpm, 5min). The pellet was resuspended in mast cell medium at 1×10^6 cells/mL and plated into 96 well U-bottom tissue culture plates (Costar 3799), 100 μ L/well. Cells were incubated overnight at 37°C, 5% CO₂.

5 Mouse, rat and human IL-33 were diluted to 200ng/mL (2 times final assay concentration) in mast cell medium. These were further diluted 1:10 in mast cell medium to generate a 6-point dose response curve.

10 100 μ L mouse, rat, or human IL-33 diluted over the range 0.002-200ng/mL (2 times final concentration) was added in duplicate to the mouse mast cell culture plate. Cells were incubated for 4 hrs at 37°C, 5% CO₂. Plates were centrifuged (350g, 5mins) to pellet the cells. The supernatant was collected and frozen at -80°C for cytokine analysis. The final concentration range of IL-33 tested was 0.001-100ng/mL.

Mouse, rat and human IL-33 were diluted to 40ng/mL and 4ng/mL (4x the final assay concentration) in mast cell medium.

15 The antibody was diluted to 6 μ g/mL (4x the final assay top concentration) in mast cell medium followed by 1 in 4 serial dilutions to generate a 6-point response curve. The diluted antibody was then mixed 1:1 with mouse, rat, or human IL-33 at 40ng/mL or 4ng/mL. The pre-complexed mixtures, now at 2x final assay concentration, were incubated at room temperature for 30mins. The negative control was medium alone. The positive control was mouse, rat, or
20 human IL-33 diluted 1:1 with medium.

A further control was 22A06-458 diluted 1:1 with medium (top concentration only).

25 100 μ L of the 22A06-458-IL-33 complex or controls was added in duplicate to the mast cell tissue culture plate. Cells were incubated for 4hrs at 37°C, 5% CO₂. Plates were centrifuged at 350g for 5mins to pellet the cells. The supernatant was collected and frozen at -80°C for cytokine analysis. The final concentration range of antibody tested was 1.5-1500ng/mL (0.01-10nM).

30 The capture antibody coupled beads were added to the assay plate followed by the standards and samples. Following an incubation at 4°C overnight the plate was washed with kit assay buffer. The biotinylated detection antibody was added per well and the plate incubated at room temperature for 30 minutes. The plate was washed again, the Streptavidin-PE added per well and incubated at room temperature for 30 minutes. After a final wash the beads were re-

suspended in assay read buffer. The plate was read on the Luminex Flexmap 3D using Luminex xPONENT software v4.2.

Mean fluorescent intensity values from Luminex xPONENT were exported into BioPlex Manager v6.1. In this software cytokine levels were back-calculated from the standard curve.

5 Data was calculated as percent inhibition of the control response (10ng/mL or 1ng/mL IL-33 stimulation). IC50 values were calculated from a non-linear regression four parameter fit curve drawn in GraphPad Prism v5.1.

B. Results

10 After 4 weeks in culture 73.6% and 90.2% of the mouse bone marrow cells showed positive expression of FcεR1 and CD117. This positive expression showed that the mouse bone marrow cells had differentiated into mast cells.

Mouse, rat, and human IL-33 all stimulated cytokine release (TNF-α, IL-6, IL-18, and IL-13) from mouse bone marrow derived mast cells showing similar concentration response curves. From these plots, IL-33 concentrations were chosen for the 22A06-458 blocking experiment.

15 Maximal (10ng/mL) and sub-maximal (1ng/mL) IL-33 concentrations were selected for all three species (**Figure 5**).

22A06-458 was able to completely block production of human IL-33 stimulated IL-6, TNF-α, IL-13, and IL-18 at both 1ng/mL and 10ng/mL IL-33 concentrations. The antibody did not inhibit cytokines stimulated by 10ng/mL mouse or rat IL-33 even at the top concentration of 20 10nM. At 1ng/mL mouse or rat IL-33 only minimal activity was observed, with maximal inhibition of only around 20-30% at 10ng/mL. Inhibition was expressed as the percentage decrease compared to IL-33 at 1ng/mL (**Figure 6**) or 10ng/mL (**Figure 7**). Each condition was tested in duplicate, and the results are from one experiment.

25 The IC50 values for 22A06-458 against each cytokine and IL-33 concentration are summarized in **Table 22** and **Table 23**.

Table 22. *Determination of Potency: Inhibition of 1ng/mL human IL-33 induced cytokine production complexed with 22A06-458*

	IL-6	TNF-α	IL-13	IL-18
IC50 pM	31.21	37.35	28.01	61.26

pIC50	10.51	10.43	0.55	10.21
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Table 23. *Determination of Potency: Inhibition of 10ng/mL human IL-33 induced cytokine production complexed with 22A06-458*

	IL-6	TNF-α	IL-13	IL-18
IC50 pM	~566	~581	~567	~631
pIC50	~9.25	~9.24	~9.25	~9.20

5 **Example 19 - Pharmacokinetics and pharmacodynamics of IL-33 binding protein in cynomolgus monkeys**

A study was carried out to investigate the pharmacokinetics and pharmacodynamics of 22A06-458 in cynomolgus monkeys following single intravenous administration or single subcutaneous administration.

10 A. Methods

Following either a single intravenous administration or single subcutaneous administration of 22A06-458 at 10 mg/kg, serum samples were taken from each animal at the following nominal times: pre-dose, 0.25, 3, 6, 24, 48, 96, 168, 336, 504, 672, 1008, 1344, 1680, 2016, 2352, and 2688 hours after dosing. All samples were stored at approximately -80°C until
15 analyzed.

Serum samples were analyzed for 22A06-458 using a validated analytical method based on an antigen capture immunoassay on the GYROLAB platform. The capture method used a biotinylated human IL-33 (in-house) to capture 22A06-458. An Alexa labeled anti-human IgG (Fc specific) (Southern Biotech #9040-1, labeled in-house) was used as the detection antibody.
20 The lower limit of quantification (LLQ) was 0.3 µg/mL and the higher limit of quantification (HLQ) was 100 µg/mL using a 3 µL aliquot of cynomolgus monkey serum diluted 1/50 with REXXIP A buffer (Gyros Protein Technologies #P0004820).

Serum samples with 22A06-458 concentration levels less than 10µg/mL were analyzed for anti-22A06-458 antibodies using a bridging method with acid dissociation on the Gyrolab
25 platform.

Serum samples were diluted in ADA buffer (Gyros Protein Technologies #P0004820) before being loaded into a 96-well plate and placed into the GYROLAB Workstation. The GYROLAB ADA workflow and Mixing CD (Gyros Protein Technologies #P0020455) uses automated acid pre-treatment followed by immunoassay. Briefly, samples were loaded into the CD mixing chamber, before the addition of acid. Following acid treatment, neutralization buffer containing a mix of biotinylated and Alexa labeled 22A06-458 (in-house) was added. Samples were then flowed onto the streptavidin coated capture column before being read.

