ANTI-TL1A/ANTI-TNF–α BISPECIFIC ANTIGEN BINDING PROTEINS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of Application No. PCT/US2016/052006, filed September 15, 2016, and U.S. Provisional Application Nos.: 62/268,432, filed December 16, 2015 and 62/333,063, filed May 6, 2016. Each of the foregoing applications is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0002] This invention relates to biopharmaceuticals, particularly to therapeutic antigen binding proteins, methods of use thereof, pharmaceutical compositions thereof, and processes of making them. In particular, this invention relates to therapeutic antigen binding proteins that are capable of binding the cytokines TL1A and TNF–α .

BRIEF DESCRIPTION OF THE SEQUENCE LISTING

[0003] Incorporated herein by reference in its entirety is a Sequence Listing entitled, "A-1937-WO-PCT_ST25.txt", comprising SEQ ID NO:1 through SEQ ID NO:2136, which includes nucleic acid and/or amino acid sequences disclosed herein. The Sequence Listing has been submitted herein in ASCII text format via EFS, and thus constitutes both the paper and computer readable form thereof. The Sequence Listing was first created using PatentIn on November 22, 2016, and is 3.45 MB in size.

BACKGROUND OF THE INVENTION

[0004] Cytokines are soluble, small proteins that mediate a variety of biological effects concerning the immune system. Such biological effects include induction of immune cell proliferation, development, differentiation, and/or migration; regulation of the growth and differentiation of many cell types; an inflammatory response through local or systemic accumulation of immune cells; and host-protective effects. See, for example, Arai et al., Annu. Rev. Biochem. 59:783 (1990); Mosmann, Curr. Opin. Immunol. 3:311 (1991); Paul et al., Cell, 76:241 (1994)). Such immune effects can produce pathological consequences when the effect leads to excessive and/or chronic inflammation, as in autoimmune disorders (such as multiple sclerosis) and cancer/neoplastic diseases. Oppenheim et al., eds., Cytokine Reference,

Academic Press, San Diego, Calif. (2001); von Andrian et al., New Engl. J. Med., 343:1020 (2000); Davidson et al., New Engl. J. Med., 345:340 (2001); Lu et al., Mol. Cancer Res., 4:221 (2006); Dalgleish et al., Cancer Treat Res., 130:1 (2006).

[0005] TLIA (TNFSF15) is a cytokine, a TNF–α family member involved in T cell activation (Richard et al., J Leukoc Biol. 2015 Sep, 98(3):333-45). It is the ligand for Death Receptor 3 (DR3), also known as TNFRSF25. TL1A is mainly expressed at low basal level in monocytes, dendritic cells and endothelial cells, but highly induced after immune complex and cytokine and microbes stimulation. Multiple genome-wide association studies (GWAS) demonstrated TL1A single nucleotide polymorphisms (SNPs) associated with Crohn's disease in various ethnic populations. In addition, TL1A inflammatory bowel disease (IBD) risk SNPs were reported to be associated with Crohn's disease severity (Hirano, IBD 19: 526, 2013). In the preclinical studies, TL1A protein treatment exacerbated colitis development in the colitis prone mdr1a-/- mice, but not in the wild-type mice. Altogether, human genomic data and preclinical colitis model data demonstrate that TL1A plays an important role in IBD development, and its blockage will be beneficial for IBD treatment.

[0006] Tumor Necrosis Factor-α (TNF–α) is a cytokine involved in the regulation of various physiological and pathological process (Buetler, et al., J Rheumatol Suppl. 57: 16-21, 1999). It is implicated in tumor regression, septic shock, cachexia, and a number of inflammatory and autoimmune conditions. Fransen et al. (June 1985), "Molecular cloning of mouse tumor necrosis factor cDNA and its eukaryotic expression," Nucleic Acids Res. **13** (12): 4417–29; Kriegler et al. (April 1988), "A novel form of TNF–α /cachectin is a cell surface cytotoxic transmembrane protein: ramifications for the complex physiology of TNF–α ," Cell **53** (1): 45–53. TNF–α inhibitors are a class of therapeutics approved to treat rheumatoid arthritis, psoriatic arthritis, juvenile idiopathic arthritis, ankylosing spondylitis, plaque psoriasis, Crohn's disease, and ulcerative colitis (Sethi et al., Adv Exp Med Biol. 2009;647:37-51). TNF–α inhibitors include etanercept, adalimumab, certolizumab pegol, infliximab, and golimumab.

[0007] Approximately 50% of patients respond to treatment with TNF inhibitors. However, only approximately 40% of those patients maintain responses after one year of treatment. Therefore, there is strong need for therapeutics with large effect size with durable response. We hypothesize that targeting multiple inflammatory pathways, such as TNF and TL1A, will likely achieve larger effect size with anticipated safety profile.

SUMMARY OF THE INVENTION

[0008] This invention relates to the development of antigen binding proteins, particularly fully human antibodies that specifically bind TL1A. Preferred antigen binding proteins have strong binding affinity to both human and cynomolgus TL1A, and block TL1A mediated NF-kB activation and T cell activation. These antigen binding proteins have potential to be used as therapeutics for treatment of inflammatory bowel disease (IBD) and other autoimmune and inflammatory conditions.

[0009] The invention further relates to bispecific antigen binding proteins, particularly bispecific antibodies. Bispecific antigen binding proteins of the invention comprise a TL1A binding entity and a TNF–α binding entity. TL1A blocking proteins and TNF–α blocking proteins have different effects in studies measuring inhibition of induction of cytokines IFNγ, IL-5, IL-6, IL-8, and IL-10. TL1A but not TNF-α induces T cell activation in vivo. TL1A and TNF–α induce NF-κB in different cell types in human peripheral blood monocytes (PBMCs). TL1A and TNF-α further induce different cytokines in human PBMCs. TL1A and TNF–α induce some of the same genes but in different cell types (16 overlapping genes induced by TL1A in whole blood and TNF-α in NCM460 cells; 13 overlapping genes induced by TL1A in whole blood and TNF-α in PBMCs). There is a strong genetic association of TL1A with inflammatory bowel disease (IBD) and anti-TNF agents are clinically validated for treatment of IBD. Such bispecific antigen binding proteins thus are useful for treatment of IBD and other autoimmune and inflammatory conditions. For these and other reasons, there is a benefit to bispecific antigen binding proteins that specifically bind TL1A and TNF–α .

[0010] One format for such bispecific antigen binding proteins is heterodimeric immunoglobulins (hetero Ig). Such hetero Ig antigen binding proteins have one heavy chain-light chain pair directed to TL1A and another directed to TNF–α .

[0011] Another format for such bispecific antigen binding proteins is IgG-scFv molecules. In an IgG-scFv, each heavy chain of an antibody that specifically binds one target is linked to a single chain antibody (scFv) that specifically binds the other target. The scFv portion can be linked to the heavy chain of the IgG portion directly or through a peptide linker. In the IgG-scFv format, the IgG is directed to TL1A and the scFv portion to TNF–α or vice-versa. Bispecific antigen binding proteins in the IgG-scFv format have the advantage of being bivalent for both of their target antigens.

[0012] A further format for such bispecific antigen binding proteins is IgG-Fab molecules. In this format, the antigen binding protein has a structure such that a Fab molecule that binds to one target is fused to each heavy chain of an IgG molecule that binds to another target. One chain of a Fab portion can be linked directly or through a peptide linker to the C-terminus of a heavy chain of the IgG portion. The resulting molecule has the advantage of being tetravalent and bispecific. All variations of the IgG-Fab format preferably comprise the CDR sequences of Tables 21.2A and 21.2B hereinafter. Preferred heavy and light chain sequences of IgG-Fab molecules appear in Table 21.1 hereinafter.

[0013] Other formats for bispecific antigen binding proteins are within the scope of this invention. One such format is IgG-Fab molecules. In this format, each heavy chain of an antibody that specifically binds to one target is linked to a Fab fragment that specifically binds to the other target. In the IgG-Fab format, the IgG is directed to TL1A and the Fab portion to TNF–α or vice-versa. Bispecific antigen binding proteins in the IgG-Fab format have the advantage of being bivalent for both of their target antigens. Other formats for bispecific antigen binding proteins within the scope of this invention are described infra.

[0014] The invention also relates to isolated nucleic acids encoding the TL1A-specific antigen binding proteins of the invention, as well as vectors comprising the nucleic acids, host cells comprising the vectors, and methods of making and using the TL1A-specific antigen binding proteins.

[0015] The invention also relates to isolated nucleic acids encoding the bispecific antigen binding proteins of the invention, as well as vectors comprising the nucleic acids, host cells comprising the vectors, and methods of making and using the bispecific antigen binding proteins.

[0016] In other embodiments, the present invention provides compositions comprising the TL1A-specific antigen binding proteins, the bispecific antigen binding proteins and kits comprising the anti-TL1A or bispecific antigen binding proteins, as well as articles of manufacture comprising the anti-TL1A or bispecific antigen binding proteins.

[0017] The TL1A-specific antigen binding proteins and the bispecific antigen binding proteins described herein can be used in the manufacture of pharmaceutical compositions or medicaments for the treatment of conditions associated with TL1A and/or, in the case of bispecific antigen binding proteins, conditions associated with TNF-α. Thus, the present invention also provides pharmaceutical compositions comprising a TL1A-specific antigen binding protein or a bispecific antigen binding protein and a pharmaceutically acceptable diluent, excipient or carrier.

[0018] The invention further relates to methods of treatment using the TL1A-specific antigen binding proteins. The TL1A-specific antigen binding proteins of the present invention are useful for the inhibition of the proinflammatory cytokine TL1A. The antibodies can be used to reduce, limit, neutralize, or block the proinflammatory effects of TL1A. Thus, in some embodiments, the invention relates to the treatment of IBD and other autoimmune or inflammatory conditions using the TL1A-specific antigen binding proteins.

[0019] The invention further relates to methods of treatment using bispecific antigen binding proteins. The bispecific antigen binding proteins of the present invention are useful for the inhibition of the proinflammatory cytokines, TL1A and TNF–α. The bispecific binding proteins can be used to reduce, limit, neutralize, or block the proinflammatory effects of TL1A. Likewise, the bispecific antigen binding proteins hereof can be used to reduce, limit, neutralize, or block the proinflammatory effects of TNF–α. Thus, in some embodiments, the invention relates to the treatment of IBD and other autoimmune or inflammatory conditions using TL1A-specific/TNF-specific antigen binding proteins.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] Figure 1 shows a schematic representation of four bispecific hetero Ig formats used to generate anti-TL1A/anti-TNF-α bispecific antigen binding proteins. As shown, a heavy chain directed to one antigen is disulfide-bonded to a heavy chain directed to a different antigen. A light chain for each antigen is disulfide-bonded to the heavy chain for the corresponding antigen. Figure 1 shows a preferred embodiment, in which the heavy and light chains comprise charge mutations to aid in correct association of the heavy and light chains. The Kabat-Eu numbering scheme is used to denote the positions of charge pair mutations within each of the chains and for all sequences throughout this specification. This IgG-like bispecific antigen binding protein format is a heterotetramer comprising two different light chains and two different heavy chains. HC1 and LC1 refer to the heavy chain and light chain, respectively, of one Fab binding arm and HC2 and LC2 refers to the heavy chain and light chain, respectively, of the second Fab binding arm. For example, in the schematic, HC1 and LC1 correspond to the anti-TL1A receptor binding arm and HC2 and LC2 correspond to the anti-TNF-α binding arm. However, the two binding arms can be switched such that HC1 and LC1 correspond to the anti-TNF-α binding arm and HC2 and LC2 correspond to the anti-TL1A receptor binding arm.

[0021] Figure 2 shows a schematic representation of the IgG-scFv format used to generate anti-TL1A/anti-TNF-α bispecific antigen binding proteins. As shown, the structure

incorporates a full tetrameric IgG directed to one antigen. A single-chain variable fragment (scFv), which comprises variable domains from a second antibody linked together by a glycine-serine linker, is fused to the carboxyl terminus of the heavy chain of a first antibody through a peptide linker to produce a modified heavy chain. Although the VH-VL orientation of the variable domains within the scFv is shown, the variable domains may also be organized in a VL-VH orientation. The complete molecule is a multimer comprising two heavy chains (but one unique heavy chain sequence) and two light chains (but one unique light chain sequence) from the first antibody.

[0022] Figure 3 concerns MabSelect SuRe affinity chromatography of an anti-TL1A/anti-TNF–α Hetero-Ig. It shows a representative FPLC protein A affinity capture chromatogram of an anti-TL1A/anti-TNF-α hetero-Ig. The protein was eluted with a step gradient of 100 mM acetic acid (conductivity: black trace, dashed), pH 3.6 and pooled based on the A280 (black trace, solid).

[0023] Figure 4 shows a representative FPLC SP high performance sepharose purification chromatogram of an anti-TL1A/anti-TNF-α hetero-Ig. Protein was eluted with an increasing salt gradient (conductivity : black trace, dashed) and was pooled based on the A280 elution profile (black trace, solid) and Caliper LabChip analysis of fractions.

[0024] Figure 5 shows a representative FPLC SP high performance sepharose purification chromatogram of an anti-TL1A/anti-TNF-α hetero-Ig. Protein was eluted with decreasing ammonium sulfate gradient (conductivity : black trace, dashed) and pooled based on the A280 elution profile (black trace, solid) and Caliper LabChip analysis of fractions.

[0025] Figure 6 concerns Caliper analysis of a TL1A/TNF–α hetero-Ig. It shows non-reduced and reduced Caliper analysis of an anti-TL1A/anti-TNF–α hetero-Ig.

[0026] Figure 7 concerns SE-HPLC analysis of an anti-TL1A/anti-TNF–α hetero-Ig. It shows size exclusion chromatography on 30 µg of the final anti-TL1A/anti-TNF-α hetero-Ig product injected onto a Sepax Zenix-C SEC-300 column (7.8 x 300 mm) in 50 mM NaH_2PO_4, 250 mM NaCl, pH 6.9 at 1 ml/min, observing the absorbance at 280 nm (black trace).

[0027] Figure 8 concerns LC-MS of Non-Reduced Hetero-Ig (Theoretical mass: 145495 Da). Figure 8 shows mass analysis of 20 µg non-reduced anti-TL1A/anti-TNF–α hetero-Ig eluted from a reverse-phase HPLC gradient using an Agilent Zorbax 300SB-C8 column (2.1 x 50 mm 3.5 µm) and mobile phases of 0.1% TFA and 90% n-Propanol/0.1% TFA (mobile phases A and B, respectively), equipped with an Agilent 6230 ESI-TOF Mass Spectrometer.

[0028] Figure 9 shows mass analysis of 20 μg TL1A/TNF–α hetero-Ig after limited Lysyl Endoproteinase C digestion for 30 minutes in 100 mM TRIS, pH 8. Reverse-phase HPLC was conducted using an Agilent Zorbax 300SB-C8 column (2.1 x 50 mm 3.5 μm) and mobile phases of 0.1% TFA and 90% n-propanol/0.1% TFA (mobile phases A and B respectively) and mass detection on an Agilent 6230 ESI-TOF Mass Spectrometer. Theoretical masses for TL1A Fab, TNF Fab, and Fc are 47350 Da, 48002 Da, and 50175 Da, respectively.

[0029] Figure 10 concerns MabSelect SuRe affinity chromatography of a anti-TL1A/anti-TNF–α IgG-scFv. It shows a representative FPLC protein A affinity capture chromatogram of an anti-TL1A/anti-TNF-α IgG-scFv. The protein was eluted with a step gradient of 100 mM acetic acid, pH 3.6 and pooled based on the A280 (black trace).

[0030] Figure 11 concerns SP Sepharose High Performance chromatography of an anti-TL1A/anti-TNF–α IgG-scFv. It shows a representative FPLC Superdex 200 purification chromatogram of an anti-TL1A/anti-TNF–α IgG-scFv. Protein was eluted with isocratic gradient of buffer and pooled based on the A280 elution profile (black trace) and SE-HPLC analysis of fractions.

[0031] Figure 12 shows non-reduced and reduced Caliper analysis of an anti-TL1A/anti-TNF–α IgG-scFv.

[0032] Figure 13 concerns SE-HPLC analysis of an anti-TL1A/anti-TNF–α IgG-scFv. It shows size exclusion chromatography on 30 μg of the final anti-TL1A/anti-TNF–α IgG-scFv product injected onto a Sepax Zenix-C SEC-300 column (7.8 x 300 mm) in 50 mM NaH_2PO_4, 250 mM NaCl, pH 6.9 at 1 ml/min, observing the absorbance at 280 nm.

[0033] Figure 14 concerns IdeS Protease digested Ig-scFv. It shows mass analysis of 20 μg Ig-scFv using reverse-phase HPLC separation on an Agilent Zorbax 300SB column (2.1 x 50 mm, 3.5 μm) with mobile phases of 0.1% TFA and 90% n-propanol/0.1% TFA (mobile phases A and B, respectively), and detection on an Agilent 6230 ESI-TOF Mass Spectrometer.

[0034] Figure 15 shows genotyping of TL1A SNPs. To evaluate potential TL1A genotype association with expression, genomic DNA (gDNA) was isolated from healthy PBMC donors using Gentra Puregene Tissue kit from Qiagen. Genomic DNA were genotyped using TaqMan SNP genotyping assays for rs7848647, rs6478109, rs6478108, and rs3810936 assays from LifeTech and standard protocols on the Bio-Rad droplet digital PCR platform. Donors were considered homozygous risk haplotype if only risk alleles were present at all 4 genotyped SNPs (rs7848647, rs6478109, rs6478108, and rs3810936). Donors were considered homozygous non-risk haplotype if only non-risk alleles were present at all 4 genotyped SNPs.

Donors were considered heterozygous haplotype if both risk and non-risk alleles were present at all 4 genotyped SNPs. Donors that were considered "recombinant" had only homozygous risk alleles at rs7848647, rs6478109, and rs6478108, but were heterozygous (risk and non-risk alleles present) at rs3810936.

[0035] Figures 16A and 16B show higher fold induction of TL1A risk allele than non-risk allele in a heterozygous PBMC AEI Study. Frequency of risk vs. non-risk allele usage in heterozygous PBMC at basal level or after immune complex stimulation at various time points was examined by droplet digit PCR (ddPCR) using synonymous SNP (rs3810936) allelic specific fluorescent probes. The allelic expression ratio was calculated by dividing the copies/ml of the risk allele by the copies/ml of the non-risk allele. Total copy number of each allele was normalized by the input amount of cDNA (copies/ng), and the fold-induction for each allele at each time point was calculated by dividing the copies/ng at the time point of interest by the copies/ng at baseline (0 hr).

[0036] Figure 17 shows that TL1A SNPs in promoter and intron contribute to allelic expression imbalance regulation. The inventor(s) identified a small number of donors homozygous for risk alleles at rs7848647, rs6478109, and rs6478108, but heterozygous at rs3810936, likely due to recombination between rs6478109 and synonymous SNP rs3810936. Frequency of risk vs. non-risk allele usage in heterozygous or recombinant donor PBMC was examined by droplet digit PCR (ddPCR) using synonymous SNP (rs3810936) allelic specific fluorescent probes. Compared to heterozygous donors, no allelic expression imbalance was detected in these recombinant individuals before or after immune complex stimulation.

[0037] Figures 18A and 18B show that TL1A risk SNPs are associated with expression quantitative trait loci (eQTL), PBMC donors with homozygous TL1A risk allele, homozygous non-risk alleles or heterozygous alleles were treated with immune complex. Total expression of TL1A (TNFSF15) in each donor was determined by digital PCR. In brief, cDNA from each sample was mixed with ddPCRTM Supermix for Probes (Bio-Rad) and PrimeTime® qPCR assay ID Hs.PT.56a.41003970. Droplets were generated for each reaction using the QX100™ droplet generator (Bio-Rad) and subjected to thermal cycling on a C1000 Touch™ thermal cycler (Bio-Rad). Following amplification, droplet fluorescence was read on a QX100™ droplet reader (Bio-Rad). Data were analyzed using QuantaSoft software (Bio-Rad) and copies/μl of TL1A was determined and normalized for the amount of input cDNA (copies/ng). P values for differences in TL1A expression levels between different TNFSF15 haplotypes were determined using the student t test. At basal level, PBMCs from donors homozygous for TL1A risk SNPs have lower TL1A mRNA compared to non-risk homozygous donors.

However, after immune complex stimulation, PBMCs from donors homozygous for TL1A risk SNPs have higher TL1A expression compared to non-risk homozygous donors.

[0038] Figures 19A and 19B show cell-type specific regulation of TL1A synonymous SNP allelic expression imbalance. HUVEC cells from various donors were genotyped as described previously. Genotyped HUVEC cells from various donors were treated with IL-1 at various time points. Total copy number of each allele was measured as described previously. The allelic expression ratio was calculated by dividing the copies/ml of the risk allele by the copies/ml of the non-risk allele. No allelic expression imbalance was observed in HUVEC cells with or without IL-1 treatment.

[0039] Figures 20A and 20B show that prophylactivc treatment with anti-TL1A monoclonal antibody inhibited spontaneous colitis development in mdr1a$^{-/-}$ mice. Mice (n=10, 6-7 weeks age) were randomized to different groups and treated intra-peritoneally once a week with 500 μg of anti-mouse TL1A antibody, anti-IL23p19 antibody, anti-mouse IL17RA antibody, or mouse isotype control or no treatment once per week for 8 weeks. Clinical disease activity was monitored by evaluating anal inflammation and stool consistency. Sections of the intestine were subjected to histopath analysis. Statistical analysis was performed using one-way ANOVA with Dunett's compared to mIgG1 group.

[0040] Figures 21A and 21B show that TL1A exacerbated colitis in mdr1a^{+} mice. Mdr1a$^{-/-}$ mice or wild-type control mice at 6-8 weeks of age were treated intra-peritoneally once a week with 150 μg of recombinant mFc-TL1A fusion protein or isotype control three times each week for 4 weeks. Clinical disease activity was monitored by evaluating anal inflammation and stool consistency as described previously. After 4 weeks of treatment, mice were sacrificed, sections of the intestine were were subjected to histopath analysis. Statistical analysis was performed using one way ANOVA with Dunett's compared to mIgG1 group.

[0041] Figures 22A to 22F shows increased inflammatory cells in lamina propria from Fc-TL1A challenged mdr1a$^{-/-}$ mice. Mdr1a$^{-/-}$ mice or wild-type control mice at 6-8 weeks of age were treated intra-peritoneally once a week with 150 μg of recombinant mFc-TL1A fusion protein or isotype control three times each week for 4 weeks. Lamina propria lymphocytes were isolated and stained for surface antigens and analyzed by FACS. TL1A challenge in mdr1a$^{-/-}$ mice resulted in increased inflammatory cells in lamina propria.

[0042] Figures 23A to 23E show distinct cytokine induction by TL1A and TNF challenge in mice. To evaluate if TL1A and TNF challenge result in similar or different pharmacodynamics effects, C57Bl/6 mice (8 week, female) were first intraperitonially injected

with 500 μg/mice of anti mouse TL1A, anti mouse TNF-α or PBS. After 4 hours, the mice were then challenged with 100 μg/mice of TL1A or 10 μg/mice of TNF-α or without challenging. The sera were collected after 24 hours. The cytokines were measured by MSD (IL-22 was measured by ELISA).

[0043] Figures 24A to 24F shows distinct cytokine induction by TL1A and TNF treatment in human PBMC. PBMC freshly isolated from human blood were cultured in media (RPMI1640 supplemented with 10% FBS, 2 mM glutamine, 1 mM sodium pyruvate, 5 X 10^{-5} M 2-ME, and antibiotics) in the presence of 100 ng/ml of human TL1A or TNF-α. Supernatant was collected after 72 hours. The cytokines in the supernatant were measured by MSD (IL-22 was measured by ELISA).

[0044] Figure 25 depicts a schematic representation of a bispecific IgG-Fab format for an anti-TL1A anti-TNF-α bispecific antigen binding protein within the present invention. In this format, one polypeptide chain of a Fab fragment from a second antibody (e.g. the heavy chain (VH2-CH1) is fused to the carboxyl terminus of each heavy chain of a first antibody through a peptide linker to produce a modified heavy chain. The complete molecule is a homohexamer comprising two modified heavy chains, two light chains from the first antibody, and two polypeptide chains containing the other half of the Fab fragment from the second antibody (e.g. the light chain (VL2-CL)). Charge pair mutations (represented by the circles) can be introduced into the Fab regions of the first antibody (Fab 1) and/or second antibody (Fab 2) to promote correct heavy chain-light chain pairing.

[0045] Figure 26 depicts a schematic representation of a bispecific IgG-Fab format for an anti-TL1A anti-TNF-α bispecific antigen binding protein within the present invention using immunoglobulin domain crossover. In this variation of the IgG-Fab format, one polypeptide chain of a Fab fragment from a second antibody (e.g. the heavy chain (VH2-CH1) is fused to the carboxyl terminus of the heavy chain comprising a CL instead of a CH1 domain of a first antibody through a peptide linker to produce a modified heavy chain. In this way, the CH1 and the CL domains of Fab 1 are "swapped." This swap is referred to as a Fab1 swap or an N-terminal swap herein. The complete molecule is a homohexamer comprising two modified heavy chains, two light chains from the first antibody comprising a CH1 domain instead of a CL domain, and two polypeptide chains containing the other half of the Fab fragment from the second antibody (e.g. the light chain (VL2-CL)). Charge pair mutations (represented by the circles) can be introduced into the Fab regions of the first antibody (Fab 1) and/or second antibody (Fab 2) to promote correct heavy chain-light chain pairs. Not shown but also within the scope of this invention are IgG-Fab molecules in which (i) the CH1 and CL domains of Fab

2 are swapped instead of those of Fab 1 (referred to herein as a Fab 2 swap or a C-terminal swap) or (ii) both the CH1 and CL domains of Fab 1 are swapped and the CH1 and CL domains of Fab 2 are swapped (referred to herein as a dual swap).

[0046] Figure 27 compares the expression titer of IgG-Fabs based on domain swapping format.

[0047] Figure 28 compares the expression titer of IgG-Fabs based on type of charge pair mutation(s).

[0048] Figure 29 compares the purity of IgG-Fabs based on domain swapping format.

[0049] Figure 30 compares the anti-TL1A potency of IgG-Fabs based on domain swapping format.

[0050] Figure 31 compares the anti-TNF-α potency of IgG-Fabs based on domain swapping format.

[0051] Figure 32 compares the anti-TL1A potency of IgG-Fabs based on type of charge pair mutation(s).

[0052] Figure 33 compares the anti- TNF-α potency of IgG-Fabs based on type of charge pair mutation(s).

DETAILED DESCRIPTION OF THE INVENTION

Definition of terms

[0053] In the description that follows, a number of terms are used extensively. The following definitions are provided to facilitate understanding of the invention.

[0054] Unless otherwise specified, "a", "an", "the", and "at least one" are used interchangeably and mean one or more than one.

[0055] A "binding entity" as used herein means any monomeric or multimeric protein or protein fragment that specifically binds a specified target antigen. The term “binding entity" includes but is not limited to antibodies and binding parts thereof, such as immunologically functional fragments. Peptibodies and peptides are other examples of binding entities. The term "immunologically functional fragment" (or simply "fragment") of an antibody or immunoglobulin chain (heavy or light chain) binding entity, as used herein, is a species of binding entity comprising a portion (regardless of how that portion is obtained or synthesized) of an antibody that lacks at least some of the amino acids present in a full-length chain but which is still capable of specifically binding to an antigen. Such fragments are biologically active in that they bind to the target antigen and can compete with other binding entities, including intact antibodies, for binding to a given epitope. In some embodiments, the fragments are neutralizing fragments. In some embodiments, the fragments can block or reduce the

likelihood of the interaction between the target antigen (TL1A or TNF–α) and its receptor. In one aspect, such a fragment will retain at least one CDR present in the full-length light or heavy chain, and in some embodiments will comprise a single heavy chain and/or light chain or portion thereof. These biologically active fragments can be produced by recombinant DNA techniques, or can be produced by enzymatic or chemical cleavage of binding entities, including intact antibodies. Immunologically functional immunoglobulin fragments include, but are not limited to, Fab, a diabody (heavy chain variable domain on the same polypeptide as a light chain variable domain, connected via a short peptide linker that is too short to permit pairing between the two domains on the same chain), Fab', $F(ab')_2$, Fv, domain antibodies and single-chain antibodies, and can be derived from any mammalian source, including but not limited to human, mouse, rat, camelid or rabbit. It is further contemplated that a functional portion of the binding entities disclosed herein, for example, one or more CDRs, could be covalently bound to a second protein or to a small molecule to create a therapeutic agent directed to a particular target in the body, possessing bifunctional therapeutic properties, or having a prolonged serum half-life. As will be appreciated by one of skill in the art, a binding entity can include non-protein components. In some sections of the present disclosure, examples of binding entities are named herein in terms of "number/letter/number" (e.g., 23B3), with some binding entities further identified by additional letter/number combinations (e.g., VH4). In these cases, the exact name denotes a specific antibody. That is, a binding entity named 23B3 may have some degree of sequence identity with but is not the same as an antibody named 23B3 VH4 (unless they are explicitly taught to be the same in the specification).