A positive cut point value was determined by screening the study predose samples. The ADA assay cut point was defined as Mean Response+(1.645*SD). Animals with response values greater than or equal to the ADA assay cut point (0.097 response) in the screening assay were considered potentially positive.

Pharmacokinetic analysis was performed by non-compartmental pharmacokinetic analysis using WINNONLIN (WNL), Version 6.3. All computations utilized the nominal sampling times.

Following either single intravenous or subcutaneous administration, the systemic exposure to 22A06-458 was determined by calculating the area under the serum concentration-time curve (AUC) from the start of dosing to the last quantifiable time point (AUC_{0-∞}) using the linear log trapezoidal method. The maximum observed peak serum concentration (C_{max}) and the time at which it was observed (T_{max}) were determined by inspection of the observed data. In addition, the total serum clearance (CL or CL_F); volume of distribution (V_{ss} or V_{z_F}); terminal half-life (t_{1/2}) and mean residence time (MRT) were calculated.

B. Results

The mean and individual derived pharmacokinetic parameters for 22A06-458 in cynomolgus monkeys following either a single intravenous or subcutaneous administration at 10 mg/kg are presented in **Table 24**.

The individual ADA responses for the samples tested are presented in **Table 24**. There were no notable responses.

22A06-458 is cleared slowly from systemic circulation and had an estimated terminal half-life of approximately 16.5 days, which is comparable to other YTE modified monoclonal antibodies. The volume of distribution is approximately twice blood volume, suggesting the antibody is mainly confined to the systemic circulation.

Absolute bioavailability, estimated by comparing the mean $AUC_{0-\infty}$ for the subcutaneous administration with the mean $AUC_{0-\infty}$ for the intravenous route, was 74%.

Table 24. Mean and individual non-compartmental pharmacokinetic parameters for 22A06-458 in cynomolgus monkeys following single intravenous or subcutaneous administration at a target dose of 10 mg/kg

Regimen	Animal	AUC	AUCInf	Cmax	Tmax*	Half-life	Cl or Cl_F	Vss or Vz_F
		(hr* μ g/mL)	(hr* μ g/mL)	(μ g/mL)	(hr)	(hr)	(mL/hr/kg)	(mL/kg)
IV	1	57800	58000	194	0.25	340	0.17	93
	2	46600	47100	249	0.25	400	0.21	120
	3	51000	51700	234	0.25	450	0.19	130
	Mean	51800	52300	226	0.25	400	0.19	120
SC	4	41800	42400	57	96	430	0.24	140
	5	37400	37700	42	48	360	0.27	140
	6	36400	36700	51	336	370	0.27	140
	Mean	38500	38900	49.9	96	390	0.26	140

* Median Value reported

Example 20 - Inhibition of IL-33 stimulated HEK-BLUE cells by an IL-33 binding protein in PK samples and equivalent to freshly prepared IL-33 binding protein

Studies were conducted to compare whether the inhibitory profile of cyno or human IL-33 by an IL-33 binding protein present in cyno PK samples was equivalent to freshly prepared drug.

A. Methods

HEK BLUE IL-33 cells (InvivoGen #HKB-HIL-33) were cultured in a T75cm² tissue culture flask in the presence of 1x HEK-BLUE selection antibiotics until approximately 80-90% confluent. After removal of medium and a wash of the cells with PBS (12mL), the adherent cells were detached by adding back 10mL PBS and pipetting several times over the adherent cells followed by tapping the flask. The cell suspension was then transferred to a 15mL conical tube and centrifuged (350g/5 min/RT). Pelleted cells were resuspended in 1mL fresh growth media and counted using a haemocytometer.

HEK-BLUE IL-33 cells (passage <10) were diluted to a cell density of 1.25×10^6 /mL and 40 μ L seeded (50,000 cells/well) into 384 well tissue culture plate (Corning #3701).

Serum concentrations of 22A06-458 in cyno pharmacokinetic samples (2688h) are shown in **Table 25**.

Table 25. *Concentration of 22A06-458 in serum from six individual cyno monkeys*

Cyno #	µg/mL of drug	[nM] of drug
1	0.36	2.4
2	0.84	5.6
3	1.2	8
4	0.94	6.3
5	0.67	4.5
6	0.62	4.2

- 5 From the serum concentrations of 22A06-458 (**Table 25**), the highest achievable final assay concentration based on cyno #1 (i.e., the sample having the lowest available drug concentration) was 0.025µg/mL (167pM).

All serum samples were diluted with DMEM growth media to 4x final assay concentrations (FAC) of 0.1µg/mL (0.025µg/mL x4 =0.1µg/mL) as shown in **Table 26** below.

- 10 This top starting concentration was then subsequently diluted 1 in 3 in DMEM growth media (Thermo Fisher #41965-039) a further 7 times to give a total of 8 concentrations (0.076pM - 167pM). All dilutions were carried out in a U-bottom sterile polypropylene 96 well plate.

Table 26. *Cyno serum 22A06-458 concentrations and dilutions conducted to achieve a maximal assay concentration of 167pM*

Concentration in 2688 hour PK samples						
Cyno #	µg/mL	[nM]	Dilution factor	[µg/mL] 4xFAC	[nM] 4xFAC	Max FAC (pM)
1	0.36	2.4	3.6	0.1	0.67	167
*2	0.84	5.6	8.9	0.09	0.63	157
3	1.2	8	12	0.1	0.67	167
4	0.94	6.3	9.4	0.1	0.67	167
5	0.67	4.5	6.7	0.1	0.67	167

6	0.62	4.2	6.2	0.1	0.67	167
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* Due to a typographical error in the PK drug concentration, the incorrect dilution was applied to this sample thus the max final assay concentration for serum 22A06-458 was 157pM.

22A06-458 was produced at a stock concentration of 9.95mg/mL in 20mM Histidine, 180mM Trehalose, 40mM Arginine, 8mM Methionine, 0.05mM EDTA, pH 6. 20µL aliquots stored at -80°C were thawed and diluted to the appropriate concentration on day of use.

Fresh 22A06-458 was prepared to the same top concentration as the serum drug samples, i.e., fresh drug was diluted to a 4xFAC of 0.1µg/mL (0.67nM) into DMEM growth media.

Stock concentration of drug, 9.95mg/mL was diluted 1 in 100 then 1 in 100 again then 1 in 9.5 Subsequent 1 in 3 dilutions were conducted to a total of 8 concentrations (0.076pM - 167pM).

For this study the same stock cyno serum samples were used. The cyno #1 serum sample had insufficient volume remaining and thus had to be omitted from this study.

Based on the serum concentrations of 22A06-458, the highest assay concentration of the drug based on cyno #6 (i.e., sample having the lowest available drug concentration) was calculated to be 0.05 µg/mL (333pM).

All serum samples were diluted with DMEM growth media to 4x FAC of 0.2µg/mL (4x 0.05µg/mL = 0.2µg/mL) as shown in **Table 27** below. This top concentration was then subsequently diluted 1 in 3 in DMEM growth media a further 7 times to give a total of 8 concentrations (0.15pM - 333pM). All dilutions were carried out in a U-bottom sterile polypropylene 96 well plate.

Table 27. Cyno serum 22A06-458 concentrations and dilutions conducted to achieve a maximal assay concentration of 333pM

Cyno #	Concentration in 2688 hour PK sample		Dilution factor	[µg/mL] 4xFAC	[nM] 4xFAC	Max FAC (pM)
	µg/mL	[nM]				
*1	0.36	2.4	1.8	0.2	1.3	333
**2	0.84	5.6	4.45	0.19	1.26	315
3	1.2	8	6	0.2	1.3	333
4	0.94	6.3	4.7	0.2	1.3	333

5	0.67	4.5	3.35	0.2	1.3	333
6	0.62	4.2	3.1	0.2	1.3	333

* Cyno #1 could not be used due to lack of adequate sample volume

** Due to a typographical error in the PK drug concentration, the incorrect dilution factor was applied to this sample thus the max final assay concentration for serum 22A06-458 was 315pM

22A06-458 was prepared from a fresh stock sample to the same top concentration as the serum drug samples. i.e., fresh drug was diluted to a 4xFAC of 0.2µg/mL (1.3nM) into DMEM growth media.

Stock concentration of drug, 9.95mg/mL was diluted 1 in 100 then 1 in 100 again then 1 in 4.9. Subsequent 1 in 3 dilutions were conducted to a total of 8 concentrations (0.15pM - 333pM).

10 Cyno IL-33 (in-house) was used at a FAC of 75pg/mL (3.8pM) (~EC₅₀ concentration for cyno IL-33). Stock [cyno IL-33]= 5.9mg/mL; diluted to 300pg/mL (4xFAC) in DMEM growth media.

rhu IL-33 (in-house) was used at a FAC of 200pg/mL (10pM) (~EC₅₀ concentration for IL-33). Stock [rhu IL-33]= 2.1mg/mL; diluted to 800pg/mL (4xFAC) in DMEM growth media.

15 Cyno and rhu IL-33 diluted to 200pg/mL and 800pg/mL (4xFAC) respectively were mixed 1:1 (e.g., 50µL+50µL) with the prepared 4x concentrated serum 22A06-458 and freshly prepared 22A06-458, then co-incubated for 30 min/ RT. This reduced the drug and IL-33 concentrations to 2x FAC's.

20 The cyno and rhu IL-33 positive assay controls (no 22A06-458) prepared to 4x FAC were also further diluted by mixing 1:1 with DMEM growth media to mimic the pre-complexing treatment with 22A06-458 (as above) and maintain the correct assay concentration (i.e., 2x FAC).

The negative assay control consisted of DMEM growth media alone.

25 To the 384 well plate containing the 40µL of HEK BLUE cells, 40µL of pre-complexed drug- IL-33 including positive and negative controls were added to wells (duplicate wells per test treatment). The plate was then incubated at 37°C or 24h.

Following the 24h incubation period the Quanti Blue substrate reagent (InvivoGen #HB-DET2) was prepared according to manufacturer's instructions, filtered and frozen in aliquots of

10mL at -20°C. Prior to use, the Quanti Blue substrate reagent was defrosted and warmed to RT. 40µL Quanti Blue substrate was added to wells of a new 384 well plate and 10µL of HEK BLUE cell supernatants transferred from treatment plate into the Quanti Blue containing wells. The plate was then incubated at 37°C for 2h. The absorbance of the colour formation was read on a spectrophotometer plate reader (665nm).

Raw absorbance data was transferred to MS Excel, calculated, and expressed as % inhibition of the IL-33 (prepared in Media) control response using **Equation 2**. This was then plotted relative to the [22A06-458] M and a non-linear regression curve fit (three parameter) was applied using the Graph Prism software v5.0.4.

B. Results

22A06-458 from the individual cyno serum PK samples and freshly prepared sample, concentration-dependently blocked cyno IL-33 mediated stimulation of HEK BLUE cells. To illustrate the concentration dependent inhibition by 22A06-458, graphs of molar concentration of 22A06-458 were plotted against % inhibition of IL-33 (3.8pM) stimulation for each of the 6 individual cyno monkey serum samples (**Figure 8**). The inhibition curve for fresh 22A06-458 was included in each graph to illustrate the similarity in activity. Full inhibition curves were not achieved since the maximal concentration of 22A06-458 in the assay was limited to 167pM (157pM for cyno #2).

22A06-458 from individual cyno serum PK samples and freshly prepared drug sample, concentration dependently blocked rhu IL-33 mediated stimulation of HEK BLUE cells. To illustrate the concentration dependent inhibition by 22A06-458 of rhu IL-33, graphs of the molar concentration of 22A06-458 were plotted against % inhibition of rhu IL-33 (10pM) stimulation for each of the 5 individual cyno monkey serum samples (**Figure 9**). The inhibition curve for fresh 22A06-458 was included in each graph to illustrate the similarity in activity. Full inhibition curves were not achieved since the maximal concentration of 22A06-458 in this assay was limited to 333pM (315pM for cyno #2).

Example 21 – Cryo-Electron Microscopy Structure of IL-33 Binding Protein

Cryo-electron microscopy (cryo-EM) (200keV ThermoFisher Glacios transmission electron microscope equipped with an X-FEG source) was used to determine the binding mode

of the fAb of IL-33 binding protein 22A06-458 to human IL-33 and understand the mAb mode of action.

A 4.75 Å Cryo-EM structure of the fAb of anti-IL33 mAb 22A06-458 in complex with reduced IL-33 and a kappa nanobody was determined. This was of sufficient resolution to allow the interaction regions between 22A06-458 fAb (paratope) and reduced IL-33 (epitope) to be identified by Qt-PISA v2.1.0 within the CCP4 program suite (Collaborative Computational Projection Number 4, 1994).

As shown in **Figure 10**, the epitope is defined by a central region consisting of Y163-L182 (SEQ ID NO: 82) and V219-V228 (SEQ ID NO: 83). This is flanked by epitope regions consisting of S117-Y122 (SEQ ID NO: 84), V252-L258 (SEQ ID NO: 85) and L267-E269 (LSE). The paratope involves residues drawn from all six CDRs of 22A06-458.