[0056] A "TL1A binding entity" is a binding entity that specifically binds to human TL1A; i.e., a binding entity for which human TL1A is the target antigen.

[0057] A "TNF–α binding entity" is a binding entity that specifically binds to human TNF–α ; i.e., a binding entity for which human TNF–α is the target antigen.

[0058] "Antigen binding protein" refers to a protein or polypeptide that comprises an antigen-binding region or antigen-binding portion that has a strong affinity for another molecule to which it binds (antigen). Antigen-binding proteins encompass antibodies, peptibodies, antibody fragments, antibody derivatives, antibody analogs, fusion proteins, and antigen receptors including chimeric antigen receptors (CARs).

[0059] "Antibodies" (Abs) and "immunoglobulins" (Igs) are glycoproteins having the same structural characteristics. While antibodies exhibit binding specificity to a specific

antigen, immunoglobulins include both antibodies and other antibody-like molecules that lack antigen specificity. Polypeptides of the latter kind are, for example, produced at low levels by the lymph system and at increased levels by myelomas. Thus, as used herein, the term "antibody" or "antibody peptide(s)" refers to an intact antibody, an antibody that competes for specific binding with an antibody disclosed in this specification, or an antigen-binding fragment thereof that competes with the intact antibody for specific binding and includes chimeric, humanized, fully human, and bispecific antibodies. In certain embodiments, antigen-binding fragments are produced, for example, by recombinant DNA techniques. In additional embodiments, antigen-binding fragments are produced by enzymatic or chemical cleavage of intact antibodies. Antigen-binding fragments include, but are not limited to, Fab, Fab', $F(ab)^2$, $F(ab')^2$, Fv, and single-chain antibodies.

[0060] The term "isolated antibody" as used herein refers to an antibody that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

[0061] The term "agonist" refers to any compound including a protein, polypeptide, peptide, antibody, antibody fragment, large molecule, or small molecule (less than 10 kD), that increases the activity, activation or function of another molecule.

[0062] The term "antagonist" refers to any compound including a protein, polypeptide, peptide, antibody, antibody fragment, large molecule, or small molecule (less than 10 kD), that decreases the activity, activation or function of another molecule.

[0063] The term "bind(ing)" of an antigen or other polypeptide includes, but is not limited to, the binding of a ligand polypeptide of the present invention to a receptor; the binding of a receptor polypeptide of the present invention to a ligand; the binding of an antibody of the present invention to an antigen or epitope; the binding of an antigen or epitope of the present

invention to an antibody; the binding of an antibody of the present invention to an anti-idiotypic antibody; the binding of an anti-idiotypic antibody of the present invention to a ligand; the binding of an anti-idiotypic antibody of the present invention to a receptor; the binding of an anti-anti-idiotypic antibody of the present invention to a ligand, receptor or antibody, etc.

[0064] A "bispecific" antigen binding protein is a molecule with binding entities derived from both a first antigen binding protein (e.g., antibody) that specifically binds a first target molecule of interest and a second antigen binding protein (e.g., antibody) that specifically binds a second target molecule of interest. Bispecific antigen binding proteins may be produced by a variety of methods including, but not limited to, fusion of hybridomas or linking of Fab' fragments. See, e.g., Songsivilai et al., Clin. Exp. Immunol., 79:315-321 (1990); Kostelny et al., J. Immunol., 148:1547-1553 (1992), hereby incorporated by reference. Molecules in the formats depicted in Figures 1 and 2 are bispecific antigen binding proteins in accordance with this definition. Various additional formats of bispecific antigen binding proteins within this definition are disclosed in WO 2014/159725; WO 2013/041687; US Pat. No. 8,945,553; US Pat. No. 8,945,553; US Pat. No. 8,258,268; US Pat. App. No. 2012/0195900; International patent application WO 2012/088302; US Prov. App. 62/218,977; Fischer and Leger (2007), Pathobiology 74:3-14; van Spriel et al. (2000), Immunology Today 21(8): 391-7; Kufer et al. (2004), Trends in Biotechnology 22(5): 238-44; Byrne et al. (2013), Trends in Biotechnology 31(11): 621-31; Muller and Kontermann (2010), Biodrugs 24(2): 89-98; Chames and Baty (2009), http://dx.doi.org/10.4161/mabs.1.6.10015; Kontermann (2012), http://dx.doi.org/10.4161/mabs.4.2.19000; Holliger et al. (1993), Proc. Natl. Acad. Sci. USA 90: 6444-8; Speiss et al., Mol. Immunol. (2015), 67: 95–106, http://dx.doi.org/10.1016/j.molimm.2015.01.003; Holliger et al. (1993), Proc. Natl. Acad. Sci. USA 90: 6444-8; Speiss et al., Mol. Immunol. (2015), http://dx.doi.org/10.1016/j.molimm.2015.01.003; Ridgway et al, (1996), Protein Eng. 9: 617; Gunasekaran et al (2010), J. Biol. Chem. 285:19637; Davis (2010), Protein Eng. Des. & Sel. 23:195; DiGiammarino et al. (2012), Methods Mol. Biol. 899:145, 2012); Lindhofer et al. (1995), J. Immunol. 155: 219: Schaefer et al. (2011), Proc. Natl. Acad. Sci. USA, 108: 11187; Regula et al, US Patent Application No: 2010/0322934; Bostrom et al. (2009), Science 323:1610; US Pat. App. 2011/0076722; Rossi et al. (2008), Cancer Res. 68:8384; each of which is hereby incorporated by reference.

[0065] The term "epitope" refers to the portion of an antigen to which an antibody specifically binds. Thus, the term "epitope" includes any protein determinant capable of specific binding to an immunoglobulin or T-cell receptor. Epitopic determinants usually consist

of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. More specifically, the term "IL-17 epitope", "TNF–α epitope" and/or "TNF–α /p19 epitope" as used herein refers to a portion of the corresponding polypeptide having antigenic or immunogenic activity in an animal, preferably in a mammal, and most preferably in a mouse or a human. An epitope having immunogenic activity is a portion of, for example, an IL-17A or IL-17F or TNF–α /p19 polypeptide that elicits an antibody response in an animal. An epitope having antigenic activity is a portion of, for example, an IL-17A or IL-17F or TNF–α /p19 polypeptide to which an antibody immunospecifically binds as determined by any method well known in the art, for example, by immunoassays, protease digest, crystallography or HID-Exchange. Antigenic epitopes need not necessarily be immunogenic. Such epitopes can be linear in nature or can be a discontinuous epitope. Thus, as used herein, the term "conformational epitope" refers to a discontinuous epitope formed by a spatial relationship between amino acids of an antigen other than an unbroken series of amino acids.

[0066] The term "immunoglobulin" refers to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes. One form of immunoglobulin constitutes the basic structural unit of an antibody. This form is a tetramer and consists of two identical pairs of immunoglobulin chains, each pair having one light and one heavy chain. In each pair, the light and heavy chain variable regions are together responsible for binding to an antigen, and the constant regions are responsible for the antibody effector functions.

[0067] Full-length immunoglobulin "light chains" (about 25 Kd or about 214 amino acids) are encoded by a variable region gene at the NH2-terminus (about 110 amino acids) and a kappa or lambda constant region gene at the COOH-terminus. Full-length immunoglobulin "heavy chains" (about 50 Kd or about 446 amino acids), are similarly encoded by a variable region gene (about 116 amino acids) and one of the other aforementioned constant region genes (about 330 amino acids). Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, and define the antibody's isotype as IgG (such as IgG1, IgG2, IgG3 and IgG4), IgM, IgA, IgD and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. (See generally, Fundamental Immunology (Paul, W., ed., 2nd Edition, Raven Press, NY (1989)), Chapter 7 (incorporated by reference in its entirety for all purposes).

[0068] An immunoglobulin light or heavy chain variable region consists of a "framework" region interrupted by three hypervariable regions. Thus, the term "hypervariable region" refers to the amino acid residues of an antibody which are responsible for antigen binding. The hypervariable region comprises amino acid residues from a "Complementarity Determining Region" or "CDR" (Kabat et al., Sequences of Proteins of Immunological Interest, 5th Edition, Public Health Service, National Institutes of Health, Bethesda, Md. (1991)) and/or those residues from a "hypervariable loop" (Chothia et al., J. Mol. Biol. 196: 901-917 (1987)) (both of which are incorporated herein by reference). "Framework Region" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined. The sequences of the framework regions of different light or heavy chains are relatively conserved within a species. Thus, a "human framework region" is a framework region that is substantially identical (about 85% or more, usually 90-95% or more) to the framework region of a naturally occurring human immunoglobulin. The framework region of an antibody, that is the combined framework regions of the constituent light and heavy chains, serves to position and align the CDR's. The CDR's are primarily responsible for binding to an epitope of an antigen. Accordingly, the term "humanized" immunoglobulin refers to an immunoglobulin comprising a human framework region and one or more CDR's from a non-human (usually a mouse or rat) immunoglobulin. The non-human immunoglobulin providing the CDR's is called the "donor" and the human immunoglobulin providing the framework is called the "acceptor". Constant regions need not be present, but if they are, they must be substantially identical to human immunoglobulin constant regions, i.e., at least about 85-90%, preferably about 95% or more identical. Hence, all parts of a humanized immunoglobulin, except possibly the CDR's, are substantially identical to corresponding parts of natural human immunoglobulin sequences. Further, one or more residues in the human framework region may be back mutated to the parental sequence to retain optimal antigen-binding affinity and specificity. In this way, certain framework residues from the non-human parent antibody are retained in the humanized antibody in order to retain the binding properties of the parent antibody while minimizing its immunogenicity. The term "human framework region" as used herein includes regions with such back mutations. A "humanized antibody" is an antibody comprising a humanized light chain and a humanized heavy chain immunoglobulin. For example, a humanized antibody would not encompass a typical chimeric antibody as defined above, e.g., because the entire variable region of a chimeric antibody is non-human.

[0069] As used herein, the term “modified heavy chain” refers to a fusion protein comprising an immunoglobulin heavy chain, particularly a human IgG1 or human IgG2 heavy

chain, and a functional antibody fragment (e.g., a Fab or scFv) or portion thereof (e.g. immunoglobulin light chain or Fd fragment), wherein the fragment or portion thereof is fused at its N-terminus, optionally through a peptide linker, to the C-terminus of the heavy chain.

[0070] The term "humanized" immunoglobulin refers to an immunoglobulin comprising a human framework region and one or more CDR's from a non-human, e.g., mouse, rat or rabbit, immunoglobulin. The non-human immunoglobulin providing the CDR's is called the "donor" and the human immunoglobulin providing the framework is called the "acceptor". Constant regions need not be present, but if they are, they must be substantially identical to human immunoglobulin constant regions, i.e., at least about 85-90%, preferably about 95% or more identical. Hence, all parts of a humanized immunoglobulin, except possibly the CDR's and possibly a few back-mutated amino acid residues in the framework region (e.g., 1-15 residues), are substantially identical to corresponding parts of natural human immunoglobulin sequences. A "humanized antibody" is an antibody comprising a humanized light chain and a humanized heavy chain immunoglobulin. For example, a humanized antibody would not encompass a typical chimeric antibody as defined above, e.g., because the entire variable region of a chimeric antibody is non-human.

[0071] The terms "human antibody" and “fully human antibody” each refer to an antibody that has an amino acid sequence of a human immunoglobulin, including antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulins and that do not express endogenous immunoglobulins; for example, Xenomouse antibodies and antibodies as described by Kucherlapati et al. in U.S. Pat. No. 5,939,598.

[0072] The term "genetically altered antibodies" means antibodies wherein the amino acid sequence has been varied from that of a native antibody. Because of the relevance of recombinant DNA techniques in the generation of antibodies, one need not be confined to the sequences of amino acids found in natural antibodies; antibodies can be redesigned to obtain desired characteristics. The possible variations are many and range from changing just one or a few amino acids to complete redesign of, for example, the variable and/or constant region. Changes in the constant region will, in general, be made in order to improve or alter characteristics, such as complement fixation, interaction with membranes and other effector functions. Changes in the variable region will be made in order to improve the antigen binding characteristics.

[0073] A "Fab fragment" is comprised of one light chain and the C_{H1} and variable regions of one heavy chain. The heavy chain of a Fab molecule cannot form a disulfide bond with another heavy chain molecule.

[0074] A "Fab' fragment" contains one light chain and one heavy chain that contains more of the constant region, between the C_{H1} and C_{H2} domains, such that an interchain disulfide bond can be formed between two heavy chains to form a $F(ab')_2$ molecule.

[0075] A "$F(ab')_2$ fragment" contains two light chains and two heavy chains containing a portion of the constant region between the C_{H1} and C_{H2} domains, such that an interchain disulfide bond is formed between two heavy chains.

[0076] The term "native Fc" refers to molecule or sequence comprising the sequence of a non-antigen-binding fragment resulting from digestion of whole antibody, whether in monomeric or multimeric form. The original immunoglobulin source of the native Fc is preferably of human origin and may be any of the immunoglobulins, although IgG1, IgG2 and IgG4 are preferred. Native Fc's are made up of monomeric polypeptides that may be linked into dimeric or multimeric forms by covalent (i.e., disulfide bonds) and non-covalent association. The number of intermolecular disulfide bonds between monomeric subunits of native Fc molecules ranges from 1 to 4 depending on class (e.g., IgG, IgA, IgE) or subclass (e.g., IgG1, IgG2, IgG3, IgG4, IgA1, IgGA2). One example of a native Fc is a disulfide-bonded dimer resulting from papain digestion of an IgG (see Ellison et al. (1982), Nucleic Acids Res. 10: 4071-9). The term "native Fc" as used herein is generic to the monomeric, dimeric, and multimeric forms.

[0077] The term "Fc variant" refers to a molecule or sequence that is modified from a native Fc but still comprises a binding site for the salvage receptor, FcRn. International applications WO 97/34631 (published 25 September 1997) and WO 96/32478 describe exemplary Fc variants, as well as interaction with the salvage receptor, and are hereby incorporated by reference. Thus, the term "Fc variant" comprises a molecule or sequence that is humanized from a non-human native Fc. Furthermore, a native Fc comprises sites that may be removed because they provide structural features or biological activity that are not required for the fusion molecules of the present invention. Thus, the term "Fc variant" comprises a molecule or sequence that lacks one or more native Fc sites or residues that affect or are involved in (1) disulfide bond formation, (2) incompatibility with a selected host cell (3) N-terminal heterogeneity upon expression in a selected host cell, (4) glycosylation, (5) interaction with complement, (6) binding to an Fc receptor other than a salvage receptor, or (7) antibody-dependent cellular cytotoxicity (ADCC). Fc variants are described in further detail hereinafter.

[0078] The term “Fc domain” encompasses native Fc and Fc variant molecules and sequences as defined above. As with Fc variants and native Fc’s, the term “Fc domain” includes molecules in monomeric or multimeric form, whether digested from whole antibody or produced by other means.

[0079] The term “multimer” as applied to Fc domains or molecules comprising Fc domains refers to molecules having two or more polypeptide chains associated covalently, noncovalently, or by both covalent and non-covalent interactions. IgG molecules typically form dimers; IgM, pentamers; IgD, dimers; and IgA, monomers, dimers, trimers, or tetramers. Multimers may be formed by exploiting the sequence and resulting activity of the native Ig source of the Fc or by derivatizing (as defined below) such a native Fc.

[0080] The term “dimer” as applied to Fc domains or molecules comprising Fc domains refers to molecules having two polypeptide chains associated covalently or non-covalently. Thus, exemplary dimers within the scope of this invention are as shown in Figure 2.

[0081] The terms "Fv fragment" and “single chain antibody” refer to polypeptides containing antibody variable regions from both heavy and light chains but lacking constant regions. Like a whole antibody, it is able to bind selectively to a specific antigen. With a molecular weight of only about 25 kDa, Fv fragments are much smaller than common antibodies (150-160 kD) which are composed of two heavy protein chains and two light chains, and even smaller than Fab fragments (about 50 kDa, one light chain and half a heavy chain).

[0082] A "single domain antibody" is an antibody fragment consisting of a single domain Fv unit, e.g., V_H or V_L. Like a whole antibody, it is able to bind selectively to a specific antigen. With a molecular weight of only 12-15 kDa, single-domain antibodies are much smaller than common antibodies (150-160 kDa) which are composed of two heavy protein chains and two light chains, and even smaller than Fab fragments (.about.50 kDa, one light chain and half a heavy chain) and single-chain variable fragments (.about.25 kDa, two variable domains, one from a light and one from a heavy chain). The first single-domain antibodies were engineered from heavy-chain antibodies found in camelids. Although most research into single-domain antibodies is currently based on heavy chain variable domains, light chain variable domains and nanobodies derived from light chains have also been shown to bind specifically to target epitopes.

[0083] The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

[0084] In some embodiments, the anti-TL1A antigen binding protein, bispecific antigen binding protein or functional fragment of either thereof from which the anti-TL1A binding domain is derived selectively inhibits human TL1A relative to TNF superfamily ligands. An antibody or functional fragment thereof "selectively inhibits" a specific receptor or ligand relative to other receptors or ligands when the IC50 of the antibody in an inhibition assay of the specific receptor is at least 50-fold lower than the IC50 in an inhibition assay of another "reference" ligand or receptor. An "IC50" is the dose/concentration required to achieve 50% inhibition of a biological or biochemical function. With radioactive ligands, IC50 is the concentration of a competing ligand that displaces 50% of the specific binding of the radioactive ligand. The IC50 of any particular substance or antagonist can be determined by constructing a dose-response curve and examining the effect of different concentrations of the drug or antagonist on reversing agonist activity in a particular functional assay. IC50 values can be calculated for a given antagonist or drug by determining the concentration needed to inhibit half of the maximum biological response of the agonist. Thus, the IC50 value for any anti-TL1A antibody or functional fragment thereof can be calculated by determining the concentration of the antibody or fragment needed to inhibit half of the maximum biological response of TL1A in activating the human TL1A receptor in any functional assay, such as the assay described in Example 14 hereinafter. An antibody or functional fragment thereof that selectively inhibits a specific ligand or receptor is understood to be a neutralizing antibody or neutralizing fragment with respect to that ligand or receptor. Thus, in some embodiments, the anti-TL1A antibody or functional fragment thereof from which the anti-TL1A binding domain of the bispecific antigen binding proteins of the invention is derived is a neutralizing antibody or fragment of human TL1A.

[0085] The variable regions or CDR regions of any anti-TL1A antibody or functional fragment thereof can be used to construct the anti-TL1A binding entity of any of the bispecific antigen binding proteins described herein. Likewise, the variable regions or CDR sequences of any anti-TNF-α antibody or functional fragment thereof can be used to construct the anti-TNF-α binding entity of any of the bispecific antigen binding proteins described herein. For instance, the anti-TL1A binding domain of the bispecific antigen binding proteins of the invention may comprise VH and/or VL regions or one or more CDRs from any of the anti-human TL1A antibodies described in US Pat. No. 7,820,798; US Pat. App. 2008/0003221; US Pat. No. 8,263,743; US Pat. App. 2011/0217310; US Pat. App. 2012/0263718; US Pat. App. 2014/0308271; WO 2012/161856; WO 2013/044298; WO 2005/018571; US Pat. App. 2014/0120109; US Pat. No. 6,521,422; US Pat. App. 2014/0255302; and US Pat. App.

2015/0132311; each of which is hereby incorporated by reference in its entirety. In some embodiments, the anti-TL1A antibody from which the anti-TL1A binding entity is derived cross-blocks one or more of the human anti-TL1A antibodies described in one of the references just mentioned or one or more of the human anti-TL1A antibodies described below. The terms "cross-block," "cross-blocked," and "cross-blocking" are used interchangeably herein to mean the ability of an antibody to interfere with the binding of other antibodies or binding fragments to a target (e.g. human TL1A). The extent to which an antibody or binding fragment is able to interfere with the binding of another to a target (e.g., human TL1A) and therefore whether it can be said to cross-block, can be determined using competition binding assays. In some embodiments, a cross-blocking antibody or binding fragment thereof reduces human TL1A binding of a reference antibody between about 40% and 100%, such as about 60% and about 100%, specifically preferably between about 70% and 100%, and more specifically preferably between about 80% and 100%. A particularly suitable quantitative assay for detecting cross-blocking uses a Biacore machine which measures the extent of interactions using surface plasmon resonance technology. Another suitable quantitative cross-blocking assay uses a FACS-based approach to measure competition between antibodies in terms of their binding to human TL1A. An exemplary assay regarding such cross-blocking appears in US Pat. App. 2015/0132311, example 2, paragraphs [0922]-[0924], hereby incorporated by reference.

[0086] The term "nucleic acid" or "nucleic acid molecule" refers to polynucleotides, such as deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), oligonucleotides, fragments generated by the polymerase chain reaction (PCR), and fragments generated by any of ligation, scission, endonuclease action, and exonuclease action. Nucleic acid molecules can be composed of monomers that are naturally-occurring nucleotides (such as DNA and RNA), or analogs of naturally-occurring nucleotides (e.g., .alpha.-enantiomeric forms of naturally-occurring nucleotides), or a combination of both. Modified nucleotides can have alterations in sugar moieties and/or in pyrimidine or purine base moieties. Sugar modifications include, for example, replacement of one or more hydroxyl groups with halogens, alkyl groups, amines, and azido groups, or sugars can be functionalized as ethers or esters. Moreover, the entire sugar moiety can be replaced with sterically and electronically similar structures, such as aza-sugars and carbocyclic sugar analogs. Examples of modifications in a base moiety include alkylated purines and pyrimidines, acylated purines or pyrimidines, or other well-known heterocyclic substitutes. Nucleic acid monomers can be linked by phosphodiester bonds or analogs of such linkages. Analogs of phosphodiester linkages include phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoranilidate,

phosphoramidate, and the like. The term "nucleic acid molecule" also includes so-called "peptide nucleic acids", which comprise naturally-occurring or modified nucleic acid bases attached to a polyamide backbone. Nucleic acids can be either single stranded or double stranded.

[0087] The term "complement of a nucleic acid molecule" refers to a nucleic acid molecule having a complementary nucleotide sequence and reverse orientation as compared to a reference nucleotide sequence.

[0088] The term "degenerate nucleotide sequence" denotes a sequence of nucleotides that includes one or more degenerate codons as compared to a reference nucleic acid molecule that encodes a polypeptide. Degenerate codons contain different triplets of nucleotides, but encode the same amino acid residue (i.e., GAU and GAC triplets each encode Asp).

[0089] An "isolated nucleic acid molecule" is a nucleic acid molecule that is not integrated in the genomic DNA of an organism. For example, a DNA molecule that encodes a growth factor that has been separated from the genomic DNA of a cell is an isolated DNA molecule. Another example of an isolated nucleic acid molecule is a chemically-synthesized nucleic acid molecule that is not integrated in the genome of an organism. A nucleic acid molecule that has been isolated from a particular species is smaller than the complete DNA molecule of a chromosome from that species

[0090] A "nucleic acid molecule construct" is a nucleic acid molecule, either single- or double-stranded, that has been modified through human intervention to contain segments of nucleic acid combined and juxtaposed in an arrangement not existing in nature

[0091] "Complementary DNA (cDNA)" is a single-stranded DNA molecule that is formed from an mRNA template by the enzyme reverse transcriptase. Typically, a primer complementary to portions of mRNA is employed for the initiation of reverse transcription. Those skilled in the art also use the term "cDNA" to refer to a double-stranded DNA molecule consisting of such a single-stranded DNA molecule and its complementary DNA strand. The term "cDNA" also refers to a clone of a cDNA molecule synthesized from an RNA template.

[0092] A "promoter" is a nucleotide sequence that directs the transcription of a structural gene. Typically, a promoter is located in the 5' non-coding region of a gene, proximal to the transcriptional start site of a structural gene. Sequence elements within promoters that function in the initiation of transcription are often characterized by consensus nucleotide sequences. These promoter elements include RNA polymerase binding sites, TATA sequences, CAAT sequences, differentiation-specific elements (DSEs; McGehee et al., Mol. Endocrinol., 7:551 (1993)), cyclic AMP response elements (CREs), serum response elements (SREs;

Treisman, Seminars in Cancer Biol., 1:47 (1990)), glucocorticoid response elements (GREs), and binding sites for other transcription factors, such as CRE/ATF (O'Reilly et al., J. Biol. Chem., 267:19938 (1992)), AP2 (Ye et al., J. Biol. Chem., 269:25728 (1994)), SP1, cAMP response element binding protein (CREB; Loeken, Gene Expr., 3:253 (1993)) and octamer factors (see, in general, Watson et al., eds., Molecular Biology of the Gene, 4th Edition, The Benjamin/Cummings Publishing Company, Inc. (1987), and Lemaigre et al., Biochem. J., 303:1 (1994)). If a promoter is an inducible promoter, then the rate of transcription increases in response to an inducing agent. In contrast, the rate of transcription is not regulated by an inducing agent if the promoter is a constitutive promoter. Repressible promoters are also known.

[0093] A "regulatory element" is a nucleotide sequence that modulates the activity of a core promoter. For example, a regulatory element may contain a nucleotide sequence that binds with cellular factors enabling transcription exclusively or preferentially in particular cells, tissues, or organelles. These types of regulatory elements are normally associated with genes that are expressed in a "cell-specific", "tissue-specific", or "organelle-specific" manner.

[0094] An "enhancer" is a type of regulatory element that can increase the efficiency of transcription, regardless of the distance or orientation of the enhancer relative to the start site of transcription.

[0095] "Heterologous DNA" refers to a DNA molecule, or a population of DNA molecules, that does not exist naturally within a given host cell. DNA molecules heterologous to a particular host cell may contain DNA derived from the host cell species (i.e., endogenous DNA) so long as that host DNA is combined with non-host DNA (i.e., exogenous DNA). For example, a DNA molecule containing a non-host DNA segment encoding a polypeptide operably linked to a host DNA segment comprising a transcription promoter is considered to be a heterologous DNA molecule. Conversely, a heterologous DNA molecule can comprise an endogenous gene operably linked with an exogenous promoter. As another illustration, a DNA molecule comprising a gene derived from a wild-type cell is considered to be heterologous DNA if that DNA molecule is introduced into a mutant cell that lacks the wild-type gene.

[0096] An "expression vector" is a nucleic acid molecule encoding a gene that is expressed in a host cell. Typically, an expression vector comprises a transcription promoter, a gene, and a transcription terminator. Gene expression is usually placed under the control of a promoter, and such a gene is said to be "operably linked to" the promoter. Similarly, a regulatory element and a core promoter are operably linked if the regulatory element modulates the activity of the core promoter.

[0097] A "recombinant host" is a cell that contains a heterologous nucleic acid molecule, such as a cloning vector or expression vector. In the present context, an example of a recombinant host is a cell that produces an antagonist of the present invention from an expression vector. In contrast, such an antagonist can be produced by a cell that is a "natural source" of said antagonist, and that lacks an expression vector.

[0098] The terms "amino-terminal" and "carboxyl-terminal" are used herein to denote positions within polypeptides. Where the context allows, these terms are used with reference to a particular sequence or portion of a polypeptide to denote proximity or relative position. For example, a certain sequence positioned carboxyl-terminal to a reference sequence within a polypeptide is located proximal to the carboxyl terminus of the reference sequence, but is not necessarily at the carboxyl terminus of the complete polypeptide.

[0099] A "fusion protein" is a hybrid protein expressed by a nucleic acid molecule comprising nucleotide sequences of at least two genes. For example, a fusion protein can comprise at least part of a IL-17RA polypeptide fused with a polypeptide that binds an affinity matrix. Such a fusion protein provides a means to isolate large quantities of IL-17A using affinity chromatography.