This Cryo-EM fAb epitope shows good agreement with that determined by HDX-MS protection mapping of IL33 binding described in **Example 7**, namely in the regions Y164-L182 (SEQ ID NO: 86) and V219-C227 (SEQ ID NO: 87) as shown in **Figure 11**. Enhanced protection of the two central regions of Cryo-EM epitope were observed, consistent with these being the most likely to experience solvent protection on 22A06-458 binding.

As shown in **Figure 12** and **Figure 13**, the overlay of the Cryo-EM structure with published crystal structures (PDB: 4KC3 (Liu, 2013) and 5VI4 (Günther, 2017)), respectively, reveals that 22A06-458 blocks both domain 3 (D3) and domain 2 (D2) of the IL-1 Receptor Accessory Protein (IL-1RAcP) from binding to IL-33, effectively obstructing all IL-33 binding sites for this receptor. However, 22A06-458 only hinders D3 of the ST2 receptor, also known as Interleukin 1 receptor type 1 (IL1R1), from interacting with IL-33. This suggests that domain 1 (D1) and D2 of ST2 might still be able to interact with the 22A06-458 fAb-IL33 complex.

This aligns with assay observations showing that the competition between 22A06-458 and ST2 for IL-33 can be influenced by the order of addition. If ST2 is preincubated with IL-33, it prevents 22A06-458 from binding because D1 of ST2 directly blocks 22A06-458 binding. However, if 22A06-458 is preincubated with IL-33, it only prevents D1 of ST2 from binding and ST2 can still interact with IL-33 at the D2 and D3 sites.

Example 22 – Developability Analysis

A number of assays, including affinity, developability, and solubility studies, were carried out on 22A06-458, 22A06-429, 22A06-481 and 22A06-502.

22A06-429 had the highest affinity, followed by 22A06-481, 22A06-458, and 22A06-502. Following the developability analysis, it was determined that 22A06-458 and 22A06-502 were the most desirable candidates to progress due to less aggregation and greater stability under stressed conditions.

5

SEQUENCE LISTINGS

SEQ ID NO	Sequence Identifier
1	CDRH1 of 22A06-429, 22A06-458, 22A06-481, and 22A06-502 amino acid sequence
2	CDRH2 consensus amino acid sequence
3	CDRH2 of 22A06-429, 22A06-481, and 22A06-502 amino acid sequence
4	CDRH2 of 22A06-458 amino acid sequence
5	CDRH3 consensus amino acid sequence
6	CDRH3 of 22A06-429 amino acid sequence
7	CDRH3 of 22A06-458 and 22A06-502 amino acid sequence
8	CDRH3 of 22A06-481 amino acid sequence
9	CDRL1 consensus amino acid sequence
10	CDRL1 of 22A06-429 and 22A06-458 amino acid sequence
11	CDRL1 of 22A06-481 amino acid sequence
12	CDRL1 of 22A06-502 amino acid sequence
13	CDRL2 consensus amino acid sequence
14	CDRL2 of 22A06-429 and 22A06-458 amino acid sequence
15	CDRL2 of 22A06-481 amino acid sequence
16	CDRL2 of 22A06-502 amino acid sequence
17	CDRL3 consensus amino acid sequence
18	CDRL3 of 22A06-429, 22A06-458 and 22A06-481 amino acid sequence
19	CDRL3 of 22A06-502 amino acid sequence
20	VH consensus amino acid sequence
21	VH of 22A06-429 amino acid sequence
22	VH of 22A06-458 amino acid sequence
23	VH of 22A06-481 amino acid sequence
24	VH of 22A06-502 amino acid sequence
25	VL consensus amino acid sequence

26	VL of 22A06-429 and of 22A06-458 amino acid sequence
27	VL of 22A06-481 amino acid sequence
28	VL of 22A06-502 amino acid sequence
29	HC consensus amino acid sequence
30	HC of 22A06-429 amino acid sequence
31	HC of 22A06-458 amino acid sequence
32	HC of 22A06-481 amino acid sequence
33	HC of 22A06-502 amino acid sequence
34	LC consensus amino acid sequence
35	LC of 22A06-429 and of 22A06-458 amino acid sequence
36	LC of 22A06-481 amino acid sequence
37	LC of 22A06-502 amino acid sequence
38	VH consensus nucleic acid sequence 1
39	VH consensus nucleic acid sequence 2
40	VH consensus nucleic acid sequence 3
41	VH consensus nucleic acid sequence 4
42	VH consensus nucleic acid sequence 5
43	VH consensus nucleic acid sequence 6
44	VH of 22A06-429 nucleic acid sequence
45	VH of 22A06-458 nucleic acid sequence
46	VH of 22A06-481 nucleic acid sequence
47	VH of 22A06-502 nucleic acid sequence
48	VL consensus nucleic acid sequence 1
49	VL consensus nucleic acid sequence 2
50	VL consensus nucleic acid sequence 3
51	VL consensus nucleic acid sequence 4
52	VL consensus nucleic acid sequence 5
53	VL consensus nucleic acid sequence 6
54	VL consensus nucleic acid sequence 7

55	VL consensus nucleic acid sequence 8
56	VL of 22A06-429 and of 22A06-458 nucleic acid sequence
57	VL of 22A06-481 nucleic acid sequence
58	VL of 22A06-502 nucleic acid sequence
59	HC consensus nucleic acid sequence 1
60	HC consensus nucleic acid sequence 2
61	HC consensus nucleic acid sequence 3
62	HC consensus nucleic acid sequence 4
63	HC consensus nucleic acid sequence 5
64	HC consensus nucleic acid sequence 6
65	HC of 22A06-429 nucleic acid sequence
66	HC of 22A06-458 nucleic acid sequence
67	HC of 22A06-481 nucleic acid sequence
68	HC of 22A06-502 nucleic acid sequence
69	LC consensus nucleic acid sequence 1
70	LC consensus nucleic acid sequence 2
71	LC consensus nucleic acid sequence 3
72	LC consensus nucleic acid sequence 4
73	LC consensus nucleic acid sequence 5
74	LC consensus nucleic acid sequence 6
75	LC consensus nucleic acid sequence 7
76	LC consensus nucleic acid sequence 8
77	LC of 22A06-429 and of 22A06-458 nucleic acid sequence
78	LC of 22A06-481 nucleic acid sequence
79	LC of 22A06-502 nucleic acid sequence
80	IL-33 ₁₁₃₋₂₇₀ residues 66-85 amino acid sequence
81	IL-33 ₁₁₃₋₂₇₀ residues 120-129 amino acid sequence
82	IL-33 residues 163-182 amino acid sequence
83	IL-33 residues 219-228 amino acid sequence