[00100] The term "receptor" denotes a cell-associated protein that binds to a bioactive molecule termed a "ligand." This interaction mediates the effect of the ligand on the cell. Receptors can be membrane bound, cytosolic or nuclear; monomeric (e.g., thyroid stimulating hormone receptor, beta-adrenergic receptor) or multimeric (e.g., PDGF receptor, growth hormone receptor, IL-3 receptor, GM-CSF receptor, G-CSF receptor, erythropoietin receptor and IL-6 receptor). Membrane-bound receptors are characterized by a multi-domain structure comprising an extracellular ligand-binding domain and an intracellular effector domain that is typically involved in signal transduction. In certain membrane-bound receptors, the extracellular ligand-binding domain and the intracellular effector domain are located in separate polypeptides that comprise the complete functional receptor. In general, the binding of ligand to receptor results in a conformational change in the receptor that causes an interaction between the effector domain and other molecule(s) in the cell, which in turn leads to an alteration in the metabolism of the cell. Metabolic events that are often linked to receptor-ligand interactions include gene transcription, phosphorylation, dephosphorylation, increases in cyclic AMP production, mobilization of cellular calcium, mobilization of membrane lipids, cell adhesion, hydrolysis of inositol lipids and hydrolysis of phospholipids.

[00101] The term "expression" refers to the biosynthesis of a gene product. For example, in the case of a structural gene, expression involves transcription of the structural gene into mRNA and the translation of mRNA into one or more polypeptides.

[00102] The term "complement/anti-complement pair" denotes non-identical moieties that form a non-covalently associated, stable pair under appropriate conditions. For instance, biotin and avidin (or streptavidin) are prototypical members of a complement/anti-complement pair. Other exemplary complement/anti-complement pairs include receptor/ligand pairs, antibody/antigen (or hapten or epitope) pairs, sense/antisense polynucleotide pairs, and the like. Where subsequent dissociation of the complement/anti-complement pair is desirable, the complement/anti-complement pair preferably has a binding affinity of less than 10^9 M^{-1}.

[00103] A "detectable label" is a molecule or atom which can be conjugated to an antibody moiety to produce a molecule useful for diagnosis. Examples of detectable labels include chelators, photoactive agents, radioisotopes, fluorescent agents, paramagnetic ions, or other marker moieties.

[00104] The term "affinity tag" is used herein to denote a polypeptide segment that can be attached to a second polypeptide to provide for purification or detection of the second polypeptide or provide sites for attachment of the second polypeptide to a substrate. In principal, any peptide or protein for which an antibody or other specific binding agent is available can be used as an affinity tag. Affinity tags include a polyhistidine tract, protein A (Nilsson et al., EMBO J., 4:1075 (1985); Nilsson et al., Methods Enzymol., 198:3 (1991)), glutathione S transferase (Smith et al., Gene, 67:31 (1988)), Glu-Glu affinity tag (Grussenmeyer et al., Proc. Natl. Acad. Sci. USA, 82:7952 (1985)), substance P, FLAG® peptide (Hopp et al., Biotechnology, 6:1204 (1988)), streptavidin binding peptide, or other antigenic epitope or binding domain. See, in general, Ford et al., Protein Expression and Purification, 2:95 (1991). DNA molecules encoding affinity tags are available from commercial suppliers (e.g., Pharmacia Biotech, Piscataway, N.J.).

[00105] The term "acidic residue" refers to amino acid residues having sidechains comprising acidic groups. Exemplary acidic residues include D and E.

[00106] The term "amide residue" refers to amino acids having sidechains comprising amide derivatives of acidic groups. Exemplary residues include N and Q.

[00107] The term "aromatic residue" refers to amino acid residues having sidechains comprising aromatic groups. Exemplary aromatic residues include F, Y, and W.

[00108] The term "basic residue" refers to amino acid residues having sidechains comprising basic groups. Exemplary basic residues include H, K, and R.

[00109] The term "hydrophilic residue" refers to amino acid residues having sidechains comprising polar groups. Exemplary hydrophilic residues include C, S, T, N, and Q.

[00110] The term "non-functional residue" refers to amino acid residues having sidechains that lack acidic, basic, or aromatic groups. Exemplary non-functional amino acid residues include M, G, A, V, I, L and norleucine (Nle).

[00111] Both the EU index as in Kabat et al. (1991), Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD and AHo numbering schemes (Honegger and Plückthun (2001), J Mol Biol. 8;309(3): 657-70) can be used in the present invention. Amino acid positions and CDRs and FRs of a given antibody may be identified using either system. For example, EU heavy chain positions 39, 44, 183, 356, 357, 370, 392, 399, and 409 are equivalent to AHo heavy chain positions 46, 51, 230, 484, 485, 501, 528, 535, and 551, respectively. Similarly, EU light chain positions 38, 100, and 176 are equivalent to AHO light chain positions 46 141, and 230, respectively. Tables (i), (ii) and (iii) below demonstrate the equivalence between numbering positions for v1, v2, and v3 versions of charge positions to aid correct assembly of, for example, IgG-Fab bispecific antigen binding proteins.

Table (i)- v1

Chain	Domain	Mutation	AHo #	EU #	Kabat #
LC-E	Constant	E	230	176	176
LC-K	Constant	K	230	176	176
HC-E	CH1	E	230	183	188
HC-K	CH1	K	230	183	188

Table (ii) - v2

Chain	Domain	Mutation	AHo #	EU #	Kabat #
LC-E	Variable	E	46	38	38
	Constant	E	230	176	176
LC-K	Variable	K	46	38	38
	Constant	K	230	176	176
HC-E	Variable	E	46	39	39
	CH1	E	230	183	188
HC-K	Variable	K	46	39	39
	CH1	K	230	183	188

Table (iii) - v3

Chain	Domain	Mutation	AHo #	EU #	Kabat #
LC-E	Variable	E	141	100	100

	Constant	E	230	176	176
LC-K	Variable	K	141	100	100
	Constant	K	230	176	176
HC-E	Variable	E	51	44	44
	CH1	E	230	183	188
HC-K	Variable	K	51	44	44
	CH1	K	230	183	188

Bispecific antigen binding protein formats of the invention

[00112] One aspect of the invention concerns novel TL1A-specific antigen binding proteins and antibodies. Such antibodies are useful to treat conditions known in the art and described hereinafter that are amenable to treatment by inhibition of TL1A biological activity.

[00113] Another aspect of the invention concerns bispecific antigen binding proteins in which one light chain-heavy chain pair specifically binds TL1A and another light chain-heavy chain pair binds TNF–α . Such bispecific antigen binding proteins can be whole antibodies (see Figure 1) or $F(ab')_2$ fragments. In this aspect of the invention, the TL1A binding entity is monovalent for TL1A and the TNF–α binding entity is monovalent for TNF–α .

[00114] In another bispecific antigen binding protein embodiment, the TL1A binding entity and the TNF–α binding entity are arranged in an overall structure referred to herein as IgG-scFv. In this embodiment, a first binding entity has the structure of an antibody-- i.e., two pairs of immunoglobulin chains, each pair having one light chain and one heavy chain. A second binding entity comprises the structure of a pair of Fv units-- i.e., each member of the pair has a variable domain from a heavy chain and a variable domain from a light chain linked in tandem as a single chain. In the IgG-scFv configuration, each Fv unit is covalently bound to the C-terminus of a heavy chain constant domain (Fc) of the first binding entity (see Figure 2). Each Fv unit can be linked to the Fc domain of the first binding entity directly or through a linker. In this embodiment of the invention, each binding entity is bivalent for its target antigen. In a preferred embodiment, the TL1A binding entity has the structure of an antibody and the TNF–α binding entity has the structure of a pair of Fv units.

[00115] The invention further concerns bispecific antibodies with mutations to aid correct heavy-heavy and heavy-light chain pairing. Such mutations are described in US 8,592,562; WO 2009/089004; WO 2006/106905; WO 2014/4082179; WO 2014/081955; Speiss et al., Mol. Immunol. (2015), http://dx.doi.org/10.1016/j.molimm.2015.01.003; each of which is hereby incorporated by reference.

[00116] The invention further concerns a TL1A binding entity and a TNF–α binding entity arranged in other bispecific antigen binding protein formats known in the art. Specifically, the invention concerns the TL1A and TNF–α binding entities in the formats described in: WO 2014/159 725; WO 2013/041687; US Pat. No. 8,945,553; US Pat. No. 8,945,553; US Pat. No. 8,258,268; US Pat. App. No. 2012/ 0195900; International patent application WO 2012/088 302; US Prov. App. 62/218,977; Fischer and Leger (2007), Pathobiology 74:3-14; van Spriel et al. (2000), Immunology Today 21(8): 391-7; Kufer et al. (2004), Trends in Biotechnology 22(5): 238-44; Byrne et al. (2013), Trends in Biotechnology 31(11): 621-31; Muller and Kontermann (2010), Biodrugs 24(2): 89-98; Chames and Baty (2009), http://dx.doi.org/10.4161/mabs.1.6.10015; Kontermann (2012), http://dx.doi.org/10.4161/mabs.4.2.19000; Holliger et al. (1993), Proc. Natl. Acad. Sci. USA 90: 6444-8; Speiss et al., Mol. Immunol. (2015), http://dx.doi.org/10.1016/j.molimm.2015.01.003; WO 2009/089004, published 16 July 2009; Holliger et al. (1993), Proc. Natl. Acad. Sci. USA 90: 6444-8; Speiss et al., Mol. Immunol. (2015), http://dx.doi.org/10.1016/j.molimm.2015.01.003; Ridgway et al, (1996), Protein Eng. 9: 617; Gunasekaran et al (2010), J. Biol. Chem. 285:19637; Davis (2010), Protein Eng. Des. & Sel. 23:195; DiGiammarino et al. (2012), Methods Mol. Biol. 899:145, 2012); Lindhofer et al. (1995), J. Immunol. 155: 219: Schaefer et al. (2011), Proc. Natl. Acad. Sci. USA, 108: 11187; Regula et al, US Patent Application No: 2010/0322934; Bostrom et al. (2009), Science 323:1610; US Pat. App. 2011/0076722; Rossi et al. (2008), Cancer Res. 68:8384; each of which is hereby incorporated by reference.

Preferred embodiments

[00117] The amino acid sequences of the antigen binding proteins and binding entities are preferably based upon the sequences of human and/or humanized monoclonal antibodies against TL1A and TNF–α . The invention also comprises sequences having at least 90%, at least 95%, or at least 99% sequence identity to the preferred sequences set forth hereinafter. The amino acid sequences shown in the following tables are preferred for the antigen binding proteins of this invention, including bispecific antigen binding proteins in any format.

TL1A-specific antigen Binding Proteins

[00118] TL1A-specific antigen binding proteins and antigen binding entities preferably comprise the complementarity determining region (CDR) sequences derived from preferred TL1A antibodies disclosed herein. Table A shows the preferred CDR sequences, alongside the preferred antibodies from which they were derived. Throughout, LCDR1, LCDR2, and LCDR3

refer to the light chain CDRs; HCDR1, HCDR2, and HCDR3 to the heavy chain CDRs, Throughout, the sequence identification number (SEQ ID NO) for each sequence appears in parentheses after the sequence in the table.

[00119] **Table A: Preferred TL1A-binding CDR sequences**

Source Antibody designation	LCDR1 (SEQ ID NO)	LCDR2 (SEQ ID NO)	LCDR3 (SEQ ID NO)	HCDR1 (SEQ ID NO)	HCDR2 (SEQ ID NO)	HCDR3 (SEQ ID NO)
3C6	RTSQDIRD DLG (98)	DASSLQS (100)	LQHNSYPP T (102)	SYGMH (164)	VISYDGNN KLYTDSVK G (166)	PTVTLYYY YGMDV (168)
2G11	RASQSINN YLN (110)	ATSSLQS (106)	QQSYSTPR T (108)	SYFWS (170)	YIYYSGST NYNPSLKS (172)	EIGSYYGF DY (174)
9C8	RASQSINN YLN (110)	AASSLQS (112)	QQSYSTPR T (108)	SYFWS (170)	YIYYSGNT KYNPSLKS (178)	ETGSYYGF DY (180)
23B3	KSSQSVLY SSNNKNYL V (116)	WASTRES (118)	QQYYKTPL T (120)	TNSVAWN (182)	RTYYRSK WYNDYAV SVKS (184)	EDGDSYYR YGMDV (186)
23B3 VH4	RSSQSVLY SSNNKNYL V (128)	WASTRES (118)	QQYYKTPL T (120)	TNSVAWN (182)	RTYYRSK WYNDYAV SLKS (196)	EDGDSYYR YGMDV (186)
23B3 VH3	RSSQSVLY SSNNKNYL V (128)	WASTRES (118)	QQYYKTPL T (120)	TNSVAWN (182)	RTYYRSK WYNDYAV SVKG (202)	EDGDSYYR YGMDV (186)
3B3	RASQSVRS SYLA (122)	GASSRAT (124)	QQYGSSPT (126)	GYYWN (188)	EINHAGNT NYNPSLKS (190)	GYCRSTTC YFDY (192)
5G4	RASQSVRS SYLA (122)	GASSRAT (124)	QQYGSSPT (126)	GYYWN (188)	EINHSGITN YNPSLKS (483)	GYCRSTTC YFDY (192)
17E9	RASQGISN DLA (469)	AASSLQS (112)	LQHNSYPP T (102)	SYGMH (164)	VMSYDGN NKLYADSV KG (485)	DETETLYY YYGIDV (489)
53D3	RSSQSLLY SNGYNYLD (683)	LGSSRAS (685)	MQPLQTPL T (687)	TYYMS (777)	SISSSSSFI YYADSVKG (779)	DRIAAPGT YYYYGMD V (781)

54E5	KSSQNILYSSNNKNYLA (689)	WASTRES (118)	QQYYSIPWT (693)	TYGMH (783)	VIWYDGSNKDYADSVKG (785)	EERDSYYHYGMDV (787)
56E1	KSSQSVLYSSNNKNYLA (235)	WASTRES (118)	QQYYSIPWT (693)	SYGMH (164)	VIWYDGSNKDYADSVKG (785)	EERDSYYHYGMDV (787)
57A8	TGSSSNIGAGYNVH (697)	GNNNRPS (699)	QSYDSSLSGWV (701)	SYVMS (792)	VISGSGGSTYYADSVKG (794)	GGTNYYYYYSGMDV (796)
58G5	TGSSSNIGAGYDVH (146)	GNSHRPS (705)	QSYDSSLSGYV (707)	NYAMS (798)	VISGRGGSTYYADSVKG (800)	DGYSSAWYFDY (802)
60G11	TGSGSNIGAGYDVH (709)	GNSHRPS (705)	QSYDSSLSGYV (707)	NYAMN (804)	VISGRGGSTYYADSVKG (800)	DGYSSAWFFDY (807)
73C2	RASQSFSSYLN (713)	AASSLQS (112)	QQSYSTPRS (717)	SSSATWN (809)	RTYQRSKWNNDYAVSVKS (811)	EVVAGPRWFDP (813)
76A4	RASQSVTSYLN (719)	TASSLQS (721)	QQSYSTPRS (717)	SNSATWN (815)	RTYYRSKLYNDYAVSVKS (817)	EVVAGPRWFDP (813)
77D12	SESNSDIGTNAVN (723)	SNNKRPS (725)	ATWDDNLNGPL (727)	GFYMH (819)	WINPDSGGTNYAQKFQG (821)	EGIAVALTY (823)
87H11	RASQSISSYLN (229)	VASSLQS (731)	QQSYSNPQECS (733)	GYYWS (265)	EINHSGRTNYNPSLKS (827)	DSGWHFSFDI (829)
88H9	RASQGIRNDLG (226)	AASGLQG (737)	LQHNSYPTWT (739)	SYGMH (164)	VIWFDGSNEYYADSVKG (832)	ERWFGELLDY (834)
89H9	RASQSISYYLN (741)	AASSLQS (112)	QQSYSSIT (745)	SYAMS (836)	GISGGGGSTYYADSVKG (838)	EMAGAFDI (840)
91D7	RASQNISSYLN (747)	TASSLQS (749)	QQSYSNPPESS (751)	GYYWS (265)	EINPVGRTNYKPSLKS (843)	DNGWHYAFDI (845)
91F10_LC1	RSSQSLVYSDGNTYLN (753)	KVSNWDS (755)	MQGTHWP (757)	AYYMH (847)	WINPNSGGTNYAQKFRG (849)	GGSWEGFDY (851)

91F10_LC2	RASQSISS YLN (229)	AASRLQS (761)	QQSDSTPI T (763)	AYYMH (847)	WINPNSGG TNYAQKFR G (849)	GGSWEGF DY (851)
91G8	RASQSISS YLN (229)	AASSLQS (112)	QQSFSTIT (767)	SYAMS (836)	GISGSGGS RYYADSVK G (853)	EVAGAFDI (855)
92D3	RASQSISS YLN (229)	GASRLQS (769)	QQSDTTPI T (771)	GYYMH (857)	WIIPNSGG TNYAQKFQ G (859)	GSSWEGF DY (861)
92E5	RASQSISH YLN (773)	AASSLQS (112)	QQSFSSIT (775)	SYAMS (836)	GISGRGGS TYYADSVK G (864)	EVAGAFDI (855)

[00120] Each of the foregoing sequences is encoded by the nucleic acid sequence immediately preceding it in the Sequence Listing. Throughout, the sequences from antibodies 9C8 and 3B3 are preferred.

[00121] In the antigen binding proteins of this invention, it is preferable to avoid isomerization sites. The two-amino acid sequences DG, DH, DS, and DT are known isomerization sites. The present invention relates also to antigen binding proteins in which the source antibody sequences, including the sequences of CDRs, are modified to eliminate isomerization sites. With that consideration, the invention relates to antigen binding proteins wherein HCDR2 is a modified form of the HCDR2 from source antibody 3C6 having the sequence VISYDXNNKLYTDXVKG (SEQ ID NO: 204) wherein each X is independently a residue other than G, H, S, or T (i.e., A, C, D, E, F, I, K, L, M, N, P, Q, R, V, W, or Y), with A preferred. The invention further relates to antigen binding proteins wherein HCDR2 is a modified form of the HCDR2 from source antibody 3C6 in which the acidic residue D is replaced with E so as to remove the isomerization sites, resulting in the sequence VISYEGNNKLYTESVKG (SEQ ID NO: 678). The invention further relates to antigen binding proteins wherein HCDR3 is a modified form of HCDR3 from source antibody 23B3 having the sequence EDGDXYYRYGMDV (SEQ ID NO: 676). The invention further relates to antigen binding proteins wherein the isomerization site of HCDR3 from source antibody 23B3 is removed by replacing the acidic residue D with E, resulting in the sequence EEGESYYRYGMDV (SEQ ID NO: 657).

[00122] In the antigen binding proteins of this invention, it is preferable to avoid deamidation sites. The two-amino acid sequences NG, NH, NS, and NT are known deamidation sites. The present invention relates also to antigen binding proteins in which the source antibody sequences, including the sequences of CDRs, are modified to eliminate deamidation

sites. One way to eliminate the deamidation sites is to replace the second amino acid residue in the sites NG, NH, NS, and NT with a residue other than G, H, S, or T (i.e., A, C, D, E, F, I, K, L, M, N, P, Q, R, V, W, or Y). A preferred way to eliminate deamidation sites is to replace the N in NG, NH, NS and NT with Q. With that consideration, the invention relates to antigen binding proteins having the following sequences in addition to those listed in Table A:

[00123] LCDR3 may have the sequence LQHQSYPPT (SEQ ID NO: 631) based on modification of the sequence from the source antibody 3C6;

[00124] HCDR1 may have the sequences TQSVAWN (SEQ ID NO: 632) based on modification of the sequence from the source antibody 23B3 VH4;

[00125] HCDR2 may have the sequences YIYYSGQTKYNPSLKS (SEQ ID NO: 633) or EIQHAGQTNYNPSLKS (SEQ ID NO: 677) based on modification of the sequences from the source antibodies 9C8 and 3B3, respectively.

[00126] Antigen binding proteins of this invention may further result from substitution of an acidic residue for another acidic residue, an amide residue for another amide residue, and likewise for aromatic residues, basic residues, hydrophilic residues, non-functional residues, neutral polar residues, and polar hydrophobic residues. Such substitutions of non-functional residues of the source antibody CDRs results in the sequences of the invention shown in Table B, wherein each X is independently M, G, A, V, I, or L.

[00127] **Table B: TL1A-binding CDR sequences with substitutions**

Source Antibody designation	LCDR1 (SEQ ID NO)	LCDR2 (SEQ ID NO)	LCDR3 (SEQ ID NO)	HCDR1 (SEQ ID NO)	HCDR2 (SEQ ID NO)	HCDR3 (SEQ ID NO)
3C6	RTSQDXRD DLX (635)	DXSSLQS (636)	XQHQSYPP T (637), XQHNXYPP T (638), LQHQSYPP T (631),	SYXXH (639)	XXSYDXNN KXYTDXXK X (640), VISYDXNN KLYTDXVK G (204), VISYEGNN KLYTESVK G (678), XXSYEXNN KLYTESXK X (679)	PTXTXYYY YXXDV (641)

2G11	RXSQSXNNYLN (642)	XTSSXQS (643)	QQSYSTPRT (108)	SYFWS (170)	YXYYSXSTNYNPSXKS (644)	EXXSYYXFDY (645)
9C8	RXSQSXNNYXN (646)	XXSSXQS (647)	QQSYSTPRT (108)	SYFWS (170)	YXYYSXQTKYNPSXKS (648), YXYYSXNXKYNPSXKS (649), YIYYSGQTKYNPSLKS (633)	ETXSYYXFDY (650)
23B3	KSSQSVXYSSNNKNYXX (651)	WXSTRES (652)	QQYYKTPXT (653)	TQSXXWN (654), TNXXXWN (655), TQSVAWN (632)	RTYYRSKWYNDYXVSXKS (656)	EDXDSYYRYXXDV (657), EDGDXYYRYGMDV (676), EEGESYYRYGMDV (680), EEXESYYRYXXDX (681)
23B3 VH4	RSSQSVXYSSNNKNYXX (658)	WXSTRES (652)	QQYYKTPXT (653)	TQSXXWN (654), TNXXXWN (655), TQSVAWN (632)	RTYYRSKWYNDYXXSXKS (659)	EDXDSYYRYXXDV (657), EDGDXYYRYGMDV (676), EEGESYYRYGMDV (680), EEXESYYRYXXDX (681)
23B3 VH3	RSSQSVXYSSNNKNYXX (658)	WXSTRES (652)	QQYYKTPXT (653)	TQSXXWN (654), TNXXXWN (655),	RTYYRSKWYNDYXXSVKX (660)	EDXDSYYRYXXDV (657), EDGDXYYR

				TQSVAWN (632)		YGMDV (676), EEGESYYR YGMDV (680), EEXESYYR YXXDX (681)
3B3	RXSQSVRS SYXX (661)	XXSSRXT (662)	QQYXSSPT (663)	XYYWN (664)	EXQHXXQT NYNPSXKS (665), EXNHXXNT NYNPSXKS (666), EIQHAGQT NYNPSLKS (677)	XYCRSTTC YFDY (667)
5G4	RXSQSXRS SYXX (668)	XXSSRXT (662)	QQYXSSPT (663)	XYYWN (664)	EXNXSXXT NYNPSXKS (114), EIQHSGITN YNPSLKS (227), XQHSXXTN YNPSXKS (230)	XYCRSTTC YFDY (667)
17E9	RXSQXXSN DX (646)	XXSSXQS (647)	XQHQSYPP T (637), XQHNSYPP T (233)	SYXXH (639)	VMSYDXN NKLYADXV KG (691), VMSYEGN NKLYAESV KG (715), XXSYDXNN KXYXDSXK X (669), XXSYEXNN KXYXESXK X (711)	DETETXYY YYXXDX (671)

53D3	RSSQSXXY SNXYNYXD (759), RSSQSLLY SQGYNYLD (743), RSSQSXXY SQXYNYXD (765)	XXSSRXS (789)	XQPXQTPX T (940)	TYYXS (955)	SISSSSSFI YYADXVKG (971), SISSSSSFI YYAESVKG (972), SXSSSSSF XYYXDSXK X (973), SXSSSSSF XYYXESXK X (974), SXSSSSSF XYYXDXXK X (975)	DRXXXPXT YYYYXXDX (976)
54E5	KSSQNXLY SSNNKNYX X (977)	WXSTRES (652)	QQYYSXP WT (978)	TYXXH (979)	VIWYDXSN KDYADXVK G (980), VIWYEGSN KDYAESVK G (981), XXWYDXS NKDYXDSX KX (982), XXWYDXS NKDYXDXV KG (983), XXWYEXS NKDYXESX KG (984)	EERDSYYH YXXDX (985)
56E1	KSSQSXXY SSNNKNYX X (986)	WXSTRES (652)	QQYYSXP WT (978)	SYXXH (639)	VIWYDXSN KDYADXVK G (980), VIWYEGSN KDYAESVK G (981), XXWYDXS NKDYXDSX KX (982), XXWYDXS NKDYXDXV KG (983), XXWYEXS NKDYXESX KG (984)	EERDSYYH YXXDX (985)
57A8	TXSSSNXX XXYNXH (987)	XNNNRPS (988)	QSYDXSLS GWV (989), QSYESSLS GWV (990), QSYDSSXS XWX (991), QSYDXSXS XWV (992), QSYESSXS XWV (993)	SYXXS (994)	VISGSGGS TYYADXVK G (995), VISGSGGS TYYAESVK G (996), XXSXSXXS TYYXDSXK X (997),XXSX SXXSTYYX DXXKX (998), XXSXSXXS	XXTNYYYY YSXXDX (1000)

					TYYXESXKX (999)	
58G5	TXSSSNXXXXYDXH (1001)	XNXHRPS (1002), GQSHRPS (1003), XQSHRPS (1004)	QSYDXSLSGYV (1005),QSYESSLSGYV (1006), QSYDSSXSXYX (1007), QSYDXSXSXYX (1008), QSYESSXSXYX (1009)	NYXXS (1010)	XXSXRXXSTYYXDSXKX (1011)	DXYSSAWYFDY (1012), EGYSSAWYFDY (1013), DXYSSXWYFDY (1014), EXYSSXWYFDY (1015)
60G11	TXSXSNXXXXYDXH (1016)	XNSHRPS (1002), GQSHRPS (1003), XQSHRPS (1004)	QSYDSSXSXYX (1007)	NYXXN (1017)	XXSXRXXSTYYXDSXKX (1011)	DXYSSAWFFDY (1018), EGYSSAWFFDY (1019), DXYSSXWFFDY (1020)
73C2	RXSQSFSSYXN (1021)	XXSSXQS (647)	[none]	SSSXTWN (1022)	RTYQRSKWNNDYXXSXKS (1023)	EXXXXPRWFDP (1024)
76A4	RXSQSXTSYXN (1025)	TXSSXQS (1026)	[none]	SNSXTWN (1027), SQSATWN (1028) SQSXTWN (1029)	RTYYRSKXYNDYXXSXKS (1030)	EXXXXPRWFDP (1024)
77D12	SESNXDXXTNXXN (1031), SESQSDIGTNAVN (1032) SESQSDXXTNXXN (1033)	[none]	XTWDDNXNXPX (1034), ATWDDNLQGPL (1035) XTWDDNXQXPX (1036)	XFYXH (1037)	WINPDXGGTNYAQKFQG (1038), WINPESGGTNYAQKFQG (1039), WXNPDSXXTNYXQKFQX (1040), WXNPDXXXTNYXQKFQX (1041), WXNPESXXTNYXQKFQX (1042)	EXXXXXXTY (1043)
87H11	RXSQSXSSYXN (1044)	XXSSXQS (647)	[none]	XYYWS (1045)	EXNXSXRTNYNPSXKS (1046), EIQHSGRTNYNPSLKS (1047) EXQHSXRTNYNPSXKS (1048)	DXGWHFSFDI (1049), ESGWHFSFDI (1050), DSXWHFSFDX (1051), ESXWHFSFDX (1052)

88H9	RXSQXXRNDLX (1053)	XXSXXQX (1054)	XQHNSYPTWT (1055), LQHQSYPTWT (1056) XQHQSYPTWT (1057)	SYXXH (639)	VIWFDXSNEYYADXVKG (1058), VIWFEGSNEYYADSVKG (1059), VIWFDGSNEYYAESVKG (1060), VIWFEGSNEYYAESVKG (1061), XWFDXSNEYYXDSXKX (1062)	ERWFXEXXDY (1063)
89H9	RXSQSXSYYXN (1064)	XXSSXQS (647)	QQSYSSXT (1065)	SYXXS (994)	GISGGGGSTYYADXVKG (1066),GISGGGGSTYYAESVKG (1067), XXSXXXXSTYYXDSXKX (1068), XXSXXXXSTYYXDXXKX (1069),XXSXXXXSTYYXESXKX (1070)	EXXXXFDX (1071)
91D7	RXSQNXSSYXN (1072)	TXSSXQS (1073)	[none]	XYYWS (1045)	EXNPXXRTNYKPSXKS (1074)	DNXWHYXFDX (1075), DQGWHYAFDI (1076), DQXWHYXFDX (1077)
91F10_LC1	RSSQSXXYSDXNTYXN (1078), RSSQSLVYSDGQTYLN (1079), RSSQSXXYSDXQTYXN (1080)	KVSNWDX (1081),KVSNWES (1082), KXSNWDS (1083), KXSNWDX (1084), KXSNWES (1085)	XQXTHWP (1086)	XYYXH (1087)	WXNPNXXXTNYXQKFRX (1088), WINPQSGGTNYAQKFRG (1089), WXNPQSXXTNYXQKFRX (1090)	XXSWEXFDY (1091)
91F10_LC2	RXSQSXSSYXN (1044)	XXSRXQS (1092)	QQSDSTPXT (1093)	XYYXH (1087)	WXNPNXXXTNYXQKFRX (1088), WINPQSGGTNYAQKFRG (1089), WXNPQSXXTNYXQKFRX (1090)	XXSWEXFDY (1091)

91G8	RXSQSXSS YXN (1044)	XXSSXQS (647)	QQSFSTXT (1094)	SYXXS (994)	GISGSGGS RYYADXVK G (1095), XXSXSXXS RYYXDSXK X (1096), XXSXSXXS RYYXDXXK X (1097)	EXXXXFDX (1071)
92D3	RXSQSXSS YXN (1044)	XXSRXQS (1098)	QQSDXTPI T (1099), QQSDTTPX T (1100), QQSDXTPX T (1101)	XYYXH (1087)	WXXPNXX XTNYXQKF QX (1102), WIIPQSGG TNYAQKFQ G (1103), WXXPQSX XTNYXQKF QX (1104)	XSSWEXFD Y (1105)
92E5	RXSQSXSH YXN (1106)	XXSSXQS (647)	QQSFSSXT (1107)	SYXXS (994)	GISGRGGS TYYADXVK G (1108), GISGRGGS TYYAESVK G (1109). XXSXRXXS TYYXDSVK X (1110), XXSXRXXS TYYXDXVK X (1111),XXS XRXXSTYY XESVKX (1112)	EXXXXFDX (1071)

[00128] Also preferred are antigen binding proteins comprising the CDR germline sequences related to the antibodies shown in Table A. Such sequences are shown in Table C.