84	IL-33 residues 117-122 amino acid sequence
85	IL-33 residues 252-258 amino acid sequence
86	IL-33 residues 164-182 amino acid sequence
87	IL-33 residues 219-227 amino acid sequence

SEQ ID NO:1

SYGMH

5 SEQ ID NO:2

EINPHGGSTSYAQKFXG

X Pos 16: K or Q

SEQ ID NO:3

10 EINPHGGSTSYAQKFQG

SEQ ID NO:4

EINPHGGSTSYAQKFKG

15 SEQ ID NO:5

PSAAYSHYLGJDX

X Pos 13: I, L, or M

SEQ ID NO:6

20 PSAAYSHYLGIDM

SEQ ID NO:7

PSAAYSHYLGIDL

25 SEQ ID NO:8

PSAAYSHYLGLDI

SEQ ID NO:9

RXSQGISXWLX

X Pos 2: A or T

X Pos 8: P or S

5 X Pos 11: A or S

SEQ ID NO:10

RASQGISSWLA

10 SEQ ID NO:11

RASQGISPWLA

SEQ ID NO:12

RTSQGISSWLS

15

SEQ ID NO:13

AXSXLQS

X Pos 2: A or G

X Pos 4: R or S

20

SEQ ID NO:14

AASRLQS

SEQ ID NO:15

25 AGSSLQS

SEQ ID NO:16

AASSLQS

30 SEQ ID NO:17

QQAXVXPLT

X Pos 4: H or N

X Pos 6: F or L

SEQ ID NO:18

5 QQANVFPLT

SEQ ID NO:19

QQAHVLPPLT

10 SEQ ID NO:20

QVQLVQSGAEVKKPGASVKVSCKASGYTFISYGMHWVRQAPGQGLEWMGEINPHGGSTSYAQKF

XGRVTMTRDTSTSTVYMEISSLRSEDTAVYYCARPSAAYSHYLGJDXWGRGTLVTVSS

X Pos 65: K or Q

X Pos 111: I, L, or M

15

SEQ ID NO:21

QVQLVQSGAEVKKPGASVKVSCKASGYTFISYGMHWVRQAPGQGLEWMGEINPHGGSTSYAQKF

QGRVTMTRDTSTSTVYMEISSLRSEDTAVYYCARPSAAYSHYLGIDMWGRGTLVTVSS

20 SEQ ID NO:22

QVQLVQSGAEVKKPGASVKVSCKASGYTFISYGMHWVRQAPGQGLEWMGEINPHGGSTSYAQKF

KGRVTMTRDTSTSTVYMEISSLRSEDTAVYYCARPSAAYSHYLGIDLWGRGTLVTVSS

SEQ ID NO:23

25 QVQLVQSGAEVKKPGASVKVSCKASGYTFISYGMHWVRQAPGQGLEWMGEINPHGGSTSYAQKF

QGRVTMTRDTSTSTVYMEISSLRSEDTAVYYCARPSAAYSHYLGLDIWGRGTLVTVSS

SEQ ID NO:24

QVQLVQSGAEVKKPGASVKVSCKASGYTFISYGMHWVRQAPGQGLEWMGEINPHGGSTSYAQKF

30 QGRVTMTRDTSTSTVYMEISSLRSEDTAVYYCARPSAAYSHYLGIDLWGRGTLVTVSS

SEQ ID NO:25

DIQMTQSPSSVSASVGDRVTTITCRXSQGISXWLXWYQQKPGKAPKLLIYAXSXLQSGVPSRFSG
SGSGTDFLTITISLQPEDFATYYCQQAXVXPLTFGGGTKVEIK

X Pos 25: A or T

5 X Pos 31: P or S

X Pos 34: A or S

X Pos 51: A or G

X Pos 53: R or S

X Pos 92: H or N

10 X Pos 94: F or L

SEQ ID NO:26

DIQMTQSPSSVSASVGDRVTTITCRASQGISSWLAWYQQKPGKAPKLLIYAASRLQSGVPSRFSG
SGSGTDFLTITISLQPEDFATYYCQQANVFPLTFGGGTKVEIK

15

SEQ ID NO:27

DIQMTQSPSSVSASVGDRVTTITCRASQGISPWLAWYQQKPGKAPKLLIYAGSSSLQSGVPSRFSG
SGSGTDFLTITISLQPEDFATYYCQQANVFPLTFGGGTKVEIK

20 SEQ ID NO:28

DIQMTQSPSSVSASVGDRVTTITCRTSQGISSWLSWYQQKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFLTITISLQPEDFATYYCQQAHLVPLTFGGGTKVEIK

SEQ ID NO:29

25 QVQLVQSGAEVKKPGASVKVSCKASGYTFISYGMHWVRQAPGQGLEWMGEINPHGGSTSYAQKF
XGRVTMTRDTSTSTVYMEISSLRSEDTAVYYCARPSAAYSHYLGJDXWGRGTLVTVSSASTKGP
SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKHTCTPPCPAPELLGGPSVFLFPPKPKDTL
YITREPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
30 GKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV

EWESNGQPENNYKTTTPVLDSGSEFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSL
SPGK

X Pos 65: K or Q

X Pos 111: I, L, or M

5

SEQ ID NO:30

QVQLVQSGAEVKKPGASVKVSCKASGYTFISYGMHWVRQAPGQGLEWMGEINPHGGSTSYAQKF
QGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARPSAAYSHYLGIDMWGRGTLVTVSSASTKGP
SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
10 VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL
YITREPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTTPVLDSGSEFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSL
SPGK

15

SEQ ID NO:31

QVQLVQSGAEVKKPGASVKVSCKASGYTFISYGMHWVRQAPGQGLEWMGEINPHGGSTSYAQKF
KGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARPSAAYSHYLGIDLWGRGTLVTVSSASTKGP
SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
20 VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL
YITREPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTTPVLDSGSEFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSL
SPGK

25

SEQ ID NO:32

QVQLVQSGAEVKKPGASVKVSCKASGYTFISYGMHWVRQAPGQGLEWMGEINPHGGSTSYAQKF
QGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARPSAAYSHYLGLDIWGRGTLVTVSSASTKGP
SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
30 VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL
YITREPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN

GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTPVLDSGSEFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGK

5 SEQ ID NO:33

QVQLVQSGAEVKKPGASVKVSCKASGYTFISYGMHWVRQAPGQGLEWMGEINPHGGSTSYAQKF
QGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARPSAAYSHYLGIDLWGRGTLVTVSSASTKGP
SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL
10 YITREPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTPVLDSGSEFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGK

15 SEQ ID NO:34

DIQMTQSPSSVSASVGDRVTITCRXSQGISXWLXWYQQKPGKAPKLLIYAXSXLQSGVPSRFSG
SGSGTDFTLTISSLPEDFATYYCQQAXVXPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSG
TASVVCLLNMFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSLSSTLTLSKADYEKHKVY
ACEVTHQGLSSPVTKSFNRGEC

- 20 X Pos 25: A or T
X Pos 31: P or S
X Pos 34: A or S
X Pos 51: A or G
X Pos 53: R or S
25 X Pos 92: H or N
X Pos 94: F or L

SEQ ID NO:35

- DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQKPGKAPKLLIYAASRLQSGVPSRFSG
30 SGSGTDFTLTISSLPEDFATYYCQQANVFPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSG

TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVY
ACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:36

5 DIQMTQSPSSVSASVGDRVTITCRASQGISPWLAWYQQKPGKAPKLLIYAGSSSLQSGVPSRFSG
SGSGTDFTLTISSSLQPEDFATYYCQQANVFPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSG
TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVY
ACEVTHQGLSSPVTKSFNRGEC

10 SEQ ID NO:37

DIQMTQSPSSVSASVGDRVTITCRTSQGISSWLSWYQQKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSSLQPEDFATYYCQQAHLPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSG
TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVY
ACEVTHQGLSSPVTKSFNRGEC

15

SEQ ID NO:38

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20 marggcagggtgacctgacaagggacaccagcaccagcaccgtgtacatggagctgagcagcc
tgaggagcgaggacaccgcccgtgtattactgcgcaaggcccagcgccgcctacagccactacct
gggcmthgacmnttggggcaggggcaccctggtgaccgtgagctct

SEQ ID NO:39

25 caggtgcagctggtgcagagcggcgccgaagtgaaaaagcccgcgccagcgtgaaggctcagct
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marggcagggtgacctgacaagggacaccagcaccagcaccgtgtacatggagctgagcagcc
tgaggagcgaggacaccgcccgtgtattactgcgcaaggcccagcgccgcctacagccactacct
30 gggcmthgacttrtggggcaggggcaccctggtgaccgtgagctct

SEQ ID NO:40

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5 marggcagggtgacctgacaagggacaccagcaccagcaccgtgtacatggagctgagcagcc
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gggcytgacmnttggggcaggggcaccctggtgaccgtgagctct

SEQ ID NO:41

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marggcagggtgacctgacaagggacaccagcaccagcaccgtgtacatggagctgagcagcc
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15 gggcytgacmnttggggcaggggcaccctggtgaccgtgagctct

SEQ ID NO:42

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20 gggcctcgagtggatgggagatcaacccccacggaggcagcaccagctacgcccagaagttc
marggcagggtgacctgacaagggacaccagcaccagcaccgtgtacatggagctgagcagcc
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25 SEQ ID NO:43

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30 tgaggagcgaggacaccgccgtgtattactgcgcaaggcccagcgccgcctacagccactacct
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SEQ ID NO:44

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5 gggcctcgagtggatgggcgagatcaacccccacggaggcagcaccagctacgcccagaagttc
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10 SEQ ID NO:45

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SEQ ID NO:46

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cagggcagggtgacctgacaagggacaccagcaccagcaccgtgtacatggagctgagcagcc
tgaggagcagggacaccgccgtgtattactgcgcaaggcccagcgcgcctacagccactacct
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25

SEQ ID NO:47

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30 cagggcagggtgacctgacaagggacaccagcaccagcaccgtgtacatggagctgagcagcc

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SEQ ID NO:48

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10 g

SEQ ID NO:49

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15 ccccaagctcctgatctacgccgsnagctcnctgcagagcggcgtgccctccagggttagcggc
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20 SEQ ID NO:50

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agcgggaagcggcaccgacttcaccctgaccatcagcagcctgcagcccaggacttcgccacct
25 actactgccagcagggccmaygtgytncccctgaccttcggcgggcggcacaaaagtcgagatcaa
g

SEQ ID NO:51

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5 SEQ ID NO:52

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SEQ ID NO:53

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20

SEQ ID NO:54

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25 agcggaagcggcaccgacttcacctgaccatcagcagcctgcagcccaggacttcgccacct
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SEQ ID NO:55

30 gacatccagatgaccagagccccagctcagtgagcgctagcgtgggcgacaggggtgaccatca
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5

SEQ ID NO:56

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10

SEQ ID NO:57

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20

SEQ ID NO:58

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25

SEQ ID NO:59

30

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SEQ ID NO:60

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15 tcccctggcaag

SEQ ID NO:61

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25 ccccaagctcctgatctacgccgsnagctcnctgcagagcggcgtgccctccaggtttagcggc
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30 tggacaatgccctgcagagcggcaacagccaggagagcgtgaccgagcaggacagcaaggactc
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gcctgtgaggtgaccaccagggcctgtccagccccgtgaccaagagcttcaaccggggcgagt
gc

SEQ ID NO:77

5 gacatccagatgaccagagccccagctcagtgagcgctagcgtggggcgacaggggtgaccatca
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10 gcgtacggtggccgccccagcgtgttcatcttccccccagcgatgagcagctgaagagcggc
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15 gc

SEQ ID NO:78

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20 ccccaagctcctgatctacgccggcagcagcctgcagagcggcgtgccctccagggttagcggc
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25 tggacaatgccctgcagagcgggaacagccaggagagcgtgaccgagcaggacagcaaggactc
cacctacagcctgagcagcacctgacctgagcaaggccgactacgagaagcacaaagggtgtac
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gc

30 SEQ ID NO:79

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5 actactgccagcaggccacgtgctgcccctgaccttcggcgggcgacaaaaagtcgagatcaa
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tggacaatgccttgcagagcggcaacagccaggagagcgtgaccgagcaggacagcaaggactc
cacctacagcctgagcagcacctgacctgagcaaggccgactacgagaagcacaaggtgtac
10 gcctgtgaggtgaccaccagggcctgtccagccccgtgaccaagagcttcaaccggggcgagt
gc

SEQ ID NO:80

YESQHPSNESGDGVDGKMLM
15

SEQ ID NO:81

FVLHNMHSNC

SEQ ID NO:82

20 YYESQHPSNESGDGVDGKML

SEQ ID NO:83

VLHNMHSNCV

25 SEQ ID NO:84

SPITEY

SEQ ID NO:85

VDSSENL
30

SEQ ID NO:86

YESQHPSNESGDGVDGKML

SEQ ID NO:87

VLHNMHSNC

5

CLAIMS

1. An IL-33 binding protein comprising:

- (i) CDRH1, CDRH2, and CDRH3 from SEQ ID NO:20 and CDRL1, CDRL2, and CDRL3 from SEQ ID NO:25, wherein SEQ ID NO:20 comprises:

5 QVQLVQSGAEVKKPGASVKVSCKASGYTFISYGMHWVRQAPGQGLEWMGEIN
PHGGSTSYAQKFX₁GRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARPSAAY
SHYLGX₂DX₃WGRGTLTVSS; and

- wherein SEQ ID NO:25 comprises:

10 DIQMTQSPSSVSASVGDRVITITCRX₄SQGISX₅WLX₆WYQQKPGKAPKLLIYA
X₇SX₈LQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQAX₉VX₁₀PLTFFG
GGTKVEIK; and

- wherein:

15 X₁ = K or Q; X₂ = I or L; X₃ = I, L, or M; X₄ = A or T;
X₅ = P or S; X₆ = A or S; X₇ = A or G; X₈ = R or S; X₉ =
H or N; and X₁₀ = F or L; or

- (ii) one or more CDR variants of (i), wherein the variant has 1, 2, or 3 amino acid modifications.