[00129] **Table C: Related germline CDR sequences of anti-TL1A antibodies**

Antibody designation related to germline	LCDR1 (SEQ ID NO)	LCDR2 (SEQ ID NO)	LCDR3 (SEQ ID NO)	HCDR1 (SEQ ID NO)	HCDR2 (SEQ ID NO)	HCDR3 (SEQ ID NO)
3C6	RASQGIRN DLG (226)	AASSLQS (112)	LQHNSYP WT (228)	SYAMH (253)	VISYDGSN KYYADSVK G (254)	TTVTYYYY YGMD (255)
2G11	RASQSISS YLN (229)	AASSLQS (112)	QQSYSTP WT (231)	SYYWS (256)	YIYYSGST NYNPSLKS (172)	SYYYFD (258)
9C8	RASQSISS YLN (229)	AASSLQS (112)	QQSYSTPY T (234)	SYYWS (259)	YIYYSGST NYNPSLKS (260)	LTGYFD (261)

23B3	KSSQSVLY SSNNKNYL A (235)	WASTRES (118)	QQYYSTPL T (237)	SNSAAWN (262)	RTYYRSK WYNDYAV SVKS (184)	RDGYNYYY YYYGMD (264)
23B3 VH4	RASQGISN YLA (241)	AASTLQS (242)	QKYNSAPL T (243)	SGSYYWS (268)	YIYYSGST NYNPSLKS (269)	RDGYNYYY YYYGMD (270)
23B3 VH3	RASQGISN YLA (241)	AASTLQS (242)	QKYNSAPL T (243)	SNYMS (271)	IYSGGSTY YADSVKG (272)	RDGYNYYY YYYGMD (273)
3B3	RASQSVSS SYLA (238)	GASSRAT (124)	QQYGSSPI T (240)	GYYWS (265)	EINHSGST NYNPSLKS (266)	GYCSSTSC YTYFD (267)
5G4	RASQSVSS SYLA (238)	GASSRAT (124)	QQYGSSPI T (240)	GYYWS (265)	EINHSGST NYNPSLKS (266)	GYCSSTSC YTYFDY (672)
17E9	RASQGIRN DLG (226)	AASSLQS (112)	LQHNSYPL T (674)	SYAMH (253)	VISYDGSN KYYADSVK G (254)	GTTGTYYY YYGMDV (675)
53D3	RSSQSLLH SNGYNYLD (1113)	LGSNRAS (935)	MQALQTPL T (936)	SYSMN (950)	SISSSSSYI YYADSVKG (951)	GIAAAGYY YYYGMDV (952)
54E5	KSSQSVLY SSNNKNYL A (235)	WASTRES (118)	QQYYSTP WT (938)	SYGMH (164)	VIWYDGSN KYYADSVK G (953)	EYSSSSYY YYYGMDV (954)
56E1	KSSQSVLY SSNNKNYL A (235)	WASTRES (118)	QQYYSTP WT (938)	SYGMH (164)	VIWYDGSN KYYADSVK G (953)	EYSSSSYY YYYGMDV (954)
57A8	TGSSSNIG AGYDVH (146)	GNSNRPS (148)	QSYDSSLS GWV (701)	SYAMS (836)	AISGSGGS TYYADSVK G (956)	GTTGTYYY YYGMDV (675)
58G5	TGSSSNIG AGYDVH (146)	GNSNRPS (148)	QSYDSSLS GYV (707)	SYAMS (836)	AISGSGGS TYYADSVK G (956)	RDGYNYYF DY (958)
60G11	TGSSSNIG AGYDVH (146)	GNSNRPS (148)	QSYDSSLS GYV (707)	SYAMS (836)	AISGSGGS TYYADSVK G (956)	RDGYNYYF DY (958)
73C2	RASQSISS YLN (229)	AASSLQS (112)	QQSYSTPY T (234)	SNSAAWN (262)	RTYYRSK WYNDYAV SVKS (184)	GIAVAGNW FDP (961)

76A4	RASQSISS YLN (229)	AASSLQS (112)	QQSYSTPY T (234)	SNSAAWN (262)	RTYYRSK WYNDYAV SVKS (184)	GIAVAGNW FDP (961)
77D12	SGSSSNIG SNTVN (947)	SNNQRPS (948)	AAWDDSL NGVV (949)	GYYMH (857)	WINPNSGG TNYAQKFQ G (963)	SIAARAEYF QH (964)
87H11	RASQSISS YLN (229)	AASSLQS (112)	QQSYSTPY T (234)	GYYWS (265)	EINHSGST NYNPSLKS (266)	DYGDYDAF DI (966)
88H9	RASQGIRN DLG (226)	AASSLQS (112)	LQHNSYP WT (228)	SYGMH (164)	VIWYDGSN KYYADSVK G (953)	RWLQLYFD Y (967)
89H9	RASQSISS YLN (229)	AASSLQS (112)	QQSYSTPI T (942)	SYAMS (836)	AISGSGGS TYYADSVK G (956)	VEMATIDA FDI (968)
91D7	RASQSISS YLN (229)	AASSLQS (112)	QQSYSTPY T (234)	GYYWS (265)	EINHSGST NYNPSLKS (266)	DYGDYDAF DI (966)
91F10_LC1	RSSQSLVY SDGNTYLN (753)	KVSNRDS (943)	MQGTHWP LT (944)	GYYMH (857)	WINPNSGG TNYAQKFQ G (963)	GYSYGYYF DY (969)
91F10_LC2	RASQSISS YLN (229)	AASSLQS (112)	QQSYSTPI T (942)	GYYMH (857)	WINPNSGG TNYAQKFQ G (963)	GYSYGYYF DY (969)
91G8	RASQSISS YLN (229)	AASSLQS (112)	QQSYSTPI T (942)	SYAMS (836)	AISGSGGS TYYADSVK G (956)	SIAARDAF DI (970)
92D3	RASQSISS YLN (229)	AASSLQS (112)	QQSYSTPI T (942)	GYYMH (857)	WINPNSGG TNYAQKFQ G (963)	GYSYGYYF DY (969)
92E5	RASQSISS YLN (229)	AASSLQS (112)	QQSYSTPI T (942)	SYAMS (836)	AISGSGGS TYYADSVK G (956)	SIAARDAF DI (970)
88H9	RASQGIRN DLG (226)	AASSLQS (112)	LQHNSYP WT (228)	SYGMH (164)	VIWYDGSN KYYADSVK G (953)	RWLQLYFD Y (967)
54E5	KSSQSVLY SSNNKNYL A (235)	WASTRES (118)	QQYYSTP WT (938)	SYGMH (164)	VIWYDGSN KYYADSVK G (953)	EYSSSSYY YYYGMDV (954)

56E1	KSSQSVLY SSNNKNYL A (235)	WASTRES (118)	QQYYSTP WT (938)	SYGMH (164)	VIWYDGSN KYYADSVK G (953)	EYSSSSYY YYYGMDV (954)
53D3	RSSQSLLH SNGYNYLD (1113)	LGSNRAS (935)	MQALQTPL T (936)	SYSMN (950)	SISSSSSYI YYADSVKG (951)	GIAAAGYY YYYGMDV (952)
89H9	RASQSISS YLN (229)	AASSLQS (112)	QQSYSTPI T (942)	SYAMS (836)	AISGSGGS TYYADSVK G (956)	VEMATIDA FDI (968)
91G8	RASQSISS YLN (229)	AASSLQS (112)	QQSYSTPI T (942)	SYAMS (836)	AISGSGGS TYYADSVK G (956)	SIAARDAF DI (970)
92E5	RASQSISS YLN (229)	AASSLQS (112)	QQSYSTPI T (942)	SYAMS (836)	AISGSGGS TYYADSVK G (956)	SIAARDAF DI (970)
92D3	RASQSISS YLN (229)	AASSLQS (112)	QQSYSTPI T (942)	GYYMH (857)	WINPNSGG TNYAQKFQ G (963)	GYSYGYYF DY (969)
91F10_LC2	RASQSISS YLN (229)	AASSLQS (112)	QQSYSTPI T (942)	GYYMH (857)	WINPNSGG TNYAQKFQ G (963)	GYSYGYYF DY (969)
91D7	RASQSISS YLN (229)	AASSLQS (112)	QQSYSTPY T (234)	GYYWS (265)	EINHSGST NYNPSLKS (266)	DYGDYDAF DI (966)
76A4	RASQSISS YLN (229)	AASSLQS (112)	QQSYSTPY T (234)	SNSAAWN (262)	RTYYRSK WYNDYAV SVKS (184)	GIAVAGNW FDP (961)
87H11	RASQSISS YLN (229)	AASSLQS (112)	QQSYSTPY T (234)	GYYWS (265)	EINHSGST NYNPSLKS (266)	DYGDYDAF DI (966)
73C2	RASQSISS YLN (229)	AASSLQS (112)	QQSYSTPY T (234)	SNSAAWN (262)	RTYYRSK WYNDYAV SVKS (184)	GIAVAGNW FDP (961)
91F10_LC1	RSSQSLVY SDGNTYLN (753)	KVSNRDS (943)	MQGTHWP LT (944)	GYYMH (857)	WINPNSGG TNYAQKFQ G (963)	GYSYGYYF DY (969)
60G11	TGSSSNIG AGYDVH (146)	GNSNRPS (148)	QSYDSSLS GYV (707)	SYAMS (836)	AISGSGGS TYYADSVK G (956)	RDGYNYYF DY (958)

58G5	TGSSSNIG AGYDVH (146)	GNSNRPS (148)	QSYDSSLS GYV (707)	SYAMS (836)	AISGSGGS TYYADSVK G (956)	RDGYNYYF DY (958)
57A8	TGSSSNIG AGYDVH (146)	GNSNRPS (148)	QSYDSSLS GWV (701)	SYAMS (836)	AISGSGGS TYYADSVK G (956)	GTTGTYYY YYGMDV (675)
77D12	SGSSSNIG SNTVN (947)	SNNQRPS (948)	AAWDDSL NGVV (142)	GYYMH (857)	WINPNSGG TNYAQKFQ G (963)	SIAARAEYF QH (964)

[00130] Further preferred are antigen binding proteins comprising the variable domain sequences of the preferred anti-TL1A antibodies, as shown in Table D. Throughout, "VH" as shown in Table D refers to the variable domain of the heavy chain, "VL" to that of the light chain. Molecules within this invention may incorporate modifications to the sequences shown in Table D, including truncations or substitutions for stability or other functionality or removal of hotspots (chemical or physical modification of amino acids). Also included within the invention are molecules having at least 90% sequence identity with the sequences shown in Table D.

[00131] **Table D: Variable domain sequences of preferred anti-TL1A antibodies**

Antibody designation	Amino acid sequence (SEQ ID NO)
3C6 VL	DIQMTQSPSSLSASVGDRVTITCRTSQDIRDDLGWYQQKPGKAPKRLIYDASSLQSGV PSRFSGSGSGTEFTLTISSLQPEDFATYYCLQHNSYPPTFGQGTKVEIK (6)
3C6 VH	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDG NNKLYTDSVKGRFTISRDDSKSTLYLQMNSLRAEDTAVYYCARDPTVTLYYYYGMDV WGQGTTVTVSS (8)
2G11 VL	DIQMTQSPSSLSASVGDRVTITCRASQSINNYLNWYQQKPGIAPKLLIYATSSLQSGVP SRFSGSGSGTDFSLTISSLQPEDFATYFCQQSYSTPRTFGQGTKVEIK (10)
2G11 VH	QVQLQESGPGLVKPSETLSLTCTVSGGSISSYFWSWIRQPPGKGLEWIGYIYYSGSTN YNPSLKSRVTMSIDTSKNQFSLKLSSVTAADTAVYYCAREIGSYYGFDYWGQGALVTV SS (12)
9C8 VL	DIQMTQSPSSLSASVGDRVTITCRASQSINNYLNWYQQRPGKAPKLLIYAASSLQSGV PSRFSGSGSGTDFTLTISSLQPGDFATYYCQQSYSTPRTFGQGTKLEIK (14)
9C8 VH	QVQLQESGPGLVKPSETLSLTCTVSGGSISSYFWSWIRQPPGKGLEWIGYIYYSGNTK YNPSLKSRVTISIDTSKNQFSLKLSSVTAADTAVYYCARETGSYYGFDYWGQGTLVTV SS (16)
23B3 VL	DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLVWYQQKPGQPPKLLIYWAS TRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYKTPLTFGGGTKVEIK (18)

23B3 VH	QVQLQQSGPGLVKPSQTLSLTCVISGDSVSTNSVAWNWIRQSPSRGLEWLGRTYYR SKWYNDYAVSVKSRITINPDTSKNQFSLQLNSVTPEDTAVYYCAREDGDSYYRYGMD VWGQGTTVTVSS (20)
3B3 VL	EIVLTQSPGTLSLSPGERATLSCRASQSVRSSYLAWYQQKPGQAPRLLIYGASSRATG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPTFGQGTRLEIK (22)
3B3 VH	QVQLQQWGAGLLKPSETLSLTCAVHGGSFSGYYWNWIRQPPGKGLEWIGEINHAGN TNYNPSLKSRVTISLDTSKNQFSLTLTSVTAADTAVYYCARGYCRSTTCYFDYWGQGT LVTVSS (24)
23B3 VH4 VL	DIQMTQSPSSLSASVGDRVTITCRSSQSVLYSSNNKNYLVWYQQKPGKVPKLLIYWAS TRESGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCQQYYKTPLTFGGGTKVEIK (26)
23B3 VH4 VH	QVQLQESGPGLVKPSETLSLTCTISGDSVSTNSVAWNWIRQPPGKGLEWIGRTYYRS KWYNDYAVSLKSRVTISPDTSKNQFSLKLSSVTAADTAVYYCAREDGDSYYRYGMDV WGQGTTVTVSS (28)
23B3 VH3 VL	DIQMTQSPSSLSASVGDRVTITCRSSQSVLYSSNNKNYLVWYQQKPGKVPKLLIYWAS TRESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYKTPLTFGGGTKVEIK (30)
23B3 VH3 VH	EVQLLESGGGLVQPGGSLRLSCAISGDSVSTNSVAWNWIRQAPGKGLEWVSRTYYR SKWYNDYAVSVKGRFTISPDTSKNTFYLQMNSLRAEDTAVYYCAREDGDSYYRYGM DVWGQGTTVTVSS (32)
5G4 VL	EIVLTQSPGTLSLSPGERVTLSCRASQSVRSSYLAWYQQRAGQAPRLLIYGASSRATG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPTFGQGTRLEIK (491)
5G4 VH	QVQLQQWGAGLLKPSETLSLTCDVYGGSFSGYYWNWIRQPPGKGLEWIGEINHSGIT NYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARGYCRSTTCYFDYWGQGT LVTVSS (495)
17E9 VL	DIQMTQSPSSLSASVGDRVTITCRASQGISNDLAWYQQKPGKAPKRLIFAASSLQSGV PSRFSGSGSGTEFTLTISSLQPEDFATYYCLQHNSYPPTFGGGTKVEIK (493)
17E9 VH	QVQLVESGGGVVQPGRSLRLSCAASGFTLSSYGMHWVRQAPGKGLEWVAVMSYDG NNKLYADSVKGRFTISRDNSKKTLYLQMNSLRAEDTAVYYCARDETETLYYYYGIDVW GQGTTVTVSS (497)
53D3 VL	DIVMTQSPLSLPVTPGEPASISCRSSQSLLYSNGYNYLDWYLQKPGQSPQLLIYLGSS RASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQPLQTPLTFGGGTKVEIK (866)
53D3 VH	EVQLVESGGGLVKPGGSLRLSCAASEFTFSTYYMSWVRQAPGKGLEWVSSISSSSSF IYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDRIAAPGTYYYYGMDVW GQGTTVTVSS (868)
54E5 VL	DIVMTQSPDSLAVSLGERATINCKSSQNILYSSNNKNYLAWYQQKPGQPPSLLIYWAS TRESGVPDRFSGSGSGTDFTLTLSSLQAEDVAVYFCQQYYSIPWTFGQGTKVEIK (870)

54E5 VH	QVQLVESGGGVVQPGRSLRLSCAASGFTFSTYGMHWVRQAPGKGLEWVAVIWYDG SNKDYADSVKGRFTVSRDNSRDTLYLQMNSLRAEDTAVYYCAREERDSYYHYGMDV WGQGTTVTVSS (872)
56E1 VL	DIVMTQSPDSLTVSLGEGATINCKSSQSVLYSSNNKNYLAWYQQKPGQPPSLLIYWAS TRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSIPWTFGQGTKVEIK (874)
56E1 VH	QVQLVESGGGVVQPGRSLRLSCAASGFIFSSYGMHWVRQAPGKGLEWVAVIWYDGS NKDYADSVKGRFTVSRDNSRDTLYLQMNSLRAEDTAVYYCAREERDSYYHYGMDVW GQGTTVTVSS (876)
57A8VL	QSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYNVHWYQQLPGTAPKLLIYGNNNRP SGVPDRFSGSKSGTSASLAITGLQTEDEADYYCQSYDSSLSGWVFGGGTKLTVL (878)
57A8VH	EIQLLESGGGLVQPGGSLRLSCVASGFTFSSYVMSWVRQAPGKGLEWVSVISGSGG STYYADSVKGRFTISRDNSKTTLYLQMNSLRAEDTAVYYCAKGGTNYYYYYSGMDVW GQGTTVTVSS (880)
58G5VL	QSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNSHRP SGVPDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDSSLSGYVFGTGTKVTVL (882)
58G5VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLQWVSVISGRGG STYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDGYSSAWYFDYWGQ GTLVTVSS (884)
60G11VL	QSVLTQPPSVSGAPGQRVTISCTGSGSNIGAGYDVHWYQQLPGTAPKLLIYGNSHRP SGIPDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDSSLSGYVFGTGTKVTVL (886)
60G11VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSNYAMNWVRQAPGKGLEWVSVISGRGG STYYADSVKGRFTISRDNSRNTLYLQMNSLRAEDTAIYYCAKDGYSSAWFFDYWGQG TLITVSS (888)
73C2VL	DIQMTQSPSSLSASVGDRVTITCRASQSFSSYLNWYQQKPGKAPELLIYAASSLQSGV PSRFSGSGSGTDFTLTISSLQPEDFASYFCQQSYSTPRSFGQGTKLEIK (890)
73C2VH	QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSSSATWNWIRQSPSRGLEWLGRTYQR SKWNNDYAVSVKSRITINPDTSRNQFSLQLNSVTPEDTAVYYCAREVVAGPRWFDPW GQGTLVTVSS (892)
76A4VL	DIQMTQSPSSLSASVGDRVTITCRASQSVTSYLNWYQQKPGKAPKLLIYTASSLQSGIP SRFSGSGSGTDFTLTISSLQPEDFATYFCQQSYSTPRSFGQGTKLEIK (894)
76A4VH	QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSATWNWIRQSPSRGLEWLGRTYYR SKLYNDYAVSVKSRITINPDTSKNQFSLQLNSVTPEDTAVYYCAREVVAGPRWFDPW GQGTLVTVSS (896)
77D12VL	QSVLTQPPSASGTPGQRVTISCSESNSDIGTNAVNWYQQFPGTAPKFLIYSNNKRPSG VPDRFSGSKSGTSASLAISGLQSEDEADYYCATWDDNLNGPLFGGGTKLTVL (898)

77D12VH	QVQLVQSGAEVKKPGASVRVSCKASGYTFTGFYMHWVRQAPGQGLEWMGWINPDS GGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCAREGIAVALTYWGQGT LVTVSS (900)
87H11VL	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYVASSLQSGV PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSNPQECSFGQGTKLEIK (902)
87H11VH	QEQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEWIGEINHSGR TNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARDSGWHFSFDIWDQGTM VTVSS (904)
88H9VL	DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQEKPGKAPKRLIYAASGLQGG DPSRFSGSGSGTEFTLTISSLQPEDFATYYCLQHNSYPTWTFGQGTKVEIK (906)
88H9VH	QVQLVESGAGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGMGLEWVAVIWFDG SNEYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARERWFGELLDYWGQ GTLVTVSS (908)
89H9VL	DIQMIQSPSSLSASVGDSVTITCRASQSISYYLNWYQQKPGKAPKFLIYAASSLQSGVP SRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSSITFGQGTRLEIK (910)
89H9VH	EVHLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSGISGGGG STYYADSVKGRFTISRDNSKNTLYLQMNSLRAENTAVYYCAIEMAGAFDIWGQGTMVT VSS (912)
91D7VL	DIQMTQSPSSLSASVGDRVTITCRASQNISSYLNWYQQRPGKAPNLLLFTASSLQSGV PSRFSGSGSGTDFTLTINSLQPEDFATYYCQQSYSNPPESSFGQGTKLEIK (914)
91D7VH	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEWIGEINPVGR TNYKPSLKSRVTISVDTSKNQFSLKLSSVTAADTALYYCARDNGWHYAFDIWGQGTM VTVSS (916)
91F10_LC1VL	DVVMTQSPLSLPVTLGQPASISCRSSQSLVYSDGNTYLNWFQQRPGRSPRRLIYKVS NWDSGVPDRFSGSGSGTDSTLIISRVEAEDVGVYYYMQGTHWPLGGGTKVEIK (918)
91F10_LC1VH	QGQLVQSGAEVKKPGASVKVSCQASGYTFTAYYMHWVRQAPGQGLEWMGWINPNS GGTNYAQKFRGRVTMTRDTSISTAYMELSRLRSDDTAVYYCATGGSWEGFDYWGQ GTLVTVSS (920)
91F10_LC2VL	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKFLIYAASRLQSGV PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSDSTPITFGQGTRLEIK (922)
91F10_LC2VH	QGQLVQSGAEVKKPGASVKVSCQASGYTFTAYYMHWVRQAPGQGLEWMGWINPNS GGTNYAQKFRGRVTMTRDTSISTAYMELSRLRSDDTAVYYCATGGSWEGFDYWGQ GTLVTVSS (920)
91G8VL	DIQMIQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKFLIYAASSLQSGVP SRFSGSGSGTDFTLTISSLQPEDFATYYCQQSFSTITFGQGTRLEIK (924)
91G8VH	EVQLLESGGGLVQPGGSLRLSCVASGFTFSSYAMSWVRQAPGKGLEWVSGISGSGG SRYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAIEVAGAFDIWGQGTMVT VSS (926)
92D3VL	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGTAPKFLIYGASRLQSGV PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSDTTPITFGQGTRLEIK (928)

92D3VH	QVQLVQSGAEVKKPGASVQVSCKASGYTFTGYYMHWIRQAPGQGLEWLGWIIPNSG GTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCATGSSWEGFDYWGQGT LVTVSS (930)
92E5VL	DIQMIQSPSSLSASVGDRVTITCRASQSISHYLNWYQQKPGKAPKFLIYAASSLQSGVP SRFSGSGSGTDFTLTISSLQPEDFATYYCQQSFSSITFGQGTRLEIK (932)
92E5VH	EVHLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQVPGKGLEWVSGISGRGG STYYADSVKGRFTISRDNSKNTLYLQMDSLRAEDTAVYNCAIEVAGAFDIWGQGTMVT VSS (934)

[00132] Each of the foregoing polypeptides is encoded by a nucleic acid having the nucleotide sequence immediately preceding the polypeptide sequence in the Sequence Listing. The sequences from antibodies 9C8 and 3B3 are preferred.

[00133] The full anti-TL1A antibody sequences shown in Table E are preferred for the anti-TL1A antibodies. LC refers to the antibody light chain, HC to the heavy chain. Also encompassed within the invention are molecules having at least 90% sequence identity with either or both of the light chain and heavy chain sequences in Table E.

[00134] **Table E: Preferred anti-TL1A Antibodies**

Antibody designation	Light chain SEQ ID NO	Heavy chain SEQ ID NO	iPS (molecule designation)
3C6	50	52	284112
2G11	54	56	285396
9C8	58	60	285412
23B3	62	64	290043
3B3	66	68	308844
23B3 HC4	70	72	325246
23B3 HC3	74	76	325336
5G4	455	457	284510
17E9	459	461	284078
88H9	1116	1118	427485
54E5	1120	1122	427576
60G11	1124	1126	427493
53D3	1128	1130	427504
89H9	1132	1134	427509
91D7	1136	1138	427514
57A8	1140	1142	427519
91G8	1144	1146	427524
76A4	1148	1150	427529
87H11	1152	1154	427534
58G5	1156	1158	427539

57A8	1160	1162	427519
73C2	1164	1166	427544
77D12	1168	1170	427549
56E1	1172	1174	427554
92E5	1176	1178	427559
92D3	1180	1182	427564
91F10_LC1	1184	1186	427580
91F10_LC2	1188	1190	427572

[00135] Each of the amino acid sequences in Table E is encoded by the nucleic acid sequence immediately preceding it in the Sequence Listing. The sequences from antibodies 9C8 and 3B3 are preferred.

[00136] The foregoing anti-TL1A antibody sequences can be used in bispecific antigen proteins in any of the formats described herein. Preferably, such sequences are used in hetero Ig antigen binding proteins and IgG-scFv bispecific antigen binding proteins as described in this specification.

[00137] Hetero Ig bispecific antigen binding proteins

[00138] The invention further relates to hetero Ig bispecific antigen binding proteins as shown in Figure 1 that comprise TL1A-specific binding entities. Structurally, hetero Ig bispecific antigen binding proteins comprise:

[00139] (a) a TL1A binding entity light chain variable domain comprised in a light chain separate from a TL1A binding entity heavy chain variable domain, a heavy chain variable domain directed to a different antigen (e.g., TNF–α), and a binding entity light chain variable domain directed to the different antigen;

[00140] (b) a TL1A binding entity heavy chain variable domain comprised in a heavy chain separate from the TL1A binding entity light chain variable domain, the heavy chain variable domain directed to a different antigen, and the light chain variable domain directed to a different antigen;

[00141] (c) the heavy chain variable domain directed to a different antigen comprised in a heavy chain separate from the light chain domain directed to said different antigen, the TL1A binding entity heavy chain variable domain, and the TL1A binding entity light chain variable domain;

[00142] (d) the heavy chain comprising the TL1A binding entity heavy chain variable domain covalently bound to the light chain comprising the TL1A binding entity light chain variable domain;

[00143] (e) the heavy chain comprising the heavy chain variable domain directed to said different antigen covalently bound to the light chain comprising light chain variable domain directed to the different antigen; and

[00144] (f) the heavy chain comprising the TL1A binding entity heavy chain variable domain covalently bound to the heavy chain comprising the heavy chain variable domain directed to the different antigen.