2. The IL-33 binding protein of claim 1, wherein the IL-33 binding protein comprises:

- 20 (i) CDRH1, CDRH2, and CDRH3 from any one of SEQ ID NOs:21-24 and CDRL1, CDRL2, and CDRL3 from any one of SEQ ID NOs:26-28; or
(ii) one or more CDR variants of (i), wherein the variant has 1, 2, or 3 amino acid modifications.

3. The IL-33 binding protein of claim 1 or claim 2, wherein the IL-33 binding protein comprises CDRH1, CDRH2, and CDRH3 from SEQ ID NO:21 and CDRL1, CDRL2, and CDRL3 from SEQ ID NO:26.

- 25 4. The IL-33 binding protein of claim 1 or claim 2, wherein the IL-33 binding protein comprises CDRH1, CDRH2, and CDRH3 from SEQ ID NO:22 and CDRL1, CDRL2, and CDRL3 from SEQ ID NO:26.

5. The IL-33 binding protein of claim 1 or claim 2, wherein the IL-33 binding protein comprises CDRH1, CDRH2, and CDRH3 from SEQ ID NO:23 and CDRL1, CDRL2, and CDRL3 from SEQ ID NO:27.
6. The IL-33 binding protein of claim 1 or claim 2, wherein the IL-33 binding protein comprises CDRH1, CDRH2, and CDRH3 from SEQ ID NO:24 and CDRL1, CDRL2, and CDRL3 from SEQ ID NO:28.
7. The IL-33 binding protein of claim 1 or claim 2, wherein the IL-33 binding protein comprises CDRH1 of SEQ ID NO:1, CDRH2 of SEQ ID NO:3, CDRH3 of SEQ ID NO:6, CDRL1 of SEQ ID NO:10, CDRL2 of SEQ ID NO:14, and CDRL3 of SEQ ID NO:18.
8. The IL-33 binding protein of claim 1 or claim 2, wherein the IL-33 binding protein comprises CDRH1 of SEQ ID NO:1, CDRH2 of SEQ ID NO:4, CDRH3 of SEQ ID NO:7, CDRL1 of SEQ ID NO:10, CDRL2 of SEQ ID NO:14, and CDRL3 of SEQ ID NO:18.
9. The IL-33 binding protein of claim 1 or claim 2, wherein the IL-33 binding protein comprises CDRH1 of SEQ ID NO:1, CDRH2 of SEQ ID NO:3, CDRH3 of SEQ ID NO:8, CDRL1 of SEQ ID NO:11, CDRL2 of SEQ ID NO:15, and CDRL3 of SEQ ID NO:18.
10. The IL-33 binding protein of claim 1 or claim 2, wherein the IL-33 binding protein comprises CDRH1 of SEQ ID NO:1, CDRH2 of SEQ ID NO:3, CDRH3 of SEQ ID NO:7, CDRL1 of SEQ ID NO:12, CDRL2 of SEQ ID NO:16, and CDRL3 of SEQ ID NO:19.
11. The IL-33 binding protein of any one of claims 1 to 10, wherein the IL-33 binding protein comprises a heavy chain variable (VH) domain having at least 90% identity to any one of SEQ ID NOs:20-24 and a light chain variable (VL) domain having at least 90% identity to any one of SEQ ID NOs:25-28.
12. The IL-33 binding protein of claim 11, wherein the IL-33 binding protein comprises a heavy chain variable (VH) domain having 100% identity to any one of SEQ ID NOs:20-24 and a light chain variable (VL) domain having 100% identity to any one of SEQ ID NOs:25-28.

13. The IL-33 binding protein of claim 11, wherein the IL-33 binding protein comprises a VH domain having at least 90% identity to SEQ ID NO:22 and a VL domain having at least 90% identity to SEQ ID NO:26.
14. The IL-33 binding protein of claim 11, wherein the IL-33 binding protein comprises a
5 VH domain having 100% identity to SEQ ID NO:22 and a VL domain having 100% identity to SEQ ID NO:26.
15. The IL-33 binding protein of any of the preceding claims, wherein the IL-33 binding protein is an antibody or binding fragment thereof.
16. The IL-33 binding protein of claim 15, wherein the antibody or binding fragment
10 thereof is a human IgG antibody or binding fragment thereof.
17. The IL-33 binding protein of claim 16, wherein the human IgG antibody or binding fragment thereof is a human IgG1 antibody or binding fragment thereof.
18. The IL-33 binding protein of claim 17, wherein the human IgG1 antibody or binding fragment thereof is a human IgG1 κ antibody or binding fragment thereof.
19. The IL-33 binding protein of any one of claims 15 to 18, wherein the antibody
15 comprises a modified Fc region.
20. The IL-33 binding protein of claim 19, wherein the modified Fc region comprises at least one Fc mutation to extend half-life.
21. The IL-33 binding protein of claim 20, wherein the at least one Fc mutation is YTE.
22. An IL-33 binding protein comprising a heavy chain (HC) having at least 90% identity
20 to any one of SEQ ID NOs:29-33 and a light chain (LC) having at least 90% identity to any one of SEQ ID NOs:34-37, wherein SEQ ID NO:29 comprises:
- QVQLVQSGAEVKKPGASVKVSCKASGYTFISYGMHWVRQAPGQGLEWMGEIN
PHGGSTSYAQKFX₁GRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARPSAAY
25 SHYLGX₂DX₃WGRGTLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY
FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN
VNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDITLYI
TREPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE
30 LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSK
LTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK; and

wherein SEQ ID NO:34 comprises:

DIQMTQSPSSVSASVGDRVITTCRX₄SQGISX₅WLX₆WYQQKPGKAPKLLIYA
X₇SX₈LQSGVPSRFSGSGSGTDFTLTITSSLPEDFATYYCQQA₉VX₁₀PLTFG
GGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD
NALQSGNSQESVTEQDSKSTYSLSTLTLSKADYEEKHKVYACEVTHQGLSS
PVTKSFNRGEC; and

wherein:

X₁ = K or Q; X₂ = I or L; X₃ = I, L, or M; X₄ = A or T;
X₅ = P or S; X₆ = A or S; X₇ = A or G; X₈ = R or S; X₉ =
H or N; and X₁₀ = F or L.