[00145] For such hetero Ig bispecific antibodies, the TL1A-specific binding entities are preferred to comprise one or more of the CDRs listed in Table A. The variable region domains in Table D may be employed but it is preferred that the TL1A binding entity comprise charged amino acids that aid in correct assembly of the hetero Ig bispecific antigen binding protein (see Figure 1). Variable region sequences preferred for the hetero Ig bispecific antibodies are shown in Table F. As before, VL in Table F refers to the light chain variable region, VH to the heavy chain variable region. The SEQ ID NO from the Sequence Listing appears after each sequence in Table F.

[00146] **Table F: Hetero Ig TL1A-specific variable regions**

Antibody designation	Amino acid sequence (SEQ ID NO)
3B3 variant VL	EIVLTQSPGTLSLSPGERATLSCRASQSVRSSYLAWYQQKPGQAPRLLIYGASSRATG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPTFGQGTRLEIK (288)
3B3 variant VH	QVQLQQWGAGLLKPSETLSLTCAVHGGSFSGYYWNWIRQPPGKGLEWIGEINHAGIT NYNPSLKSRVTISLDTSKNQFSLKLTSVTAADTAVYYCARGYCRSTTCYFDYWGQGTL VTVSS (290)
2G11 variant VL	DIQMTQSPSSLSASVGDRVTITCRASQSINNYLNWYQQKPGKAPKLLIYATSSLQSGV PSRFSGSGSGTDFSLTISSLQPEDFATYFCQQSYSTPRTFGQGTKVEIK (292)
2G11 variant VH	QVQLQESGPGLVKPSETLSLTCTVSGGSISSYFWSWIRQPPGKGLEWIGYIYYSGSTN YNPSLKSRVTMSIDTSKNQFSLKLSSVTAADTAVYYCAREIGSYYGFDYWGQGTLVTV SS (294)
23B3 VH4 VL	DIQMTQSPSSLSASVGDRVTITCRSSQSVLYSSNNKNYLVWYQQKPGKVPKLLIYWAS TRESGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCQQYYKTPLTFGGGTKVEIK (296)
23B3 VH4 VH	QVQLQESGPGLVKPSETLSLTCTISGDSVSTNSVAWNWIRQPPGKGLEWIGRTYYRS KWYNDYAVSLKSRVTISPDTSKNQFSLKLSSVTAADTAVYYCAREDGDSYYRYGMDV WGQGTTVTVSS (298)
23B3 VH3 VL	DIQMTQSPSSLSASVGDRVTITCRSSQSVLYSSNNKNYLVWYQQKPGKVPKLLIYWAS TRESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYKTPLTFGGGTKVEIK (300)

23B3 VH3 VH	EVQLLESGGGLVQPGGSLRLSCAISGDSVSTNSVAWNWIRQAPGKGLEWVSRTYYR SKWYNDYAVSVKGRFTISPDTSKNTFYLQMNSLRAEDTAVYYCAREDGDSYYRYGM DVWGQGTTVTVSS (302)
3C6 variant VL	DIQMTQSPSSLSASVGDRVTITCRTSQDIRDDLGWYQQKPGKAPKLLIYDASSLQSGV PSRFSGSGSGTEFTLTISSLQPEDFATYYCLQHNSYPPTFGQGTKVEIK (304)
3C6 variant VH	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDG NNKLYTDSVKGRFTISRDDSKSTLYLQMNSLRAEDTAVYYCARDPTVTLYYYYGMDV WGQGTTVTVSS (306)
3C6 VL	DIQMTQSPSSLSASVGDRVTITCRTSQDIRDDLGWYQQKPGKAPKRLIYDASSLQSGV PSRFSGSGSGTEFTLTISSLQPEDFATYYCLQHNSYPPTFGQGTKVEIK (308)
3C6 VH	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDG NNKLYTDSVKGRFTISRDDSKSTLYLQMNSLRAEDTAVYYCARDPTVTLYYYYGMDV WGQGTTVTVSS (310)

[00147] Each of the polypeptides in Table F is encoded by a nucleic acid having the sequence immediately preceding the polypeptide sequence in the Sequence Listing.

[00148] Within the scope of this invention are hetero IgG molecules with a TNF-specific binding entity, preferably together with a TL1A-specific binding entity. The TNF-specific binding entity preferably comprises one or more sequences of CDRs or variable domains of antibodies 3.2 (described hereinafter), 234 (described hereinafter), certolizumab, adalimumab, infliximab, golimumab, and the antibodies disclosed in US Pat. No. 7,285,269, which is hereby incorporated by reference. Throughout this specification, "certolizumab" refers to an antibody having variable region sequences derived from certolizumab pegol. Preferred sequences based on such CDRs are shown in Table G.

[00149] **Table G: Preferred CDR sequences from anti-TNF-α antibodies**

Antibody designation	LCDR1 (SEQ ID NO)	LCDR2 (SEQ ID NO)	LCDR3 (SEQ ID NO)	HCDR1 (SEQ ID NO)	HCDR2 (SEQ ID NO)	HCDR3 (SEQ ID NO)
adalimumab	RASQGIRN YLA (92)	AASTLQS (242)	QRYNRAPY T (96)	DYAMH (158)	AITWNSGH IDYADSVE G (160)	VSYLSTAS SLDY (162)
certolizumab	KASQNVGT NVA (152)	SASFLYS (154)	QQYNIYPL T (156)	DYGMN (218)	WINTYIGEP IYADSVKG (220)	GYRSYAM DY (222)
C234	RASQDIRN DLG (140)	AASSLQS(112)	LQHNSYPL T (144)	SYDMH (206)	VISYDGSIK YYADSVKG (208)	EVRSGSYY YYYSMDV (210)

3.2	TGSSSNIG AGYDVH (146)	GNSNRPS (148)	QSYDSSLS GSV (150)	SYWIG (212)	IIYLGDSDT RYSPSFQG (214)	SNWGLDY (216)

[00150] Each of the polypeptides in Table G is encoded by a nucleic acid having the sequence immediately preceding the sequence of the polypeptide in the Sequence Listing. The sequences in Table G may be used in any bispecific antigen binding protein format, preferably a hetero Ig or IgG-scFv format and preferably together with a TL1A antigen binding entity.

[00151] The TNF binding entities of the bispecific antigen binding proteins more preferably comprise the sequences of the variable region of selected anti-TNF antibodies. The sequences of such variable regions are set forth in Table H. As before, VL refers to the variable region of the light chain and VH refers to the variable region of the heavy chain. The invention further encompasses molecules having sequences at least about 90% identical to any of the sequences shown in Table H.

[00152] **Table H: Variable domain sequences of preferred anti-TNF-α antibodies**

Source Antibody designation	Amino acid sequence (SEQ ID NO)
adalimumab VL	DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQKPGKAPKLLIYAASTLQSGV PSRFSGSGSGTDFTLTISSLQPEDVATYYCQRYNRAPYTFGQGTKVEIK (2)
adalimumab VH	EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNS GHIDYADSVEGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAKVSYLSTASSLDYWG QGTLVTVSS (4)
certolizumab VL	DIQMTQSPSSLSASVGDRVTITCKASQNVGTNVAWYQQKPGKAPKALIYSASFLYSGV PYRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNIYPLTFGQGTKVEIK (42)
certolizumab VH	EVQLVESGGGLVQPGGSLRLSCAASGYVFTDYGMNWVRQAPGKGLEWMGWINTYI GEPIYADSVKGRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCARGYRSYAMDYWGQG TLVTVSS (44)
certolizumab variant VL	DIQMTQSPSSLSASVGDRVTITCKASQNVGTNVAWYQQKPGKAPKALIYSASFLYSGV PYRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNIYPLTFGQGTKVEIK (42)
certolizumab variant VH	EVQLVESGGGLVQPGGSLRLSCAASGYVFTDYGMNWVRQAPGKGLEWMGWINTYI GEPIYADSVKGRFTISLDTSKSTAYLQMNSLRAEDTAVYYCARGYRSYAMDYWGQGT LVTVSS (318)
3.2 VL	QSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQFPGTAPKLLIQGNSNRP SGVPDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDSSLSGSVFGGGTKLTVL (38)
3.2 VH	EVQLVQSGAEVKKPGESLKISCKTSEYSFTSYWIGWVRQMPGKGLEWMGIIYLGDSD TRYSPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARSNWGLDYWGQGTLVTV SS (40)

C234 VL	DIQMTQSPSSLSASVGDRVTITCRASQDIRNDLGWYQQKPGKAPKRLIYAASSLQSGV PSRFSGSGSGPEFTLTISSLQPEDFATYYCLQHNSYPLTFGGGTKVEIK (34)
C234 VH	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYDMHWVRQAPGKGLEWVAVISYDGS IKYYADSVKGRFTISRDNSKNTLYLQVNSLRAEDTAVYYCAREVRSGSYYYYYSMDV WGQGTTVTVSS (36)

[00153] Each of the foregoing polypeptides is encoded by a nucleic acid having the nucleotide sequence immediately preceding the polypeptide sequence in the Sequence Listing.

[00154] Preferred anti-TL1A/anti-TNF hetero IgG bispecific antibodies are shown in Table I. The hetero Ig molecule designation is based on the foregoing designations for the parental antibodies used to construct the hetero Ig molecule. The molecule designated "certolizumab/3B3 variant," for example, employs polypeptides having the sequences from antibodies designated certolizumab and 3B3 variant herein. As before, VL refers to the variable region of the light chain, VH to the variable region of the heavy chain. Such molecules may include a "C clamp," cysteine molecules substituted into the sequences to provide an additional disulfide bond for stabilization. Such cysteine substitutions are preferably introduced at the VL-VH interface, at positions 44 in the VH and 100 in the VL in the Kabat numbering scheme. Additional substitutions may be made for stability or to introduce other functionality. Embodiments of the invention include molecules with at least 90% sequence identity to any of the sequences shown in Table I.

[00155] **Table I: Variable region sequences of anti-TL1A/anti-TNF-α hetero IgG molecules**

Hetero Ig molecule designation	Anti-TNF-α binding entity		Anti-TL1A binding entity	
	VL SEQ ID NO	VH SEQ ID NO	VL SEQ ID NO	VH SEQ ID NO
Certolizumab/ 3B3 variant	42	286	288	290
Certolizumab/ 2G11 variant	42	44	292	294
Certolizumab/ 23B3 VH4	42	44	296	298
Certolizumab/ 23B3 VH3	42	44	300	302
Certolizumab/ 3C6 variant	42	44	304	306
Certolizumab/ 3C6	42	44	308	310

3.2/ 3B3 variant	312	314	288	290
3.2/ 2G11 variant	312	314	292	294
3.2/ 23B3 VH4	312	314	296	298
3.2/ 23B3 VH3	312	314	300	302
3.2/ 3C6 variant	312	314	304	306
3.2/ 3C6	312	314	308	310
Certolizumab variant/ 3B3 variant	42	318	288	290
Certolizumab variant/ 2G11 variant	42	318	292	294
Certolizumab variant/ 23B3 VH4	42	318	296	298
Certolizumab variant/ 3C6 variant	42	318	304	306
Certolizumab variant/ 3C6	42	318	308	310
C234/ 3B3 variant	320	322	288	290
C234/ 2G11 variant	320	322	292	294
C234/ 23B3 VH4	320	322	31	298
C234/ 23B3 VH3	320	322	300	302
C234/ 3C6 variant	320	322	304	306
C234/ 3C6	320	322	308	310

[00156] Each of the foregoing polypeptides is encoded by a nucleic acid having the nucleotide sequence immediately preceding the amino acid sequence of the polypeptide in the Sequence Listing.

[00157] Table J lists the full light chain and heavy chain sequences of preferred hetero Ig bispecific antigen binding proteins in accordance with this invention. The listed sequences are preferred for all hetero Ig molecules, whether or not in the listed combination. The listed combinations are further preferred. "Var" in the molecule designation refers to variant. Such molecules may include charge variants to aid in assembly, as described for example in WO 2009/089004, published 16 July 2009, hereby incorporated by reference.

[00158] **Table J: Full sequences of anti-TL1A/anti-TNF-α hetero IgG molecules**

Hetero Ig molecule designation	Anti-TNF-α binding entity		Anti-TL1A binding entity		Molecule designation (iPS no.)
	Light chain SEQ ID NO	Heavy chain SEQ ID NO	Light chain SEQ ID NO	Heavy chain SEQ ID NO	
certolizumab/ 3B3 var	132	136	134	130	340593
certolizumab/ 2G11 var	132	136	239	138	340595
certolizumab/ 23B3 VH4	132	136	253	323	340596
certolizumab/ 23B3 VH3	132	136	327	325	340597
certolizumab/ 3C6 var	132	136	331	329	340598
certolizumab/ 3C6	132	136	335	329	340599
3.2/ 3B3 var	337	339	134	130	340601
3.2/ 2G11 var	337	339	239	138	340602
3.2/ 23B3 VH4	337	339	323	253	340603
3.2/ 23B3 VH3	337	339	327	325	340604
3.2/ 3C6 var	337	339	331	329	340605
3.2/ 3C6	337	339	335	329	340606

certolizumab var/ 3B3 var	132	316	134	130	340607
certolizumab var/ 2G11 var	132	316	239	138	340608
certolizumab var/ 23B3 VH4	132	316	323	253	340609
certolizumab var/ 3C6 var	132	316	335	329	340611
certolizumab var/ 3C6	132	316	331	329	340610
certolizumab var/ 3B3 var	333	316	134	130	340612
certolizumab var/ 2G11 var	333	316	239	138	340613
certolizumab var/ 23B3 VH4	333	316	323	253	340614
certolizumab var/ 3C6 var	333	316	331	329	340615
certolizumab var/ 3C6	333	316	335	329	340616
certolizumab var/ 3B3 var	333	463	134	130	340617
Certolizumab var/ 2G11 var	333	463	239	138	340618
Certolizumab var/ 23B3 VH4	333	463	323	253	340619
certolizumab var/ 3C6 var	333	463	331	329	340620
certolizumab var/ 3C6	333	463	335	329	340621
C234/ 3B3 var	341	343	134	130	349421
C234/ 2G11 var	341	343	239	138	349425
C234/ 23B3 VH4	341	343	323	253	349428
C234/ 23B3 VH3	341	343	327	325	349431
C234/	341	343	331	329	349433

3C6 var					
C234/ 3C6	341	343	335	329	349435
certolizumab/ 3B3 var	510	514	512	555	349437
certolizumab/ 2G11 var	510	514	541	558	349439
certolizumab/ 23B3 VH4	510	514	539	562	349441
certolizumab/ 23B3 VH3	510	514	543	565	349443
Certolizumab/ 3C6 var	510	514	543	568	349445
Certolizumab/ 3C6	510	514	544	568	349448
3.2/ 3B3 var	542	571	512	555	349451
3.2/ 2G11 var	542	571	541	558	349453
3.2/ 23B3 VH4	542	571	539	562	349455
3.2/ 23B3 VH3	542	571	543	565	349456
3.2/ 3C6 var	542	571	543	568	349457
3.2/ 3C6	542	571	544	568	349458
Certolizumab var/ 3B3 var	510	573	512	555	349460
Certolizumab var/ 2G11 var	510	573	541	558	349461
Certolizumab var/ 23B3 VH4	510	573	539	562	349463
Certolizumab var/ 3C6 var	510	573	543	568	349465
Certolizumab var/ 3C6	510	573	544	568	349467
Certolizumab var/ 3B3 var	540	573	512	555	349468

Certolizumab var/ 2G11 var	540	573	541	558	349470
Certolizumab var/ 23B3 VH4	540	573	539	562	349471
Certolizumab var/ 3C6 var	540	573	543	568	349473
Certolizumab var/ 3C6	540	573	544	568	349474
Certolizumab var/ 3B3 var	540	487	512	555	349476
Certolizumab var/ 2G11 var	540	487	541	558	349479
Certolizumab var/ 23B3 VH4	540	487	539	562	349480
Certolizumab var/ 3C6 var	540	487	543	568	349482
Certolizumab var/ 3C6	540	487	544	568	349484
C234/ 3B3 var	518	522	512	555	349485
C234/ 2G11 var	518	522	541	558	349486
C234/ 23B3 VH4	518	522	539	562	349487
C234/ 23B3 VH3	518	522	543	565	349488
C234/ 3C6 var	518	522	543	568	349489
C234/ 3C6	518	522	544	568	349490
Certolizumab/ 3B3 var	545	578	546	579	349491
Certolizumab/ 2G11 var	545	578	548	582	349492
Certolizumab/ 23B3 VH4	545	578	549	585	349493
Certolizumab/ 23B3 VH3	545	578	550	588	349495
Certolizumab/	545	578	551	591	349496

3C6 var					
Certolizumab/ 3C6	545	578	552	591	349498
C234/ 3B3 var	547	595	546	579	349499
C234/ 2G11 var	547	595	548	582	349501
C234/ 23B3 VH4	547	595	549	585	349502
C234/ 23B3 VH3	547	595	550	588	349503
C234/ 3C6 var	547	595	551	591	349504
C234/ 3C6	547	595	552	591	349505
Certolizumab/ 3B3 var	132	599	134	598	349506
Certolizumb/ 2G11 var	132	599	239	601	349507
Certolizumab/ 23B3 VH4	132	599	323	603	349508
Certolizumab/ 23B3 VH3	132	599	327	605	349509
Certolizumab/ 3C6 var	132	599	331	607	349510
Certolizumab/ 3C6	132	599	335	607	349511
3.2/ 3B3 var	337	609	134	598	349512
3.2/ 2G11 var	337	609	239	601	349513
3.2/ 23B3 VH4	337	609	323	603	349514
3.2/ 23B3 VH3	337	609	327	605	349515
3.2/ 3C6 variant	337	609	331	607	349516
3.2/ 3C6	337	609	335	607	349517

Certolizumab var/ 3B3 var	132	611	134	598	349518
Certolizumab var/ 2G11 var	132	611	239	601	349519
Certolizumab var/ 23B3 VH4	132	611	323	603	349520
Certolizumab var/ 3C6 var	132	611	331	607	349521
Certolizumab var/ 3C6	132	611	335	607	349522
Certolizumab var/ 3B3 var	333	611	134	598	349523
Certolizumab var/ 2G11 var	333	611	239	601	349524
Certolizumab var/ 23B3 VH4	333	611	323	603	349525
Certolizumab var/ 3C6 var	333	611	331	607	349526
Certolizumab var/ 3C6	333	611	335	607	349527
Certolizumab var/ 3B3 var	333	613	134	598	349528
Certolizumab var/ 2G11 var	333	613	239	601	349529
Certolizumab var/ 23B3 VH4	333	613	323	603	349530
Certolizumab var/ 3C6 var	333	613	335	607	349531
Certolizumab var/ 3C6	341	615	134	598	349532
C234/ 2G11 var	341	615	239	601	349533
C234/ 23B3 VH4	341	615	323	603	349534
C234/ 23B3 VH3	341	615	327	605	349535
C234/ 3C6 var	341	615	331	607	349536
C234/	341	615	335	607	349537

3C6					
Certolizumab var/ 3C6 var	333	613	331	607	349539
Certolizumab var/ 2G11 var	132	463	239	138	361830
Certolizumab var/ 3C6 var	132	463	331	329	361831
Certolizumab var/ 3C6	132	463	335	320	361832
Certolizumab var/ 3B3 var	510	487	512	555	361833
Certolizumab var/ 2G11 var	510	487	541	558	361834
Certolizumab var/ 23B3 VH4	510	487	539	562	361835
Certolizumab var/ 3C6 var	510	487	543	568	361836
Certolizumab var/ 3C6	510	487	544	568	361837
Certolizumab var/ 3B3 var	132	613	134	598	361838
Certolizumab var/ 23B3 VH4	132	613	323	603	361839
Certolizumab var/ 3C6 var	132	613	331	607	361840
Certolizumab var/ 3C6	132	613	335	607	361841
Certolizumab var/ 3B3 var	132	463	134	130	361842
Certolizumab var/ 3B3 var	540	616	512	508	381084
Certolizumab var/ 3B3 var	540	620	512	508	381089
Certolizumab var/ 2G11 var	540	616	541	558	381094
Certolizumab var/ 2G11 var	540	620	541	558	381096
Certolizumab var/ 23B3 VH4	540	616	539	562	381098

Certolizumab var/ 23B3 VH4	540	620	539	562	381100
C234 var/ 3B3 var	538	623	512	502	381102
C234 var/ 3B3 var	537	626	512	508	381109
Certolizumab var/ 3B3 var	546	514	194	535	381208
Certolizumab var/ 3B3 var	536	616	194	535	381216
Certolizumab/ 3B3 var	536	620	194	535	381220
Certolizumab var/ 3B3 var	546	487	194	535	381222
Certolizumab/ 3B3 var	471	136	473	198	381292
Certolizumab var/ 3B3 var	479	477	473	198	381300
Certolizumab/ 3B3 var	176	136	520	198	381306
Certolizumab var/ 3B3 var	475	477	520	198	381312
Certolizumab var/ 3B3 var	471	463	473	198	381316
Certolizumab var/ 3B3 var	176	463	570	198	381318
Certolizumab var/ 3B3 var	471	465	473	198	381320
Certolizumab var/ 3B3 var	176	465	520	198	381325
certolizumab/ 3B3 variant	510	514	512	508	376541
C234/ 3B3 variant	518	522	512	508	376542
certolizumab/ 3B3 variant	510	516	512	508	376543

[00159] Each of the foregoing polypeptides is encoded by a nucleic acid having the nucleotide sequence immediately preceding the amino acid sequence of the polypeptide in the Sequence Listing, except as noted in Table J-1.

[00160] **Table J-1: Nucleic Acids Encoding Polypeptides of Table J**

Polypeptide SEQ ID NO:	Encoding Nucleic Acid SEQ ID NO:
176	526
194	627
198	529
239	236
253	250
320	332
323	257
333	464
465	528
471	531
473	530
475	534
477	532
479	533
487	515
508	618
520	527
535	629
536	630
537	625
538	622
539	559
540	560
541	556
542	570
543	563
544	569
545	576
546	574, 628
547	593
548	580
549	583
550	587
551	590
552	592
570	527

571	553
579	575
588	586
591	589
598	596
599	597
616	617
623	621
626	624

[00161] IgG-scFv Bispecific Antigen Binding Proteins

[00162] IgG-scFv antigen binding proteins have the structure shown in Figure 2. Such molecules comprise the heavy and light chains of an antibody (the IgG portion of the molecule), which comprises a first antigen binding entity of the IgG-scFv molecule. Such molecules further comprise an scFv--a fusion protein having in one chain the heavy chain variable domain and light chain variable domain of a second antibody. This scFv portion of the molecule comprises a second antigen binding entity of the IgG-scFv molecule. An scFv portion is fused to each heavy chain of the IgG portion of the molecule.

[00163] An IgG-scFv antigen binding protein herein comprises an anti-TL1A binding entity, particularly the amino acid sequences of anti-TL1A binding entities as described hereinabove. Preferred IgG-scFv antigen binding proteins comprise the sequences of anti-TL1A CDRs as described in Tables A, B and C, with those of Table A most preferred. More preferred IgG-scFv antigen binding proteins comprise the anti-TL1A variable domain sequences of Table D. Also preferred are IgG-scFv antigen binding proteins having the full antibody sequences of Table E.

[00164] This specification further relates to IgG-scFv molecules comprising an anti-TNF-α antigen binding entity, preferably comprising CDR sequences as described in Table G. Further preferred are IgG-scFv molecules comprising the sequences of anti-TNF-α variable domains as described in Table H. Further preferred are IgG-scFv molecules comprising the full amino acid sequences of anti-TNF-α antibodies as described in Table K.

[00165] **Table K: Preferred anti-TNF-α Antibodies**

Antibody designation	Light chain SEQ ID NO	Heavy chain SEQ ID NO	iPS no.
Adalimumab	46	48	104628
C234	78	80	330194

3.2	84	86	330237
certolizumab	88	90	333788

[00166] Each of the amino acid sequences in Table K is encoded by the nucleic acid sequence immediately preceding it in the Sequence Listing.

[00167] In certain embodiments, the heavy chain of the IgG portion is fused to the scFv portion via a peptide linker. In certain embodiments, the peptide linker comprises a sequence selected from the group consisting of $(Gly_3Ser)_2$, $(Gly_4Ser)_2$, $(Gly_3Ser)_3$, $(Gly_4Ser)_3$, $(Gly_3Ser)_4$, $(Gly_4Ser)_4$, $(Gly_3Ser)_5$, $(Gly_4Ser)_5$, $(Gly_3Ser)_6$, and $(Gly_4Ser)_6$.

[00168] Further preferred are IgG-scFv antigen binding proteins having the heavy and light chain sequences as disclosed in Tables L and M. The heavy chain sequences in Tables L and M include the ScFv portion of the molecule fused to the heavy chain of the IgG portion of the molecule. In the molecule designation in the left column of these tables, the first antibody name identifies the IgG portion of the molecule, such that the IgG-scFv molecule comprises the full heavy and light chain sequences of the noted antibody. The second antibody name identifies the scFv portion of the molecule, such that the heavy chain sequence comprises the heavy chain variable domain and the light chain variable domain sequences as disclosed previously herein. Thus, "adalimumab IgG-9C8 scFv" includes the light chain of adalimumab and the heavy chain of adalimumab fused to heavy chain variable domain and light chain variable domain of antibody 9C8. "CC" in the molecule designations in Tables L and M refers to a cysteine clamp; molecules that include CC in their designation have been modified to have an additional cysteine disulfide bond. Such cysteine substitutions are preferably introduced at the VL-VH interface, at positions 44 in the VH and 100 in the VL in the Kabat numbering scheme. "Var" in Table L means variant.

[00169] **Table L: Preferred TL1A binding entity IgG- TNF binding entity scFv antigen binding proteins**

Molecule designation	heavy chain SEQ ID NO	light chain SEQ ID NO	iPS
9C8 IgG-adalimumab scFv	355	447	370012
9C8 IgG-3.2 scFv	357	447	370013
9C8 IgG-C234 scFv	359	318	370014
9C8 IgG-adalimumab scFv+CC	361	447	370015
9C8 IgG-3.2 scFv+CC	363	447	370016
9C8 IgG-C234 scFv+CC	365	58	370017

23B3 VH4 IgG-adalimumab scFv	367	70	370018
23B3 VH4 IgG-3.2 scFv	369	70	370019
23B3 VH4 IgG-C234 scFv	371	70	370020
23B3 VH4 IgG-adalimumab scFV+CC	373	70	370021
23B3 VH4 IgG-3.2 scFv+CC	375	70	370022
23B3 VH4 IgG-C234 scFv+CC	377	70	370023
3B3 var 2 IgG-adalimumab scFv	391	523	371218
3B3v2 IgG-3.2 scFv	393	523	371219
3B3v2 IgG-C234 scFv	395	523	371220
3B3v2 IgG-adalimumab scFv+CC	397	523	371221
3B3v2 IgG-3.2 scFv+CC	399	523	371222
3B3v2 IgG-C234 scFv+CC	401	523	371223
3B3 var IgG-3.2 var scFv	403	523	381031
3B3 var IgG-3.2 var scFv	405	523	381035
9C8 var IgG-3.2 var scFv	407	447	381040
9C8 var IgG-3.2 var scFv	409	447	381044
3B3 var IgG-3.2 var scFv+CC	411	523	381048
3B3 var IgG-3.2 var scFv+CC	413	523	381053
3B3 var IgG-C234 var scFv	415	523	381058
3B3 var IgG-C234 var scFv	417	523	381062
23B3 VH4 var IgG-adalimumab var scFv	419	70	381066
23B3 VH4 var IgG-adalimumab var scFv	421	70	381070
23B3 VH4 var IgG-adalimumab var scFv+CC	423	70	381074
23B3 VH4 var IgG-adalimumab var scFv+CC	425	70	381079

[00170] Each of the polypeptides in Table L is encoded by a nucleic acid having the nucleotide sequence immediately preceding the polypeptide sequence in the Sequence Listing.

[00171] **Table M: Preferred TNF-α binding entity IgG- TL1A binding entity scFv antigen binding proteins**

Molecule designation	heavy chain SEQ ID NO	light chain SEQ ID NO	iPS
Adalimumab IgG-9C8 scFv	82	46	369989
Adalimumab IgG-23B3 VH4 scFv	284	46	369990
Adalimumab IgG-9C8 scFv+CC	286	46	369992
Adalimumab IgG-9C8 var scFv+CC	441	453	381505
Adalimumab IgG-23B3 VH4 scFv+CC	312	46	369993
3.2 IgG-9C8 scFv	314	84	369995

3.2 IgG-23B3 VH4 scFv	320	84	369996
3.2 IgG-9C8 scFv+CC	322	84	369998
3.2 IgG-23B3 VH4 scFv+CC	345	84	369999
C234 IgG-9C8 scFv	347	78	370001
C234 IgG-23B3 VH4 scFv	349	78	370002
C234 IgG-9C8 scFv+CC	351	78	370004
C234 IgG 23B3 VH4 scFv+CC	353	78	370005
Adalimumab IgG-3B3v2 scFv	379	46	371212
Adalimumab IgG-3Bv2 scFv+CC	381	46	371213
3.2 IgG-3B3v2 scFv	383	84	371214
3.2 IgG-3B3v2 scFv+CC	385	84	371215
C234 IgG-3B3v2 scFv	387	78	371216
C234 IgG-3B3v2 scFv+CC	389	78	371217
3.2 var IgG-9C8 var scFv	427	449	381327
3.2 var IgG-9C8 var scFv	429	449	381331
C234 var IgG-9C8 var scFv	431	451	381485
C234 var IgG-9C8 var scFv+CC	433	451	381489
C234 var IgG-3B3 var scFv	435	451	381493
C234 var IgG-3B3 var scFv+CC	437	451	381497
Adalimumab var IgG-9C8 var scFv	439	453	381501
Adalimumab var IgG-9C8 var scFv+CC	441	453	381505
Adalimumab var IgG-3B3 var scFv	443	453	381509
Adalimumab var IgG-3B3 var scFv+CC	445	453	381513

[00172] Each of the polypeptides in Table M is encoded by a nucleic acid having the nucleotide sequence immediately preceding the polypeptide sequence in the Sequence Listing.

[00173] IgG-Fab bispecific antigen binding proteins

[00174] In the IgG-Fab format, the bispecific, multivalent antigen binding protein comprises (i) a first polypeptide comprising a first heavy chain (VH2-CH1-CH2-CH3) from a first antibody, wherein the first heavy chain is fused at its carboxyl terminus (optionally through a peptide linker) to a polypeptide comprising VH2-CH1 domains of a second antibody to form a modified heavy chain, (ii) a second polypeptide comprising a light chain from a first antibody (VL1-CL) and (iii) a third polypeptide comprising VL2-CL domains of the second antibody. The CL and CH1 domains of the first antibody may be switched ("swapped") in some embodiments between the first and second polypeptide. In such embodiments, the second polypeptide comprises VL1-CH1, while the first polypeptide comprises VH1-CL-CH2-CH3-VH2-CH1 with VH1-CL-CH2-CH3 fused at its C-terminus to VH2-CH1 optionally through a peptide linker. The third polypeptide comprises VL2-CL. Alternatively, the CL and CH1

domains of the second antibody may be switched in some embodiments between the first and third polypeptides. In such embodiments, the third polypeptide comprises VL2-CH1, while the first polypeptide comprises VH1-CH1-CH2-CH3-VH2-CL wherein VH1-CH1-CH2-CH1 is fused at its C-terminus to VH2-CL optionally through a peptide linker. The second polypeptide comprises VL1-CL. In yet another embodiment, the CL and CH1 domains of both antibodies are switched between the first, second and third polypeptides. In such embodiments, the first polypeptide comprises VH1-CL-CH2-CH3-VH2-CL, with VH1-CL-CH2-CH3 optionally fused at its C-terminus to VH2-CL, the second polypeptide comprises VL1-CH1, and the third polypeptide comprises VL2-CH1. Within the scope of this invention are such IgG-Fab molecules in which the first antibody comprises a TL1A binding entity and the second antibody comprises a TNF-α binding entity. Likewise, also within the scope of this invention are such IgG-Fab molecules in which the first antibody comprises a TNF-α binding entity and the second antibody comprises a TL1A binding entity. An expression having the same meaning as the last two sentences is that the first antibody comprises one of a TL1A binding entity or a TNF-α binding entity and the second antibody comprises the other.

[00175] In one aspect of the IgG-Fab format within this invention, the bispecific, tetravalent antigen binding protein comprises a) a first heavy chain of a first antibody (VH1), wherein the first antibody specifically binds to a first antigen, and wherein the first heavy chain is fused through its C-terminus to the N-terminus of a moiety comprising a second heavy chain of a second antibody (VH2), wherein the second antibody specifically binds to a second antigen; b) two light chains of the first antibody of a); and c) two light chains of the second antibody of a). Within this invention, the first antibody may specifically bind TL1A and the second may specifically bind TNF-α or vice-versa.

[00176] In another aspect of the IgG-Fab format within this invention, the bispecific antigen binding protein comprises (i) a first binding domain that specifically binds to a first antigen comprising a first light chain immunoglobulin variable region (VL1) and a first heavy chain immunoglobulin variable region (VH1); (ii) a second binding domain that specifically binds to a second antigen comprising a second light chain immunoglobulin variable region (VL2) and a second heavy chain immunoglobulin variable region (VH2); and (iii) a human immunoglobulin Fc region, wherein one of the binding domains is positioned at the amino terminus of the Fc region and the other binding domain is positioned at the carboxyl terminus of the Fc region, wherein the carboxyl-terminal binding domain is a Fab and is fused through a peptide linker to the carboxyl terminus of the Fc region, and wherein the Fab is fused to the Fc

region through the amino terminus of the VH region of the Fab. Within this invention, the first antigen may be TL1A and the second TNF-α or vice-versa.

[00177] In another aspect of the IgG-Fab format, the bispecific, tetravalent antigen binding protein comprises:

[00178] a) a first polypeptide comprising a first heavy chain of a first antibody comprising a first heavy chain variable region (VH1) and a first CH1 domain, wherein the first antibody specifically binds to a first antigen, and wherein the first heavy chain is fused through its C-terminus to the N-terminus of a polypeptide comprising a second heavy chain variable region of a second antibody (VH2), wherein the VH2 is fused through its C-terminus to the N-terminus of a second CH1 domain, and wherein the second antibody specifically binds to a second antigen; wherein

[00179] i) the VH1 or first CH1 domain comprises at least one amino acid substitution to introduce a charged (e.g., positively charged) amino acid at a residue selected from the group consisting of positions 39, 44, and 183 using EU numbering; and

[00180] ii) the VH2 or second CH1 domain comprises at least one amino acid substitution to introduce a charged (preferably oppositely charged from that of i) above) amino acid at a residue selected from the group consisting of a residue that corresponds to positions 39, 44, and 183 using EU numbering, wherein the charge is the opposite of the substituted residue of the VH1 or first CH1 of the first heavy chain; and

[00181] b) a second polypeptide comprising a first light chain of the first antibody of a), wherein the first light chain comprises a first light chain variable region (VL1) and a first CL region; and wherein the VL1 or first CL domain comprises at least one amino acid substitution to introduce a charged amino acid at a residue selected from the group consisting of positions 38, 100, and 176 using EU numbering, wherein the charge at position 38 is the opposite of the substituted residue of the VH1 or first CH1 of the first heavy chain at position 39; the charge at position 100 is the opposite of the substituted residue of the VH1 or first CH1 of the first heavy chain at position 44; the charge at position 176 is the opposite of the substituted residue of the VH1 or first CH1 of the first heavy chain at position 183; and

[00182] c) a third polypeptide comprising a second light chain of the second antibody of a), wherein the second light chain comprises a second light chain variable region (VL2) and a second CL region; and wherein the VL2 or second CL domain comprises at least one amino acid substitution to introduce a charged amino acid at a residue selected from the group

consisting of positions 38, 100, and 176 using EU numbering, wherein the charge at position 38 is the opposite of the substituted residue of the VH2 or second CH1 of the second heavy chain at position 39; the charge at position 100 is the opposite of the substituted residue of the VH2 or second CH1 of the second heavy chain at position 44; the charge at position 176 is the opposite of the substituted residue of the VH2 or second CH1 of the second heavy chain at position 183.

[00183] Within this invention, the first antigen in subparagraph a) above may be one of TL1A and TNF-α and the second antigen may be the other. Another way of stating this same binding specificity is to say that the first antibody in subparagraph a) comprises one of a TL1A binding entity or a TNF-α binding entity and the second antibody comprises the other.

[00184] In some embodiments of the bispecific antigen binding proteins of the invention in which the carboxyl-terminal binding domain is a Fab fragment, the binding domain positioned at the amino terminus of the Fc region (i.e., the amino-terminal binding domain) is also a Fab fragment. The amino-terminal Fab fragment can be fused to the amino terminus of the Fc region through a peptide linker or an immunoglobulin hinge region described herein. In some embodiments, the amino-terminal Fab fragment is joined to the amino terminus of the Fc region through a human IgG1 hinge region. In other embodiments, the amino-terminal Fab fragment is joined to the amino terminus of the Fc region through a human IgG2 hinge region. In one embodiment, the amino-terminal Fab fragment is fused to the Fc region through the carboxyl terminus of the CH1 region of the Fab.

[00185] In some embodiments, the bispecific antigen binding protein of the invention comprises a first antibody that specifically binds to a first target where one polypeptide chain (e.g. the heavy chain (VH2-CH1)) of a Fab fragment from a second antibody that specifically binds to a second target is fused to the carboxyl terminus of the heavy chain of the first antibody. The bispecific antigen binding protein in such embodiments also comprises a polypeptide chain containing the other half of the Fab fragment from the second antibody (e.g., the light chain (VL2-CL)). This format is referred to herein as the "IgG-Fab" format, and one embodiment of this type of molecule is shown schematically in Figure 25. Thus, in certain embodiments, the present invention includes a bispecific, multivalent antigen binding protein comprising: (i) a light chain from a first antibody, (ii) a heavy chain from the first antibody, wherein the heavy chain is fused at its carboxyl terminus through a peptide linker to a first polypeptide comprising VH-CH1 domains of a second antibody to form a modified heavy chain, and (iii) a second polypeptide comprising VL-CL domains of the second antibody. When dimerized, the bispecific antigen binding protein is a homohexamer comprising two modified

heavy chains, two light chains from the first antibody, and two polypeptide chains containing the other half of the Fab fragment from the second antibody (the Fd fragment). In one embodiment, the first polypeptide, which is fused to the carboxyl terminus of the heavy chain, comprises VH and CH1 domains from the second antibody, and the second polypeptide comprises VL and CL domains from the second antibody.

[00186] In certain embodiments, the antigen binding proteins of the invention comprise (i) a first binding domain that specifically binds a first target antigen, (ii) a second binding domain that specifically binds to a second target antigen, and (iii) a human immunoglobulin Fc region, wherein one of the binding domains is positioned at the amino terminus of the Fc region and the other binding domain is positioned at the carboxyl terminus of the Fc region. In some such embodiments, each of the first and second binding domains comprises immunoglobulin variable regions. For instance, in certain embodiments, the first binding domain comprises a first light chain variable region (VL1) and a first heavy chain variable region (VH1) from an anti-first target antigen antibody and the second binding domain comprises a second light chain variable region (VL2) and a second heavy chain variable region (VH2) from an anti-second target antigen antibody.

[00187] In certain embodiments of the bispecific antigen binding proteins of the invention, the binding domain positioned at the amino terminus of the Fc region (i.e. the amino-terminal binding domain) is a Fab fragment fused to the amino terminus of the Fc region through a peptide linker described herein or through an immunoglobulin hinge region. An "immunoglobulin hinge region" refers to the amino acid sequence connecting the CH1 domain and the CH2 domain of an immunoglobulin heavy chain. The hinge region of human IgG1 is generally defined as the amino acid sequence from about Glu216 or about Cys226, to about Pro230. Hinge regions of other IgG isotypes may be aligned with the IgG1 sequence by placing the first and last cysteine residues forming inter-heavy chain disulfide bonds in the same positions and are determinable to those of skill in the art. In some embodiments, the amino-terminal binding domain is joined to the amino terminus of the Fc region through a human IgG1 hinge region. In other embodiments, the amino-terminal binding domain is joined to the amino terminus of the Fc region through a human IgG2 hinge region. In one embodiment, the amino-terminal binding domain (e.g. Fab fragment) is fused to the Fc region through the carboxyl terminus of the CH1 region of the Fab.

[00188] In some embodiments of the antigen binding proteins of the invention, the binding domain positioned at the carboxyl terminus of the Fc region (i.e. the carboxyl-terminal binding domain) is a Fab fragment. In such embodiments, the Fab is fused or otherwise

connected to the carboxyl terminus of the Fc region (e.g. the carboxyl terminus of the CH3 domain) through a peptide linker through the amino terminus of the VH region of the Fab fragment. Thus, in one embodiment, the Fab is fused to an Fc region through the amino terminus of the VH region of the Fab such that the resulting fusion protein comprises, from N-terminus to C-terminus, a CH2 domain, a CH3 domain, a peptide linker, a VH region, and a CH1 region.

[00189] In certain embodiments, the first heavy chain of an antigen binding protein of the present invention is fused to the VH2 via a peptide linker. In certain embodiments, the peptide linker comprises a sequence selected from the group consisting of $(Gly_3Ser)_2$, $(Gly_4Ser)_2$, $(Gly_3Ser)_3$, $(Gly_4Ser)_3$, $(Gly_3Ser)_4$, $(Gly_4Ser)_4$, $(Gly_3Ser)_5$, $(Gly_4Ser)_5$, $(Gly_3Ser)_6$, and $(Gly_4Ser)_6$ These sequences can also be written as:

[00190] GGGSGGGS (SEQ ID NO: 673),

[00191] GGGGSGGGGS (SEQ ID NO: 695),

[00192] GGGSGGGSGGGS (SEQ ID NO: 703),

[00193] GGGGSGGGGSGGGGS (SEQ ID NO: 729),

[00194] GGGSGGGSGGGSGGGS (SEQ ID NO: 735),

[00195] GGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 825),

[00196] GGGSGGGSGGGSGGGSGGGS (SEQ ID NO: 937),

[00197] GGGGSGGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 939),

[00198] GGGSGGGSGGGSGGGSGGGSGGGS (SEQ ID NO: 941), and

[00199] GGGGSGGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 945).

[00200] The peptide linker joining the Fc region to the carboxyl-terminal Fab can be any of the peptide linkers described herein. In particular embodiments, the peptide linker joining the Fc region to the carboxyl-terminal Fab fragment is at least 5 amino acids in length. In other embodiments, the peptide linker joining the Fc region to the carboxyl-terminal Fab fragment is at least 8 amino acids in length. Particularly suitable peptide linkers for joining the Fc region to the carboxyl-terminal Fab fragment are glycine-serine linkers, such as $(Gly_xSer)_n$ wherein x=3 or 4 and n= 2, 3, 4, 5 or 6. In one embodiment, the peptide linker connecting the Fc region to the carboxyl-terminal Fab fragment is a L10 $(G_4S)_2$ linker. In another embodiment, the peptide linker connecting the Fc region to the carboxyl-terminal Fab fragment is a L9 or G_3SG_4S linker (GGGSGGGGS, SEQ ID NO: 946).

[00201]

Chain mutations

[00202] In particular embodiments, the bispecific antigen binding proteins described herein comprise a Fc region from a human IgG1 antibody modified to remove effector functions. Such modified IgG1 Fc regions comprise substitutions R292C and V302C. In such embodiments, the Fc region may also comprise substitution N297G (known as a SEFL2 scaffold),

[00203] Preferred embodiments further include mutations in the heavy and light chains to aid in correct assembly of a hetero IgG bispecific antigen binding protein. An approach for promoting heterodimer formation to the exclusion of homodimer formation entails utilizing an electrostatic steering mechanism (see Gunasekaran et al. (2010), J. Biol. Chem., Vol. 285: 19637-19646, which is hereby incorporated by reference in its entirety). This approach involves introducing or exploiting charged residues in the CH3 domain in each heavy chain so that the two different heavy chains associate through opposite charges that cause electrostatic attraction. Homodimerization of the identical heavy chains are disfavored because the identical heavy chains have the same charge and therefore are repelled. This same electrostatic steering technique can be used to prevent mispairing of light chains with the non-cognate heavy chains by introducing residues having opposite charges in the correct light chain – heavy chain pair at the binding interface. The electrostatic steering technique and suitable charge pair mutations for promoting heterodimers and correct light chain-heavy chain pairing is described in WO2009/089004 and WO2014/081955, both of which are hereby incorporated by reference in their entireties.

[00204] In embodiments in which the bispecific antigen binding proteins of the invention are heterodimeric antibodies comprising a first light chain (LC1) and first heavy chain (HC1) from a first antibody that specifically binds to a first target antigen and a second light chain (LC2) and second heavy chain (HC2) from a second antibody that specifically binds to a second target, HC1 or HC2 may comprise one or more amino acid substitutions to replace a positively-charged amino acid with a negatively-charged amino acid. For instance, in one embodiment, the CH3 domain of HC1 or the CH3 domain of HC2 comprises an amino acid sequence differing from a wild-type IgG amino acid sequence such that one or more positively-charged amino acids (e.g., lysine, histidine and arginine) in the wild-type human IgG amino acid sequence are replaced with one or more negatively-charged amino acids (e.g., aspartic acid and glutamic acid) at the corresponding position(s) in the CH3 domain. In these and other embodiments, amino acids (e.g. lysine) at one or more positions selected from 370, 392 and 409 (EU numbering system) are replaced with a negatively-charged amino acid (e.g., aspartic acid and glutamic acid). An amino acid substitution in an amino acid sequence is typically

designated herein with a one-letter abbreviation for the amino acid residue in a particular position, followed by the numerical amino acid position relative to an original sequence of interest, which is then followed by the one-letter symbol for the amino acid residue substituted in. For example, "T30D" symbolizes a substitution of a threonine residue by an aspartate residue at amino acid position 30, relative to the original sequence of interest. Another example, "S218G" symbolizes a substitution of a serine residue by a glycine residue at amino acid position 218, relative to the original amino acid sequence of interest.

[00205] In certain embodiments, HC1 or HC2 of the heterodimeric antibodies may comprise one or more amino acid substitutions to replace a negatively-charged amino acid with a positively-charged amino acid. For instance, in one embodiment, the CH3 domain of HC1 or the CH3 domain of HC2 comprises an amino acid sequence differing from wild-type IgG amino acid sequence such that one or more negatively-charged amino acids in the wild-type human IgG amino acid sequence are replaced with one or more positively-charged amino acids at the corresponding position(s) in the CH3 domain. In these and other embodiments, amino acids (e.g., aspartic acid or glutamic acid) at one or more positions selected from 356, 357, and 399 (EU numbering system) of the CH3 domain are replaced with a positively-charged amino acid (e.g., lysine, histidine and arginine).

[00206] In particular embodiments, the heterodimeric antibody comprises a first heavy chain comprising negatively-charged amino acids at positions 392 and 409 (e.g., K392D and K409D substitutions), and a second heavy chain comprising positively-charged amino acids at positions 356 and 399 (e.g., E356K and D399K substitutions). In other particular embodiments, the heterodimeric antibody comprises a first heavy chain comprising negatively-charged amino acids at positions 392, 409, and 370 (e.g., K392D, K409D, and K370D substitutions), and a second heavy chain comprising positively-charged amino acids at positions 356, 399, and 357 (e.g., E356K, D399K, and E357K substitutions). In related embodiments, the first heavy chain is from an anti-first target antigen antibody and the second heavy chain is from an anti-second target antigen antibody.

[00207] To facilitate the association of a particular heavy chain with its cognate light chain, both the heavy and light chains may contain complementary amino acid substitutions. As used herein, "complementary amino acid substitutions" refer to a substitution to a positively-charged amino acid in one chain paired with a negatively-charged amino acid substitution in the other chain. For example, in some embodiments, the heavy chain comprises at least one amino acid substitution to introduce a charged amino acid and the corresponding light chain comprises at least one amino acid substitution to introduce a charged amino acid, wherein the

charged amino acid introduced into the heavy chain has the opposite charge of the amino acid introduced into the light chain. In certain embodiments, one or more positively-charged residues (e.g., lysine, histidine or arginine) can be introduced into a first light chain (LC1) and one or more negatively-charged residues (e.g., aspartic acid or glutamic acid) can be introduced into the companion heavy chain (HC1) at the binding interface of LC1/HC1, whereas one or more negatively-charged residues (e.g., aspartic acid or glutamic acid) can be introduced into a second light chain (LC2) and one or more positively-charged residues (e.g., lysine, histidine or arginine) can be introduced into the companion heavy chain (HC2) at the binding interface of LC2/HC2. The electrostatic interactions will direct the LC1 to pair with HC1 and LC2 to pair with HC2, as the opposite charged residues (polarity) at the interface attract. The heavy/light chain pairs having the same charged residues (polarity) at an interface (e.g. LC1/HC2 and LC2/HC1) will repel, resulting in suppression of the unwanted HC/LC pairings.

[00208] In these and other embodiments, the CH1 domain of the heavy chain or the CL domain of the light chain comprises an amino acid sequence differing from a wild-type IgG amino acid sequence such that one or more positively-charged amino acids in a wild-type IgG amino acid sequence is replaced with one or more negatively-charged amino acids. Alternatively, the CH1 domain of the heavy chain or the CL domain of the light chain comprises an amino acid sequence differing from a wild-type IgG amino acid sequence such that one or more negatively-charged amino acids in a wild-type IgG amino acid sequence is replaced with one or more positively-charged amino acids. In some embodiments, one or more amino acids in the CH1 domain of the first and/or second heavy chain in the heterodimeric antibody at an EU position selected from F126, P127, L128, A141, L145, K147, D148, H168, F170, P171, V173, Q175, S176, S183, V185 and K213 is replaced with a charged amino acid. In certain embodiments, a heavy chain residue for substitution with a negatively- or positively-charged amino acid is S183 (EU numbering system). In some embodiments, S183 is substituted with a positively-charged amino acid. In alternative embodiments, S183 is substituted with a negatively-charged amino acid. For instance, in one embodiment, S183 is substituted with a negatively-charged amino acid (e.g. S183E) in the first heavy chain, and S183 is substituted with a positively-charged amino acid (e.g. S183K) in the second heavy chain.

[00209] In embodiments in which the light chain is a kappa light chain, one or more amino acids in the CL domain of the first and/or second light chain in the heterodimeric antibody at a position (EU numbering in a kappa light chain) selected from F116, F118, S121, D122, E123, Q124, S131, V133, L135, N137, N138, Q160, S162, T164, S174 and S176 is replaced with a charged amino acid. In embodiments in which the light chain is a lambda light

chain, one or more amino acids in the CL domain of the first and/or second light chain in the heterodimeric antibody at a position (EU numbering in a lambda chain) selected from T116, F118, S121, E123, E124, K129, T131, V133, L135, S137, E160, T162, S165, Q167, A174, S176 and Y178 is replaced with a charged amino acid. In some embodiments, a residue for substitution with a negatively- or positively- charged amino acid is S176 (EU numbering system) of the CL domain of either a kappa or lambda light chain. In certain embodiments, S176 of the CL domain is replaced with a positively-charged amino acid. In alternative embodiments, S176 of the CL domain is replaced with a negatively-charged amino acid. In one embodiment, S176 is substituted with a positively-charged amino acid (e.g. S176K) in the first light chain, and S176 is substituted with a negatively-charged amino acid (e.g. S176E) in the second light chain.

[00210] In addition to or as an alternative to the complementary amino acid substitutions in the CH1 and CL domains, the variable regions of the light and heavy chains in the heterodimeric antibody may contain one or more complementary amino acid substitutions to introduce charged amino acids. For instance, in some embodiments, the VH region of the heavy chain or the VL region of the light chain of a heterodimeric antibody comprises an amino acid sequence differing from wild-type IgG amino acid sequence such that one or more positively-charged amino acids in wild-type IgG amino acid sequence is replaced with one or more negatively-charged amino acids. Alternatively, the VH region of the heavy chain or the VL region of the light chain comprises an amino acid sequence differing from a wild-type IgG amino acid sequence such that one or more negatively-charged amino acids in a wild-type IgG amino acid sequence is replaced with one or more positively-charged amino acids.

[00211] V region interface residues (i.e., amino acid residues that mediate assembly of the VH and VL regions) within the VH region include EU positions 1, 3, 35, 37, 39, 43, 44, 45, 46, 47, 50, 59, 89, 91, and 93. One or more of these interface residues in the VH region can be substituted with a charged (positively- or negatively-charged) amino acid. In certain embodiments, the amino acid at EU position 39 in the VH region of the first and/or second heavy chain is substituted for a positively-charged amino acid, e.g., lysine. In alternative embodiments, the amino acid at EU position 39 in the VH region of the first and/or second heavy chain is substituted for a negatively-charged amino acid, e.g., glutamic acid. In some embodiments, the amino acid at EU position 39 in the VH region of the first heavy chain is substituted for a negatively-charged amino acid (e.g., G39E), and the amino acid at EU position 39 in the VH region of the second heavy chain is substituted for a positively-charged amino acid (e.g., G39K). In some embodiments, the amino acid at EU position 44 in the VH region of

the first and/or second heavy chain is substituted for a positively-charged amino acid; for example, lysine. In alternative embodiments, the amino acid at EU position 44 in the VH region of the first and/or second heavy chain is substituted for a negatively-charged amino acid; for example, glutamic acid. In certain embodiments, the amino acid at EU position 44 in the VH region of the first heavy chain is substituted for a negatively-charged amino acid (e.g., G44E), and the amino acid at EU position 44 in the VH region of the second heavy chain is substituted for a positively-charged amino acid (e.g., G44K).

[00212] V region interface residues (i.e., amino acid residues that mediate assembly of the VH and VL regions) within the VL region include EU positions 32, 34, 35, 36, 38, 41, 42, 43, 44, 45, 46, 48, 49, 50, 51, 53, 54, 55, 56, 57, 58, 85, 87, 89, 90, 91, and 100. One or more interface residues in the VL region can be substituted with a charged amino acid, preferably an amino acid that has an opposite charge to those introduced into the VH region of the cognate heavy chain. In some embodiments, the amino acid at EU position 100 in the VL region of the first and/or second light chain is substituted for a positively-charged amino acid; for example, lysine. In alternative embodiments, the amino acid at EU position 100 in the VL region of the first and/or second light chain is substituted for a negative-charged amino acid; for example,s glutamic acid. In certain embodiments, the amino acid at EU position 100 in the VL region of the first light chain is substituted for a positively-charged amino acid (e.g. G100K), and the amino acid at EU position 100 in the VL region of the second light chain is substituted for a negatively-charged amino acid (e.g. G100E).

[00213] In certain embodiments, a heterodimeric antibody of the invention comprises a first heavy chain and a second heavy chain and a first light chain and a second light chain, wherein the first heavy chain comprises amino acid substitutions at positions 44 (EU), 183 (EU), 392 (EU) and 409 (EU), wherein the second heavy chain comprises amino acid substitutions at positions 44 (EU), 183 (EU), 356 (EU) and 399 (EU), wherein the first and second light chains comprise an amino acid substitution at positions 100 (EU) and 176 (EU), and wherein the amino acid substitutions introduce a charged amino acid at the positions. In related embodiments, the glycine at position 44 (EU) of the first heavy chain is replaced with glutamic acid, the glycine at position 44 (EU) of the second heavy chain is replaced with lysine, the glycine at position 100 (EU) of the first light chain is replaced with lysine, the glycine at position 100 (EU) of the second light chain is replaced with glutamic acid, the serine at position 176 (EU) of the first light chain is replaced with lysine, the serine at position 176 (EU) of the second light chain is replaced with glutamic acid, the serine at position 183 (EU) of the first heavy chain is replaced with glutamic acid, the lysine at position 392 (EU) of the first heavy

chain is replaced with aspartic acid, the lysine at position 409 (EU) of the first heavy chain is replaced with aspartic acid, the serine at position 183 (EU) of the second heavy chain is replaced with lysine, the glutamic acid at position 356 (EU) of the second heavy chain is replaced with lysine, and/or the aspartic acid at position 399 (EU) of the second heavy chain is replaced with lysine.