23. The IL-33 binding protein of claim 22, wherein the IL-33 binding protein is an antibody comprising an HC having at least 90% identity to SEQ ID NO:31 and an LC having at least 90% identity to SEQ ID NO:35.
24. The IL-33 binding protein of claim 22, wherein the IL-33 binding protein is an antibody comprising an HC of SEQ ID NO:31 and an LC of SEQ ID NO:35.
25. A pharmaceutical composition comprising the IL-33 binding protein as defined in any one of the preceding claims and a pharmaceutically acceptable excipient.
26. A method of treating or preventing a disease or condition in a human in need thereof comprising administering to the human a therapeutically effective amount of the IL-33 binding protein of any one of claims 1 to 24, or the pharmaceutical composition of claim 25.
27. An IL-33 binding protein of any one of claims 1 to 24, or a pharmaceutical composition of claim 25, for use in treating or preventing a disease or condition.
28. Use of the IL-33 binding protein of any one of claims 1 to 24, or the pharmaceutical composition of claim 25, in the manufacture of a medicament for treating or preventing a disease or condition.
29. The method of claim 26, the IL-33 binding protein or pharmaceutical composition for use of claim 27, or the use of claim 28, wherein the disease or condition is chronic obstructive pulmonary disease (COPD), asthma, bronchitis, bronchiolitis, acute respiratory failure, inflammatory lung diseases, diabetic kidney disease, endometriosis, chronic rhinosinusitis with nasal polyps, food hypersensitivity, food

allergy, peanut allergy, allergic rhinitis, eosinophilic oesophagitis, atopic dermatitis, cystic fibrosis, or chronic urticaria.

30. The method, the IL-33 binding protein or pharmaceutical composition for use, or the use of claim 29, wherein the disease or condition is COPD.

5 31. A nucleic acid sequence or plurality of nucleic acid sequences encoding an IL-33 binding protein according to any one of claims 1 to 24.

32. An expression vector comprising the nucleic acid sequence or plurality of nucleic acid sequences of claim 31.

10 33. A host cell that comprises the nucleic acid sequence or plurality of nucleic acids of any one of claim 31, or the expression vector of claim 32.

34. A method of producing an IL-33 binding protein, comprising culturing the host cell as defined in claim 33 under conditions suitable for expression of said nucleic acid sequence, plurality of nucleic acid sequences, or vector, whereby a polypeptide comprising the IL-33 binding protein is produced.

15 35. The IL-33 binding protein produced by the method of claim 34.

36. An IL-33 binding protein that binds to human IL-33 at amino acid residues 219-227 (SEQ ID NO:87), optionally wherein the IL-33 binding protein also binds to human IL-33 at amino acid residues 164-182 (SEQ ID NO:86).

20 37. An IL-33 binding protein that binds to human IL-33 and competes for binding to the human IL-33 with a reference IL-33 binding protein that binds to the IL-33 at amino acid residues 219-227 (SEQ ID NO:87), and optionally at amino acid residues 164-182 (SEQ ID NO:86), wherein the reference IL-33 binding protein comprises:

(i) CDRH1, CDRH2, and CDRH3 from SEQ ID NO:20 and CDRL1, CDRL2, and CDRL3 from SEQ ID NO:25, wherein SEQ ID NO:20 comprises:

25 QVQLVQSGAEVKKPGASVKVSCKASGYTFISYGMHWVRQAPGQGLEWMGEIN
PHGGSTSYAQKFX₁GRVTMTRDTSTSTVYMELSSLRSED₁TAVYYCARPSAAY
SHYLGX₂DX₃WGRGTLVTVSS; and

wherein SEQ ID NO:25 comprises:

30 DIQMTQSPSSVSASVGDRTITCRX₄SQGISX₅WLX₆WYQQKPGKAPKLLIYA
X₇SX₈LQSGVPSRFSGSGSGTDFTLTIS₁SLQPEDFATYYCQQA₉VX₁₀PLTFF
GGTKVEIK; and

wherein:

$X_1 = K \text{ or } Q$; $X_2 = I \text{ or } L$; $X_3 = I, L, \text{ or } M$; $X_4 = A \text{ or } T$;
 $X_5 = P \text{ or } S$; $X_6 = A \text{ or } S$; $X_7 = A \text{ or } G$; $X_8 = R \text{ or } S$; $X_9 =$
 $H \text{ or } N$; and $X_{10} = F \text{ or } L$;

- 5 (ii) CDRH1, CDRH2, and CDRH3 from any one of SEQ ID NOs:21-24 and CDRL1, CDRL2, and CDRL3 from any one of SEQ ID NOs:26-28; or
 (iii) one or more CDR variants of (i) or (ii), wherein the variant has 1, 2, or 3 amino acid modifications.

38. An IL-33 binding protein that binds to human IL-33 and competes for binding to the
 10 human IL-33 with a reference IL-33 binding protein, wherein the reference IL-33 binding protein comprises:

- (i) CDRH1, CDRH2, and CDRH3 from SEQ ID NO:20 and CDRL1, CDRL2, and CDRL3 from SEQ ID NO:25, wherein SEQ ID NO:20 comprises:

15 QVQLVQSGAEVKKPGASVKVSCKASGYTFISYGMHWVRQAPGQGLEWMGEIN
 PHGGSTSYAQKFX₁GRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARPSAAY
 SHYLGX₂DX₃WGRGTLVTVSS; and

wherein SEQ ID NO:25 comprises:

20 DIQMTQSPSSVSASVGDRVTITCRX₄SQGISX₅WLX₆WYQQKPGKAPKLLIYA
 X₇SX₈LQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQA₉VX₁₀PLTFG
 GGTKVEIK; and

wherein:

$X_1 = K \text{ or } Q$; $X_2 = I \text{ or } L$; $X_3 = I, L, \text{ or } M$; $X_4 = A \text{ or } T$;
 $X_5 = P \text{ or } S$; $X_6 = A \text{ or } S$; $X_7 = A \text{ or } G$; $X_8 = R \text{ or } S$; $X_9 =$
 $H \text{ or } N$; and $X_{10} = F \text{ or } L$;

- 25 (ii) CDRH1, CDRH2, and CDRH3 from any one of SEQ ID NOs:21-24 and CDRL1, CDRL2, and CDRL3 from any one of SEQ ID NOs:26-28; or
 (iii) one or more CDR variants of (i) or (ii), wherein the variant has 1, 2, or 3 amino acid modifications.