[00214] In other embodiments, a heterodimeric antibody of the invention comprises a first heavy chain and a second heavy chain and a first light chain and a second light chain, wherein the first heavy chain comprises amino acid substitutions at positions 183 (EU), 392 (EU) and 409 (EU), wherein the second heavy chain comprises amino acid substitutions at positions 183 (EU), 356 (EU) and 399 (EU), wherein the first and second light chains comprise an amino acid substitution at position 176 (EU), and wherein the amino acid substitutions introduce a charged amino acid at the positions. In related embodiments, the serine at position 176 (EU) of the first light chain is replaced with lysine, the serine at position 176 (EU) of the second light chain is replaced with glutamic acid, the serine at position 183 (EU) of the first heavy chain is replaced with glutamic acid, the lysine at position 392 (EU) of the first heavy chain is replaced with aspartic acid, the lysine at position 409 (EU) of the first heavy chain is replaced with aspartic acid, the serine at position 183 (EU) of the second heavy chain is replaced with lysine, the glutamic acid at position 356 (EU) of the second heavy chain is replaced with lysine, and/or the aspartic acid at position 399 (EU) of the second heavy chain is replaced with lysine.

[00215] In still other embodiments, a heterodimeric antibody of the invention comprises a first heavy chain and a second heavy chain and a first light chain and a second light chain, wherein the first heavy chain comprises amino acid substitutions at positions 183 (EU), 392 (EU), 409 (EU), and 370 (EU), wherein the second heavy chain comprises amino acid substitutions at positions 183 (EU), 356 (EU), 399 (EU), and 357 (EU), wherein the first and second light chains comprise an amino acid substitution at position 176 (EU), and wherein the amino acid substitutions introduce a charged amino acid at the positions. In related embodiments, the serine at position 176 (EU) of the first light chain is replaced with lysine, the serine at position 176 (EU) of the second light chain is replaced with glutamic acid, the serine at position 183 (EU) of the first heavy chain is replaced with glutamic acid, the lysine at position 392 (EU) of the first heavy chain is replaced with aspartic acid, the lysine at position 409 (EU) of the first heavy chain is replaced with aspartic acid, the lysine at position 370 (EU) of the first heavy chain is replaced with aspartic acid, the serine at position 183 (EU) of the second heavy chain is replaced with lysine, the glutamic acid at position 356 (EU) of the

second heavy chain is replaced with lysine, the aspartic acid at position 399 (EU) of the second heavy chain is replaced with lysine, and/or the glutamic acid at position 357 (EU) of the second heavy chain is replaced with lysine.

[00216] Any of the constant domains can be modified to contain one or more of the charge pair mutations described above to facilitate correct assembly of a heterodimeric antibody.

[00217] The inventive heterodimeric antibodies also encompass antibodies comprising the heavy chain(s) and/or light chain(s), where one, two, three, four or five amino acid residues are lacking from the N-terminus or C-terminus, or both, in relation to any one of the heavy and light chains, e.g., due to post-translational modifications resulting from the type of host cell in which the antibodies are expressed. For instance, Chinese Hamster Ovary (CHO) cells frequently cleave off a C-terminal lysine from antibody heavy chains.

[00218] Charge pair mutations or complementary amino acid substitutions as described herein can be introduced into the Fab regions of the first antibody (Fab 1) or second antibody (Fab 2) to promote correct heavy chain-light chain pairing. For instance, in some embodiments, the amino acid at EU position 38 of the VL domain in Fab 1 is replaced with a negatively-charged amino acid (e.g. glutamic acid) and the amino acid at EU position 39 of the VH domain in Fab 1 is replaced with a positively-charged amino acid (e.g. lysine). In other embodiments, the amino acid at EU position 38 of the VL domain in Fab 1 is replaced with a positively-charged amino acid (e.g. lysine) and the amino acid at EU position 39 of the VH domain in Fab 1 is replaced with a negatively-charged amino acid (e.g. glutamic acid). In certain embodiments, the amino acid at EU position 38 of the VL domain in Fab 2 is replaced with a negatively-charged amino acid (e.g. glutamic acid) and the amino acid at EU position 39 of the VH domain in Fab 2 is replaced with a positively-charged amino acid (e.g. lysine). In other embodiments, the amino acid at EU position 38 of the VL domain in Fab 2 is replaced with a positively-charged amino acid (e.g. lysine) and the amino acid at EU position 39 of the VH domain in Fab 2 is replaced with a negatively-charged amino acid (e.g. glutamic acid).

[00219] In embodiments in which the VH-CH1 region (i.e. Fd fragment) from the second antibody is fused to the heavy chain of the first antibody, the heavy chain from the first antibody comprises a S183E mutation (EU numbering), the light chain from the first antibody comprises a S176K mutation (EU numbering), the light chain from the second antibody comprises a S176E mutation (EU numbering), and the Fd region from the second antibody (which is fused to the C-terminus of the heavy chain from the first antibody) comprises a S183K mutation (EU numbering). In other embodiments, the heavy chain from the first

antibody comprises a G44E mutation (EU) and S183E mutation (EU numbering), the light chain from the first antibody comprises a G100K mutation (EU) and S176K mutation (EU numbering), the light chain from the second antibody comprises a G100E mutation (EU) and S176E mutation (EU numbering), and the Fd region from the second antibody (which is fused to the C-terminus of the heavy chain from the first antibody) comprises a G44K mutation (EU) and S183K mutation (EU numbering). The charges in the foregoing examples may be reversed so long as the charge on the corresponding light or heavy chain is also reversed so that the correct heavy/light chain pairs have opposite charges.

[00220] In one embodiment, the present invention is directed to a bispecific, tetravalent antigen binding protein, comprising:

[00221] a) a first polypeptide comprising a first heavy chain of a first antibody comprising a first heavy chain variable region (VH1) and a first CH1 domain, wherein the first antibody specifically binds to a first antigen, and wherein the first heavy chain is fused through its C-terminus to the N-terminus of a polypeptide comprising a second heavy chain variable region of a second antibody (VH2), wherein the VH2 is fused through its C-terminus to the N-terminus of a second CH1 domain, and wherein the second antibody specifically binds to a second antigen; wherein

[00222] i) the VH1 or first CH1 domain comprises at least one amino acid substitution to introduce a charged (e.g., positively charged) amino acid at a residue selected from the group consisting of positions 39, 44, and 183 using EU numbering; and

[00223] ii) the VH2 or second CH1 domain comprises at least one amino acid substitution to introduce an oppositely charged (e.g., negatively charged) amino acid at a residue selected from the group consisting of a residue that corresponds to positions 39, 44, and 183 using EU numbering; and

[00224] b) a second polypeptide comprising a first light chain of the first antibody of a), wherein the first light chain comprises a first light chain variable region (VL1) and a first CL region; and wherein the VL1 or first CL domain comprises at least one amino acid substitution to introduce a negatively charged amino acid at a residue selected from the group consisting of positions 38, 100, and 176 using EU numbering; and

[00225] c) a third polypeptide comprising a second light chain of the second antibody of a), wherein the second light chain comprises a second light chain variable region (VL2) and a second CL region;. and wherein the VL1 or first CL domain comprises at least one amino acid substitution to introduce a positively charged amino acid at a residue selected from the group consisting of positions 38, 100, and 176 using EU numbering.

[00226] Within this invention, the first antigen in subparagraph a) above may be one of TL1A and TNF-α and the second antigen may be the other. Another way of stating this same binding specificity is to say that the first antibody in subparagraph a) comprises one of a TL1A binding entity or a TNF-α binding entity and the second antibody comprises the other.

[00227]

[00228] "Corresponds to" as it pertains to the VH2 and second CH1 domain means that the amino acid residues of the VH2 and second CH1 domain are counted from the C-terminus of the first heavy chain if there is no linker. If there is a peptide linker, the amino acid residues of the VH2 and second CH1 domain are counted from the C-terminus of the peptide linker. In neither case are the amino acid residues counted from the N-terminus of the first heavy chain. Rather, for the VH2 and second CH1 domain, counting begins at the first amino acid residue of the VH2 domain. The counting of amino acid residues is performed using the EU or AHo convention.

[00229] In certain embodiments: a) the VH1 or first CH1 domain comprises a mutation selected from the group consisting of Q39K, G44K, and S183K using EU numbering; b) the VH2 or second CH1 domain comprises a mutation selected from the group consisting of Q39E, G44E, and S183E using EU numbering; c) the VL1 or first CL domain comprises a mutation selected from the group consisting of Q38E, G100E, and S176E using EU numbering; and d) the VL2 or second CL domain comprises a mutation selected from the group consisting of Q38K, G100K, and S176K using EU numbering.

[00230] In certain embodiments: a) the VH1 comprises a Q39K mutation and the first CH1 domain comprises a S183K mutation using EU numbering; b) the VH2 comprises a Q39E mutation and the second CH1 domain comprises a S183E mutation using EU numbering; c) the VL1 comprises a Q38E mutation and the first CL domain comprises a S176E mutation using EU numbering; and d) the VL2 comprises a Q38K mutation and the second CL domain comprises a S176K mutation using EU numbering.

[00231] In certain embodiments: a) the first CH1 domain comprises G44K and S183K mutations using EU numbering; b) the second CH1 domain comprises G44E and S183E mutations using EU numbering; c) the first CL domain comprises G100E and S176E mutations using EU numbering; and d) the second CL domain comprises G100K and S176K mutations using EU numbering.

[00232] In one embodiment the present invention is directed to a bispecific, tetravalent antigen binding protein, comprising:

[00233] a) a first polypeptide comprising a first heavy chain of a first antibody comprising a first heavy chain variable region (VH1) and a first CH1 domain, wherein the first antibody specifically binds to a first antigen, and wherein the first heavy chain is fused through its C-terminus to the N-terminus of a polypeptide comprising a second heavy chain variable region of a second antibody (VH2), wherein the VH2 is fused through its C-terminus to the N-terminus of a second CH1 domain, and wherein the second antibody specifically binds to a second antigen; wherein

[00234] i) the VH1 or first CH1 domain comprises at least one amino acid substitution to introduce a negatively charged amino acid at a residue selected from the group consisting of positions 39, 44, and 183 using EU numbering; and

[00235] ii) the VH2 or second CH1 domain comprises at least one amino acid substitution to introduce a positively charged amino acid at a residue selected from the group consisting of a residue that corresponds to positions 39, 44, and 183 using EU numbering; and

[00236] b) a second polypeptide comprising a first light chain of the first antibody of a), wherein the first light chain comprises a first light chain variable region (VL1) and a first CL region; and wherein the VL1 or first CL domain comprises at least one amino acid substitution to introduce a positively charged amino acid at a residue selected from the group consisting of positions 38, 100, and 176 using EU numbering; and

[00237] c) a third polypeptide comprising a second light chain of the second antibody of a), wherein the second light chain comprises a second light chain variable region (VL2) and a second CL region;. and wherein the VL1 or first CL domain comprises at least one amino acid substitution to introduce a negatively charged amino acid at a residue selected from the group consisting of positions 38, 100, and 176 using EU numbering.

[00238] Within this invention, the first antigen in subparagraph a) above may be one of TL1A and TNF-α and the second antigen may be the other. Another way of stating this same binding specificity is to say that the first antibody in subparagraph a) comprises one of a TL1A binding entity or a TNF-α binding entity and the second antibody comprises the other.

[00239] In certain embodiments: a) the VH1 or first CH1 domain comprises a mutation selected from the group consisting of Q39E, G44E, and S183E using EU numbering; b) the VH2 or second CH1 domain comprises a mutation selected from the group consisting of Q39K, G44K, and S183K using EU numbering; c) the VL1 or first CL domain comprises a mutation selected from the group consisting of Q38K, G100K, and S176K using EU numbering; and d)

the VL2 or second CL domain comprises a mutation selected from the group consisting of Q38E, G100E, and S176E using EU numbering.

[00240] In certain embodiments: a) the first CH1 domain comprises a S183E mutation using EU numbering; b) the second CH1 domain comprises a S183K mutation using EU numbering; c) the first CL domain comprises a S176K mutation using EU numbering; and d) the second CL domain comprises a S176E mutation using EU numbering.

[00241] In certain embodiments: a) the VH1 comprises a Q39E mutation and the first CH1 domain comprises a S183E mutation using EU numbering; b) the VH2 comprises a Q39K mutation and the second CH1 domain comprises a S183K mutation using EU numbering; c) the VL1 comprises a Q38K mutation and the first CL domain comprises a S176K mutation using EU numbering; and d) the VL2 comprises a Q38E mutation and the second CL domain comprises a S176E mutation using EU numbering.

[00242] In certain embodiments: a) the first CH1 domain comprises G44E and S183E mutations using EU numbering; b) the second CH1 domain comprises G44K and S183K mutations using EU numbering; c) the first CL domain comprises G100K and S176K mutations using EU numbering; and d) the second CL domain comprises G100E and S176E mutations using EU numbering.

[00243] In one embodiment the present invention is directed to a bispecific, tetravalent antigen binding protein, comprising:

[00244] a) a first polypeptide comprising a first heavy chain of a first antibody comprising a first heavy chain variable region (VH1) and a first CH1 domain, wherein the first antibody specifically binds to a first antigen, and wherein the first heavy chain is fused through its C-terminus to the N-terminus of a polypeptide comprising a second heavy chain variable region of a second antibody (VH2), wherein the VH2 is fused through its C-terminus to the N-terminus of a second CH1 domain, and wherein the second antibody specifically binds to a second antigen; wherein

[00245] i) the VH1 or first CH1 domain comprises at least one amino acid substitution to introduce a charged amino acid at a residue selected from the group consisting of positions 39, 44, and 183 using EU numbering; and

[00246] ii) the VH2 or second CH1 domain comprises at least one amino acid substitution to introduce a charged amino acid at a residue selected from the group consisting of a residue that corresponds to positions 39, 44, and 183 using EU numbering, wherein the charge is the opposite of the substituted residue of the VH1 or first CH1 of the first heavy chain; and

[00247] b) a second polypeptide comprising a first light chain of the first antibody of a), wherein the first light chain comprises a first light chain variable region (VL1) and a first CL region; and wherein the VL1 or first CL domain comprises at least one amino acid substitution to introduce a charged amino acid at a residue selected from the group consisting of positions 38, 100, and 176 using EU numbering, wherein

[00248] the charge at position 38 is the opposite of the substituted residue of the VH1 or first CH1 of the first heavy chain at position 39; the charge at position 100 is the opposite of the substituted residue of the VH1 or first CH1 of the first heavy chain at position 44; the charge at position 176 is the opposite of the substituted residue of the VH1 or first CH1 of the first heavy chain at position 183; and

[00249] c) a third polypeptide comprising a second light chain of the second antibody of a), wherein the second light chain comprises a second light chain variable region (VL2) and a second CL region; and wherein the VL2 or second CL domain comprises at least one amino acid substitution to introduce a charged amino acid at a residue selected from the group consisting of positions 38, 100, and 176 using EU numbering, wherein

[00250] the charge at position 38 is the opposite of the substituted residue of the VH2 or second CH1 of the second heavy chain at position 39; the charge at position 100 is the opposite of the substituted residue of the VH2 or second CH1 of the second heavy chain at position 44; the charge at position 176 is the opposite of the substituted residue of the VH2 or second CH1 of the second heavy chain at position 183.

[00251] Within this invention, the first antigen in subparagraph a) above may be one of TL1A and TNF-α and the second antigen may be the other. Another way of stating this same binding specificity is to say that the first antibody in subparagraph a) comprises one of a TL1A binding entity or a TNF-α binding entity and the second antibody comprises the other.

[00252] In certain embodiments: a) the VH1 comprises a Q39E mutation and the first CH1 domain comprises a S183K mutation using EU numbering; b) the VH2 comprises a Q39K mutation and the second CH1 domain comprises a S183E mutation using EU numbering; c) the VL1 comprises a Q38K mutation and the first CL domain comprises a S176E mutation using EU numbering; and d) the VL2 comprises a Q38E mutation and the second CL domain comprises a S176K mutation using EU numbering.

[00253] In certain embodiments: a) the first CH1 domain comprises G44E and S183K mutations using EU numbering; b) the second CH1 domain comprises G44K and S183E mutations using EU numbering; c) the first CL domain comprises G100K and S176E mutations

using EU numbering; and d) the second CL domain comprises G100E and S176K mutations using EU numbering.

[00254] In certain embodiments: a) the VH1 comprises a Q39K mutation and the first CH1 domain comprises a S183E mutation using EU numbering; b) the VH2 comprises a Q39E mutation and the second CH1 domain comprises a S183K mutation using EU numbering; c) the VL1 comprises a Q38E mutation and the first CL domain comprises a S176K mutation using EU numbering; and d) the VL2 comprises a Q38K mutation and the second CL domain comprises a S176E mutation using EU numbering.

[00255] In certain embodiments: a) the first CH1 domain comprises G44K and S183E mutations using EU numbering; b) the second CH1 domain comprises G44E and S183K mutations using EU numbering; c) the first CL domain comprises G100E and S176K mutations using EU numbering; and d) the second CL domain comprises G100K and S176E mutations using EU numbering.

[00256] In one embodiment the present invention is directed to a method for preparing a bispecific, tetravalent antigen binding protein, comprising:

[00257] 1) co-expressing in a host cell;

[00258] a) a first polynucleotide wherein the first polynucleotide encodes a first polypeptide comprising a first heavy chain of a first antibody comprising a first heavy chain variable region (VH1) and a first CH1 domain, wherein the first antibody specifically binds to a first antigen, and wherein the first heavy chain is fused through its C-terminus to the N-terminus of a polypeptide comprising a second heavy chain variable region of a second antibody (VH2), wherein the VH2 is fused through its C-terminus to the N-terminus of a second CH1 domain, and wherein the second antibody specifically binds to a second antigen; wherein

[00259] i) the VH1 or first CH1 domain comprises at least one amino acid substitution to introduce a positively charged amino acid at a residue selected from the group consisting of positions 39, 44, and 183 using EU numbering; and

[00260] ii) the VH2 or second CH1 domain comprises at least one amino acid substitution to introduce a negatively charged amino acid at a residue selected from the group consisting of a residue that corresponds to positions 39, 44, and 183 using EU numbering; and

[00261] b) a second polynucleotide wherein the second polynucleotide encodes a second polypeptide comprising a light chain of the first antibody of a), wherein the light chain comprises a first light chain variable region (VL1) and a first CL region; and wherein the VL1 or first CL domain comprises at least one amino acid substitution to introduce a negatively charged amino acid at a residue selected from the group consisting of positions 38, 100, and 176 using EU numbering;

[00262] c) a third polynucleotide wherein the third polycucleotide encodes a third polypeptide comprising a light chain of the second antibody of a), wherein the light chain comprises a second light chain variable region (VL2) and a second CL region;. and wherein the VL1 or first CL domain comprises at least one amino acid substitution to introduce a positively charged amino acid at a residue selected from the group consisting of positions 38, 100, and 176 using EU numbering;

[00263] 2) cultivating the host cell under conditions such that the polypeptides are produced; and

[00264] 3) recovering from the host cell the antigen binding protein.

[00265] Within this invention, the first antigen in subparagraph a) above may be one of TL1A and TNF-α and the second antigen may be the other. Another way of stating this same binding specificity is to say that the first antibody in subparagraph a) comprises one of a TL1A binding entity or a TNF-α binding entity and the second antibody comprises the other.

[00266] In one embodiment the present invention is directed to a method for preparing a bispecific, tetravalent antigen binding protein, comprising:

[00267] 1) co-expressing in a host cell:

[00268] a) a first polynucleotide wherein the first polynucleotide encodes a first polypeptide comprising a first heavy chain of a first antibody comprising a first heavy chain variable region (VH1) and a first CH1 domain, wherein the first antibody specifically binds to a first antigen, and wherein the first heavy chain is fused through its C-terminus to the N-terminus of a polypeptide comprising a second heavy chain variable region of a second antibody (VH2), wherein the VH2 is fused through its C-terminus to the N-terminus of a second CH1 domain, and wherein the second antibody specifically binds to a second antigen; wherein

[00269] i) the VH1 or first CH1 domain comprises at least one amino acid substitution to introduce a negatively charged amino acid at a residue selected

from the group consisting of positions 39, 44, and 183 using EU numbering; and

[00270] ii) the VH2 or second CH1 domain comprises at least one amino acid substitution to introduce a positively charged amino acid at a residue selected from the group consisting of a residue that corresponds to positions 39, 44, and 183 using EU numbering; and

[00271] b) a second polynucleotide wherein the second polynucleotide encodes a second polypeptide comprising a first light chain of the first antibody of a), wherein the first light chain comprises a first light chain variable region (VL1) and a first CL region; and wherein the VL1 or first CL domain comprises at least one amino acid substitution to introduce a positively charged amino acid at a residue selected from the group consisting of positions 38, 100, and 176 using EU numbering; and

[00272] c) a third polynucleotide wherein the third polynucleotide encodes a third polypeptide comprising a second light chain of the second antibody of a), wherein the second light chain comprises a second light chain variable region (VL2) and a second CL region;. and wherein the VL1 or first CL domain comprises at least one amino acid substitution to introduce a negatively charged amino acid at a residue selected from the group consisting of positions 38, 100, and 176 using EU numbering;

[00273] 2) cultivating the host cell under conditions such that the polypeptides are produced; and

[00274] 3) recovering from the host cell the antigen binding protein.

[00275] Within this invention, the first antigen in subparagraph a) above may be one of TL1A and TNF-α and the second antigen may be the other. Another way of stating this same binding specificity is to say that the first antibody in subparagraph a) comprises one of a TL1A binding entity or a TNF-α binding entity and the second antibody comprises the other.

[00276] In one embodiment the present invention is directed to a method for preparing a bispecific, tetravalent antigen binding protein, comprising:

[00277] 1) co-expressing in a host cell:

[00278] a) a first polynucleotide wherein the first polynucleotide encodes a first polypeptide comprising a first heavy chain of a first antibody comprising a first heavy chain variable region (VH1) and a first CH1 domain, wherein the first antibody specifically binds to a first antigen, and wherein the first heavy chain is

fused through its C-terminus to the N-terminus of a polypeptide comprising a second heavy chain variable region of a second antibody (VH2), wherein the VH2 is fused through its C-terminus to the N-terminus of a second CH1 domain, and wherein the second antibody specifically binds to a second antigen; wherein

[00279] i) the VH1 or first CH1 domain comprises at least one amino acid substitution to introduce a charged amino acid at a residue selected from the group consisting of positions 39, 44, and 183 using EU numbering; and

[00280] ii) the VH2 or second CH1 domain comprises at least one amino acid substitution to introduce a charged amino acid at a residue selected from the group consisting of a residue that corresponds to positions 39, 44, and 183 using EU numbering, wherein the charge is the opposite of the substituted residue of the VH1 or first CH1 of the first heavy chain; and

[00281] b) a second polynucleotide wherein the second polynucleotide encodes a second polypeptide comprising a light chain of the first antibody of a), wherein the light chain comprises a first light chain variable region (VL1) and a first CL region; and wherein the VL1 or first CL domain comprises at least one amino acid substitution to introduce a charged amino acid at a residue selected from the group consisting of positions 38, 100, and 176 using EU numbering, wherein

[00282] the charge at position 38 is the opposite of the substituted residue of the VH1 or first CH1 of the first heavy chain at position 39; the charge at position 100 is the opposite of the substituted residue of the VH1 or first CH1 of the first heavy chain at position 44; the charge at position 176 is the opposite of the substituted residue of the VH1 or first CH1 of the first heavy chain at position 183; and

[00283] c) a third polynucleotide wherein the third polynucleotide encodes a third polypeptide comprising a light chain of the second antibody of a), wherein the light chain comprises a second light chain variable region (VL2) and a second CL region; and wherein the VL1 or first CL domain comprises at least one amino acid substitution to introduce a positively charged amino acid at a residue selected from the group consisting of positions 38, 100, and 176 using EU numbering, wherein

[00284] the charge at position 38 is the opposite of the substituted residue of the VH2 or second CH1 of the second heavy chain at position 39; the charge at position 100 is the opposite of the substituted residue of the VH2 or

second CH1 of the second heavy chain at position 44; the charge at position 176 is the opposite of the substituted residue of the VH2 or second CH1 of the second heavy chain at position 183;

[00285] 2) cultivating the host cell under conditions such that the polypeptides are produced; and

[00286] 3) recovering from the host cell the antigen binding protein.

[00287] Within this invention, the first antigen in subparagraph a) above may be one of TL1A and TNF-α and the second antigen may be the other. Another way of stating this same binding specificity is to say that the first antibody in subparagraph a) comprises one of a TL1A binding entity or a TNF-α binding entity and the second antibody comprises the other.

[00288] Additionally or alternatively, correct heavy-light chain pairing may be facilitated by swapping the CH1 and CL domains in the carboxyl-terminal Fab binding domain. By way of example, the first polypeptide, which is fused to the carboxyl terminus of the heavy chain, may comprise a VL domain and CH1 domain from the second antibody, and the second polypeptide may comprise a VH domain and CL domain from the second antibody. In another embodiment, the first polypeptide, which is fused to the carboxyl terminus of the heavy chain, may comprise a VH domain and a CL domain from the second antibody, and the second polypeptide may comprise a VL domain and CH1 domain from the second antibody.

[00289] The heavy chain constant regions or the Fc regions of the bispecific antigen binding proteins described herein may comprise one or more amino acid substitutions that affect the glycosylation and/or effector function of the antigen binding protein. One of the functions of the Fc region of an immunoglobulin is to communicate to the immune system when the immunoglobulin binds its target. This is commonly referred to as "effector function." Communication leads to antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and/or complement dependent cytotoxicity (CDC). ADCC and ADCP are mediated through the binding of the Fc region to Fc receptors on the surface of cells of the immune system. CDC is mediated through the binding of the Fc with proteins of the complement system, e.g., C1q. In some embodiments, the bispecific antigen binding proteins of the invention comprise one or more amino acid substitutions in the constant region to enhance effector function, including ADCC activity, CDC activity, ADCP activity, and/or the clearance or half-life of the antigen binding protein. Exemplary amino acid substitutions (EU numbering) that can enhance effector function include, but are not limited to, E233L, L234I, L234Y, L235S, G236A, S239D, F243L, F243V, P247I, D280H, K290S, K290E, K290N, K290Y, R292P, E294L, Y296W, S298A, S298D, S298V, S298G, S298T, T299A, Y300L, V305I,

Q311M, K326A, K326E, K326W, A330S, A330L, A330M, A330F, I332E, D333A, E333S, E333A, K334A, K334V, A339D, A339Q, P396L, or combinations of any of the foregoing.

[00290] In other embodiments, the bispecific antigen binding proteins of the invention comprise one or more amino acid substitutions in the constant region to reduce effector function. Exemplary amino acid substitutions (EU numbering) that can reduce effector function include, but are not limited to, C220S, C226S, C229S, E233P, L234A, L234V, V234A, L234F, L235A, L235E, G237A, P238S, S267E, H268Q, N297A, N297G, V309L, E318A, L328F, A330S, A331S, P331S or combinations of any of the foregoing.

[00291] Exemplary substitutions to aid in correct assembly of hetero Ig molecules are shown in Figure 1. Preferred substitutions for the hetero Ig format are shown in (v2) in Figure 1 and Table N. All positions in Table N are according to EU numbering.

Table N: Mutations in the hetero-Ig Molecules

Chain	Domain	Mutation	EU #
Light chain 1	Variable	E	38
	Constant	E	176
Light chain 2	Variable	K	38
	Constant	K	176
Heavy chain 1	Variable	K	39
	CH1	K	183
	CH3	D	392
	CH3	D	409
Heavy chain 2	Variable	E	39
	CH1	E	183
	CH3	K	356
	CH3	K	399

[00267] Preferred charged amino acids for the IgG-Fab format, as well as CH/CL domain swaps as described herein for Figure 26, are shown in Table O. "Fab 1" and "Fab 2" in Table O are as depicted in Figures 25 and 26. Fab 1 is at the N-terminus of the IgG-Fab molecule and Fab 2 is at its C-terminus. The identity and position of the charged amino acids in the Fab appear to the right of the domain listed in Table O. All positions are according to EU numbering.

[00268] **Table O: Mutations in the IgG-Fab Molecules**

Designation	Domain	Mutation	EU #	Domain	Mutation	EU #
CPMv1	Fab 1 CL	E	230	Fab 2 CL	K	230
	Fab 1 CH1	K	230	Fab 2 CH1	E	230
CPMv1 Fab 1 swap	Fab 1 CL	K	230	Fab 2 CL	K	230
	Fab 1 CH1	E	230	Fab 2 CH1	E	230

CPMv1 Fab 2 swap	Fab 1 CL	E	230	Fab 2 CL	E	230
	Fab 1 CH1	K	230	Fab 2 CH1	K	230
CPMv1 Fab 1&2 swap	Fab 1 CL	K	230	Fab 2 CL	E	230
	Fab 1 CH1	E	230	Fab 2 CH1	K	230
CPMv2	Fab 1 CL	E	230	Fab 2 CL	K	230
	Fab 1 CH1	K	230	Fab 2 CH1	E	230
	Fab 1 VL	E	46	Fab 2 VL	E	46
	Fab 1 VH	K	46	Fab 2 VH	K	46
CPMv2 Fab 1 swap	Fab 1 CL	K	230	Fab 2 CL	K	230
	Fab 1 CH1	E	230	Fab 2 CH1	E	230
	Fab 1 VL	E	46	Fab 2 VL	E	46
	Fab 1 VH	K	46	Fab 2 VH	K	46
CPMv2 Fab 2 swap	Fab 1 CL	E	230	Fab 2 CL	E	230
	Fab 1 CH1	K	230	Fab 2 CH1	K	230
	Fab 1 VL	E	46	Fab 2 VL	E	46
	Fab 1 VH	K	46	Fab 2 VH	K	46
CPMv2 Fab 1 & 2 swap	Fab 1 CL	K	230	Fab 2 CL	E	230
	Fab 1 CH1	E	230	Fab 2 CH1	K	230
	Fab 1 VL	E	46	Fab 2 VL	E	46
	Fab 1 VH	K	46	Fab 2 VH	K	46
CPMv3	Fab 1 CL	E	230	Fab 2 CL	K	230
	Fab 1 CH1	K	230	Fab 2 CH1	E	230
	Fab 1 VL	E	141	Fab 2 VL	E	51
	Fab 1 VH	K	51	Fab 2 VH	K	141
CPMv3 Fab 1 swap	Fab 1 CL	K	230	Fab 2 CL	K	230
	Fab 1 CH1	E	230	Fab 2 CH1	E	230
	Fab 1 VL	E	141	Fab 2 VL	E	51
	Fab 1 VH	K	51	Fab 2 VH	K	141
CPMv3 Fab 2 swap	Fab 1 CL	E	230	Fab 2 CL	E	230
	Fab 1 CH1	K	230	Fab 2 CH1	K	230
	Fab 1 VL	E	141	Fab 2 VL	E	51
	Fab 1 VH	K	51	Fab 2 VH	K	141
CPMv3 Fab 1 & 2 swap	Fab 1 CL	K	230	Fab 2 CL	E	230
	Fab 1 CH1	E	230	Fab 2 CH1	K	230
	Fab 1 VL	E	141	Fab 2 VL	E	51
	Fab 1 VH	K	51	Fab 2 VH	K	141

Binding affinity and biological activity

[00269] In another embodiment of the foregoing aspects of the invention, an anti-TL1A antibody (or an antigen-binding fragment thereof) or the TL1A binding entity of a hetero Ig bispecific antigen binding protein or an IgG-scFv antigen binding protein binds TL1A with a binding affinity (K_{D1}) of at least 1 x 10^{-10} M^{-1}, at least 5 x 10^{-10} M^{-1}, at least 1 x 10^{-10} M^{-1}, at least 5 x 10^{-10} M^{-1}, at least 8 x 10^{-10} M^{-1}, or at least 1 x 10^{-10} M^{-1} wherein the binding affinity is

measured by surface plasmon resonance, such as Biacore. More particularly, the invention relates to the following embodiments:

[00270] an anti-TL1A antibody (or an antigen-binding fragment thereof) binds to human TL1A when the antibody is immobilized on an SCM5 sensor chip at a binding affinity of about 1 to about 5 KD pM or to cynomolgus TL1A when the antibody is immobilized on an SCM5 sensor chip at a binding affinity of about 1^{-10} to about 7.5^{-10} M^{-1} KD;

[00271] an anti-TNF–α binding entity of a hetero Ig bispecific antigen binding protein binds to human TNF-α when the antigen binding protein is immobilized on an SCM5 sensor chip at a binding affinity of about 1^{-10} to about 5^{-10} M^{-1} KD pM, preferably about 10 to about 12 pM;

[00272] an anti-TL1A binding entity of a hetero Ig bispecific antigen binding protein binds to human TL1A when the antigen binding protein is immobilized on an SCM5 sensor chip at a binding affinity of about 2^{-10} to about 6.5^{-10} M^{-1} KD, preferably about 10 to about 11 pM;

[00273] an anti-TNF–α binding entity of an IgG-scFv bispecific antigen binding protein binds to human TNF-α when the antigen binding protein is immobilized on an SCM5 sensor chip at a binding affinity of about 4.5^{-10} to about 7.5^{-10} M^{-1} KD, preferably about 10 to about 20 pM; and

[00274] a TL1A binding entity of an IgG-scFv bispecific antigen binding protein binds to human TL1A when the antigen binding protein is immobilized on an SCM5 sensor chip at a binding affinity of about 1.5 to about 160 pM KD.

[00275] Further details on TL1A and TNF-α binding appear in Examples 13 and 15 hereinafter.

[00276] In another embodiment of the foregoing aspects of the invention, a TNF–α binding entity of an IgG-Fab bispecific antigen binding protein binds a TNF–α trimer with a binding affinity (K_{D1}) of 1×10^{-10} M^{-1}, at least 5×10^{-10} M^{-1}, at least 1×10^{-10} M^{-1}, at least 5×10^{-10} M^{-1}, at least 8×10^{-10} M^{-1}, or at least 1×10^{-10} M^{-1} wherein the binding affinity is measured by surface plasmon resonance, such as Biacore.

[00277] In other embodiments of the foregoing aspects of the invention:

[00278] an anti-TL1A antibody (or an antigen-binding fragment thereof) neutralizes or inhibits human TL1A in a TF-1 NF-κB reporter cell line with an IC_{50} of about 0.08 to about 3 nM, cynomolgus TL1A TL1A in a TF-1 NF-κB reporter cell line with an IC_{50} of about 0.375 to about 10 nM;

[00279] a TNF–α binding entity of a hetero Ig bispecific antigen binding protein neutralizes or inhibits human TNF-α in a TF-1 NF-κB reporter cell line with an IC_{50} of about 50 to 550 pM, with about 50 to about 110 pM preferred;

[00280] a TL1A binding entity of a hetero Ig bispecific antigen binding protein neutralizes or inhibits human TL1A in a TF-1 NF-κB reporter cell line with an IC_{50} of about 45 to about 3500 pM, with about 45 to 190 pM preferred;

[00281] a TNF–α binding entity of an IgG-scFv bispecific antigen binding protein neutralizes or inhibits soluble human TNF-α in a TF-1 NF-κB reporter cell line with an IC_{50} of about 20 to about 75 pM; and

[00282] a TL1A binding entity of an IgG-scFv bispecific antigen binding protein neutralizes or inhibits human TL1A in a TF-1 NF-κB reporter cell line with an IC_{50} of about 19 to about 200 pM.

[00283] Further details on TL1A inhibition and TNF-α inhibition appears in Examples 14 and 16 hereinafter.

Immunoconjugates, derivatives, variants

[00284] The antibodies and bispecific antibodies of the invention may be used alone or as immunoconjugates with a therapeutic agent (e.g., a cytotoxic agent). In some embodiments, the agent is a chemotherapeutic agent. In some embodiments, the agent is a radioisotope, including but not limited to Lead-212, Bismuth-212, Astatine-211, Iodine-131, Scandium-47, Rhenium-186, Rhenium-188, Yttrium-90, Iodine-123, Iodine-125, Bromine-77, Indium-111, and fissionable nuclides such as Boron-10 or an Actinide. In other embodiments, the agent is a toxin or cytotoxic drug, including but not limited to ricin, abrin, modified Pseudomonas enterotoxin A, Pseudomonas exotoxin, calicheamicin, adriamycin, 5-fluorouracil, diphtheria toxin, and the like. Methods of conjugation of antibodies to such agents are known in the literature, and include direct and indirect conjugation.

[00285] Suitable detectable molecules may be directly or indirectly attached to the antibodies and bispecific antibodies of the present invention. Suitable detectable molecules include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent markers, chemiluminescent markers, magnetic particles and the like. For indirect attachment of a detectable or cytotoxic molecule, the detectable or cytotoxic molecule can be conjugated with a member of a complementary/anti-complementary pair, where the other member is bound to the binding polypeptide or antibody portion. For these purposes, biotin/streptavidin is an exemplary complementary/anti-complementary pair.

[00286] The bispecific antibodies and antibodies of the invention also include derivatives that are modified, e.g., by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from binding to its epitope. Examples of suitable derivatives include but are not limited to antibodies or bispecific antibodies that are fucosylated, glycosylated, acetylated, pegylated, phosphorylated, or amidated. The antibodies and bispecific antibodies and derivatives thereof of the invention may themselves by derivatized by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other proteins, and the like. In some embodiments of the invention, at least one heavy chain of the antibody or bispecific antigen binding protein is PEGylated. In some embodiments, the PEGylation is N-linked or is linked through the sidechain of an amino acid (e.g., lysine).

[00287] Glycosylation can contribute to the effector function of antibodies, particularly IgG1 antibodies. Thus, in some embodiments, the antigen binding proteins of the invention may comprise one or more amino acid substitutions that affect the level or type of glycosylation of the binding proteins. Glycosylation of polypeptides is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tri-peptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tri-peptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose, to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

[00288] In certain embodiments, glycosylation of the antigen binding proteins described herein is increased by adding one or more glycosylation sites, e.g., to the Fc region of the binding protein. Addition of glycosylation sites to the antigen binding protein can be conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tri-peptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the starting sequence (for O-linked glycosylation sites). For ease, the antigen binding protein amino acid sequence may be altered through changes at the DNA level, particularly by mutating the DNA encoding the target polypeptide at preselected bases such that codons are generated that will translate into the desired amino acids.

[00289] The invention also encompasses production of antigen binding proteins with altered carbohydrate structure resulting in altered effector activity, including antigen binding

proteins with absent or reduced fucosylation that exhibit improved ADCC activity. Various methods are known in the art to reduce or eliminate fucosylation. For example, ADCC effector activity is mediated by binding of the antibody molecule to the FcγRIII receptor, which has been shown to be dependent on the carbohydrate structure of the N-linked glycosylation at the N297 residue of the CH2 domain. Non-fucosylated antibodies bind this receptor with increased affinity and trigger FcγRIII-mediated effector functions more efficiently than native, fucosylated antibodies. For example, recombinant production of non-fucosylated antibody in CHO cells in which the alpha-1,6-fucosyl transferase enzyme has been knocked out results in antibody with 100-fold increased ADCC activity (*see* Yamane-Ohnuki *et al.*, Biotechnol Bioeng. 87(5):614-22, 2004). Similar effects can be accomplished through decreasing the activity of alpha-1,6-fucosyl transferase enzyme or other enzymes in the fucosylation pathway, e.g., through siRNA or antisense RNA treatment, engineering cell lines to knockout the enzyme(s), or culturing with selective glycosylation inhibitors (*see* Rothman *et al.*, Mol Immunol. 26(12):1113-23, 1989). Some host cell strains, e.g. Lec13 or rat hybridoma YB2/0 cell line naturally produce antibodies with lower fucosylation levels (*see* Shields *et al.*, J Biol Chem. 277(30):26733-40, 2002 and Shinkawa *et al.*, J Biol Chem. 278(5):3466-73, 2003). An increase in the level of bisected carbohydrate, e.g. through recombinantly producing antibody in cells that overexpress GnTIII enzyme, has also been determined to increase ADCC activity (*see* Umana *et al.*, Nat Biotechnol. 17(2):176-80, 1999).

[00290] In other embodiments, glycosylation of the antigen binding proteins described herein is decreased or eliminated by removing one or more glycosylation sites, e.g., from the Fc region of the binding protein. Amino acid substitutions that eliminate or alter N-linked glycosylation sites can reduce or eliminate N-linked glycosylation of the antigen binding protein. In certain embodiments, the bispecific antigen binding proteins described herein comprise a mutation at position N297 (EU numbering), such as N297Q, N297A, or N297G. In one particular embodiment, the bispecific antigen binding proteins of the invention comprise a Fc region from a human IgG1 antibody with a N297G mutation. To improve the stability of molecules comprising a N297 mutation, the Fc region of the molecules may be further engineered. For instance, in some embodiments, one or more amino acids in the Fc region are substituted with cysteine to promote disulfide bond formation in the dimeric state. Residues corresponding to V259, A287, R292, V302, L306, V323, or I332 (EU numbering) of an IgG1 Fc region may thus be substituted with cysteine. In one embodiment, specific pairs of residues are substituted with cysteine such that they preferentially form a disulfide bond with each other, thus limiting or preventing disulfide bond scrambling. In certain embodiments pairs include,

but are not limited to, A287C and L306C, V259C and L306C, R292C and V302C, and V323C and I332C. In particular embodiments, the bispecific antigen binding proteins described herein comprise a Fc region from a human IgG1 antibody with mutations at R292C and V302C. In such embodiments, the Fc region may also comprise a N297G mutation.

[00291] Modifications of the antigen binding proteins of the invention to increase serum half-life also may desirable, for example, by incorporation of or addition of a salvage receptor binding epitope (e.g., by mutation of the appropriate region or by incorporating the epitope into a peptide tag that is then fused to the antigen binding protein at either end or in the middle, e.g., by DNA or peptide synthesis; *see, e.g.*, WO96/32478) or adding molecules such as PEG or other water soluble polymers, including polysaccharide polymers. The salvage receptor binding epitope preferably constitutes a region wherein any one or more amino acid residues from one or two loops of a Fc region are transferred to an analogous position in the antigen binding protein. In one embodiment, three or more residues from one or two loops of the Fc region are transferred. In one embodiment, the epitope is taken from the CH2 domain of the Fc region (e.g., an IgG Fc region) and transferred to the CH1, CH3, or VH region, or more than one such region, of the antigen binding protein. Alternatively, the epitope is taken from the CH2 domain of the Fc region and transferred to the CL region or VL region, or both, of the antigen binding protein. *See* International applications WO 97/34631 and WO 96/32478 for a description of Fc variants and their interaction with the salvage receptor.

[00292] The bispecific antibodies and antibodies of the invention include variants having single or multiple amino acid substitutions, deletions, additions, or replacements that retain their biological properties (e.g., blocking the binding of TL1A and/or TNF–α to their respective receptors, inhibiting the biological activity of TL1A and TNF–α). A person of ordinary skill in the art can produce variants having single or multiple amino acid substitutions, deletions, additions or replacements. These variants may include, inter alia: (a) variants in which one or more amino acid residues are substituted with conservative or non-conservative amino acids, (b) variants in which one or more amino acids are added to or deleted from the polypeptide, (c) variants in which one or more amino acids include a substituent group, and (d) variants in which the polypeptide is fused with another peptide or polypeptide such as a fusion partner, a protein tag or other chemical moiety, that may confer useful properties to the polypeptide, such as, for example, an epitope for an antibody, a polyhistidine sequence, a biotin moiety and the like. Antibodies and bispecific antibodies of the invention may include variants in which amino acid residues from one species are substituted for the corresponding residue in another species, either at the conserved or non-conserved positions. In another embodiment,

amino acid residues at non-conserved positions are substituted with conservative or non-conservative residues. The techniques for obtaining these variants, including genetic (suppressions, deletions, mutations, etc.), chemical, and enzymatic techniques, are known to the person having ordinary skill in the art.

Nucleic acids, vectors, host cells

[00293] The invention also includes isolated nucleic acids encoding the bispecific antibodies of the invention, which includes, for instance, the light chain, light chain variable region, light chain constant region, heavy chain, heavy chain variable region, heavy chain constant region, linkers, and any and all components and combinations thereof of the bispecific antibodies disclosed herein. Nucleic acids of the invention include nucleic acids having at least 80%, more preferably at least about 90%, more preferably at least about 95%, and most preferably at least about 98% homology to nucleic acids of the invention. The terms "percent similarity", "percent identity" and "percent homology" when referring to a particular sequence are used as set forth in the University of Wisconsin GCG® software program. Nucleic acids of the invention also include complementary nucleic acids. In some instances, the sequences will be fully complementary (no mismatches) when aligned. In other instances, there may be up to about a 20% mismatch in the sequences. In some embodiments of the invention are provided nucleic acids encoding both a heavy chain and a light chain of an antibody of the invention.

[00294] Nucleic acids of the invention can be cloned into a vector, such as a plasmid, cosmid, bacmid, phage, artificial chromosome (BAC, YAC) or virus, into which another genetic sequence or element (either DNA or RNA) may be inserted so as to bring about the replication of the attached sequence or element. In some embodiments, the expression vector contains a constitutively active promoter segment (such as but not limited to CMV, SV40, Elongation Factor or LTR sequences) or an inducible promoter sequence such as the steroid inducible pIND vector (Invitrogen), where the expression of the nucleic acid can be regulated. Expression vectors of the invention may further comprise regulatory sequences, for example, an internal ribosomal entry site. The expression vector can be introduced into a cell by transfection, for example.

[00295] For IgG-Fab bispecific antigen binding proteins, nucleic acids encoding each of the three components may be cloned into the same expression vector. In some embodiments, the nucleic acid encoding the light chain of the IgG-Fab molecule and the nucleic acid encoding the second polypeptide (which comprises the other half of the C-terminal Fab domain) are cloned into one expression vector, whereas the nucleic acid encoding the modified heavy chain (fusion protein comprising a heavy chain and half of a Fab domain) is cloned into a second

expression vector. In certain embodiments, all components of the bispecific antigen binding proteins described herein are expressed from the same host cell population. For example, even if one or more components is cloned into a separate expression vector, the host cell is co-transfected with both expression vectors such that one cell produces all components of the bispecific antigen binding proteins.

[00296] In another embodiment, the present invention provides an expression vector comprising the following operably linked elements; a transcription promoter; a first nucleic acid molecule encoding the heavy chain of a bispecific antigen binding protein, antibody or antigen-binding fragment of the invention; a second nucleic acid molecule encoding the light chain of a bispecific antigen binding protein, antibody or antigen-binding fragment of the invention; and a transcription terminator. In another embodiment, the present invention provides an expression vector comprising the following operably linked elements; a first transcription promoter; a first nucleic acid molecule encoding the heavy chain of a bispecific antigen binding protein, antibody or antigen-binding fragment of the invention; a first transcription terminator; a second transcription promoter a second nucleic acid molecule encoding the light chain of a bispecific antigen binding protein, antibody or antigen-binding fragment of the invention; and a second transcription terminator.

[00297] A secretory signal peptide sequence can also, optionally, be encoded by the expression vector, operably linked to the coding sequence of interest, so that the expressed polypeptide can be secreted by the recombinant host cell, for more facile isolation of the polypeptide of interest from the cell, if desired. For instance, in some embodiments, signal peptide sequences may be appended/fused to the amino terminus of any of the polypeptide sequences listed in Tables E, J, K, and L. In certain embodiments, a signal peptide having the amino acid sequence of MKHLWFFLLLVAAPRWVLS (SEQ ID NO: 499) is fused to the amino terminus of any of the polypeptide sequences in Tables D, I, J, and K. In other embodiments, a signal peptide having the amino acid sequence of METPAQLLFLLLLWLPDTTG (SEQ ID NO: 501) is fused to the amino terminus of any of the polypeptide sequences in Tables E, J, K, and L. In still other embodiments, a signal peptide having the amino acid sequence of MDMRVPAQLLGLLLLWLRGARC (SEQ ID NO: 503) is fused to the amino terminus of any of the polypeptide sequences in Tables E, J, K, and L. Each of the foregoing signal peptides is encoded by the nucleic acid having a sequence immediately preceding it in the Sequence Listing. Other suitable signal peptide sequences that can be fused to the amino terminus of the polypeptide sequences described herein include: MEAPAQLLFLLLLWLPDTTG (SEQ ID NO: 504), MEWTWRVLFLVAAATGAHS (SEQ

ID NO: 505), and MEWSWVFLFFLSVTTGVHS (SEQ ID NO: 506). Other signal peptides are known to those of skill in the art and may be fused to any of the polypeptide chains listed in Tables E, J, K and L, for example, to facilitate or optimize expression in particular host cells.

[00298] Recombinant host cells comprising such vectors and expressing the heavy and light chains are also provided.

[00299] Antibody-producing cells and bispecific antigen binding protein producing cells contain, depending on the bispecific antigen binding protein format, nucleic acids encoding the heavy chain, light chain, heavy chain-scFv construct, heavy chain-Fab heavy chain variable domain construct, and Fab light chain variable domain. Such nucleic acids can be used to produce the antibodies or bispecific antibodies of the invention in accordance with techniques known in the art. The present invention, in one embodiment, provides a method of producing a bispecific antigen binding protein or antibody comprising culturing a recombinant host cell expressing the heavy and light chains or other constructs noted above and isolating the bispecific antigen binding protein or antibody produced by the cell.

[00300] The recombinant host cell may be a prokaryotic cell, for example an E. coli cell, or a eukaryotic cell, for example a mammalian cell or a yeast cell. Yeast cells include Saccharomyces cerevisiae, Schizosaccharomyces pombe, and Pichia pastoris cells. Mammalian cells include VERO, HeLa, Chinese hamster Ovary (CHO), W138, baby hamster kidney (BHK), COS-7, MDCK, human embryonic kidney line 293, normal dog kidney cell lines, normal cat kidney cell lines, monkey kidney cells, African green monkey kidney cells, COS cells, and non-tumorigenic mouse myoblast G8 cells, fibroblast cell lines, myeloma cell lines, mouse NIH/3T3 cells, LMTK31 cells, mouse sertoli cells, human cervical carcinoma cells, buffalo rat liver cells, human lung cells, human liver cells, mouse mammary tumor cells, TRI cells, MRC 5 cells, and FS4 cells. Antibody-producing and bispecific antigen binding protein-producing cells of the invention also include any insect expression cell line known, such as for example, Spodoptera frugiperda cells. In a preferred embodiment, the cells are mammalian cells. In a most preferred embodiment, the mammalian cells are CHO cells.

[00301] The antibody-producing cells preferably are substantially free of TL1A and TNF–α binding competitors. In preferred embodiments, the antibody-producing cells comprise less than about 10%, preferably less than about 5%, more preferably less than about 1%, more preferably less than about 0.5%, more preferably less than about 0.1%, and most preferably 0% by weight TL1A or TNF–α binding competitors. In some embodiments, the antibodies and bispecific antibodies produced are substantially free of TL1A and TNF–α competitors. In

preferred embodiments, the antibodies and bispecific antibodies produced comprise less than about 10%, preferably less than about 5%, more preferably less than about 1%, more preferably less than about 0.5%, more preferably less than about 0.1%, and most preferably 0% by weight both TL1A and TNF–α binding competitors.

Purification

[00302] Methods of antibody purification are known in the art and can be employed with production of the antibodies and bispecific antibodies of the present invention. In some embodiments of the invention, methods for antibody purification include filtration, affinity column chromatography, cation exchange chromatography, anion exchange chromatography, and concentration. The filtration step preferably comprises ultrafiltration, and more preferably ultrafiltration and diafiltration. Filtration is preferably performed at least about 5-50 times, more preferably 10 to 30 times, and most preferably 14 to 27 times. Affinity column chromatography, may be performed using, for example, PROSEP® Affinity Chromatography (Millipore, Billerica, Mass.). In a preferred embodiment, the affinity chromatography step comprises PROSEP®-vA column chromatography. Eluate may be washed in a solvent detergent. Cation exchange chromatography may include, for example, SP-Sepharose Cation Exchange Chromatography. Anion exchange chromatography may include, for example but not limited to, Q-Sepharose Fast Flow Anion Exchange. The anion exchange step is preferably non-binding, thereby allowing removal of contaminants including DNA and BSA. The antibody product is preferably nanofiltered, for example, using a Pall DV 20 Nanofilter. The antibody product may be concentrated, for example, using ultrafiltration and diafiltration. The method may further comprise a step of size exclusion chromatography to remove aggregates. Further parameters of purification appear in the working examples hereinafter.

[00303] The bispecific antibodies, antibodies or antigen-binding fragments may also be produced by other methods known in the art, for example by chemical coupling of antibodies and antibody fragments.

Uses of the monospecific and bispecific antibodies of the invention

[00304] The bispecific antibodies and monospecific antibodies of the present invention are useful, for example, for the inhibition of proinflammatory cytokines, such as TL1A and TNF–α . The bispecific antibodies and monospecific antibodies of the invention can be used to treat inflammatory disorders and autoimmune diseases, such as inflammatory bowel disease (IBD), Crohn's disease (CD), ulcerative colitis (UC), irritable bowel syndrome (IBS), bladder syndrome/interstitial cystitis, urinary bowel disfunction, sepsis, uveitis, encephalomyelitis,

myasthenia gravis, Sjogren's syndrome (SS), scleroderma, multiple sclerosis (MS), cystic fibrosis (CF), inflammation in chronic kidney disease (CKD), psoriasis (Pso), psoriatic arthritis (PsA), ankylosing spondylitis (AS), rheumatoid arthritis (RA), juvenile rheumatoid arthritis (JRA), osteoarthritis (OA), spondyloarthropathy, primary sclerosing cholangitis, primary biliary cirrhosis, atherosclerosis, splenomegaly, inflammation in chronic kidney disease (CKD), atopic dermatitis (AD), eczematous dermatitis, contact dermatitis systemic sclerosis, systemic lupus erythematosus (SLE), lupus nephritis (LN), cutaneous lupus erythematosus, autoimmune thyroiditis, IgA nephropathy, diabetic kidney disease, antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis (AAV), minimal change disease (lipoid nephrosis), focal segmental glomerulosclerosis (FSGS), nephrogenic systemic fibrosis (NSF), nephrogenic fibrosing dermopathy, fibrosing cholestatic hepatitis, eosinophilic fasciitis (Shulman's syndrome), scleromyxedema (popular mucinosis), scleroderma, lichen sclerosusetatrophicus, inflammatory lung injury such as idiopathic pulmonary fibrosis, asthma, chronic obstructive pulmonary disease (COPD), airway hyper-responsiveness, chronic bronchitis, allergic asthma, eczema, Helicobacter pylori infection, intraabdominal adhesions and/or abscesses as results of peritoneal inflammation (e.g., from infection, injury, etc.), nephrotic syndrome, idiopathic demyelinating polyneuropathy, Guillain-Barre syndrome, transplant rejection, organ allograft rejection, graft vs. host disease (GVHD) (e.g., from a transplant, such as blood, bone marrow, kidney, pancreas, liver, orthotopic liver, lung, heart, intestine, small intestine, large intestine, thymus, allogeneic stem cell, reduced-intensity allogeneic, bone, tendon, cornea, skin, heart valves, veins, arteries, blood vessels, stomach and testis), IgA nephropathy, diabetic kidney disease, diabetes mellitus, minimal change disease (lipoid nephrosis), nephrogenic systemic fibrosis (NSF), nephrogenic fibrosing dermopathy, fibrosing cholestatic hepatitis, eosinophilic fasciitis (Shulman's syndrome), scleromyxedema (popular mucinosis), scleroderma, lichen sclerosusetatrophicus, Takatsuki disease (or PEP syndrome), nephrotic syndrome, POEMs syndrome, Crow-Fukase syndrome, nephrotic syndrome, , antineutrophil cytoplasmic antibodies, vasculitis, giant cell arteritis and multiple-myeloma-induced lytic bone disease, streptococcal cell wall (SCW)-induced arthritis, gingivitis/periodontitis, herpetic stromal keratitis, gluten-sensitive enteropathy restenosis, Kawasaki's disease, and immune-mediated renal diseases. The bispecific antibodies and monospecific antibodies described herein can also be used to treat cancer, including angiogenesis.

[00305] In one embodiment, the invention concerns methods of treating one or more of the aforementioned diseases and disorders in a mammal in need of such treatment by administering a therapeutically effective amount of a monospecific or bispecific antigen

binding protein of the present invention. In a preferred embodiment the mammal is a human. In another preferred embodiment, the disease or disorder is IBD, CD, or UC.

[00306] The invention further concerns the use of the bispecific and monospecific antibodies of the present invention in the treatment of inflammatory diseases characterized by the presence of elevated levels of TL1A or/(in the case of the bispecific antibodies) TNF–α , and in the treatment of cancers characterized by the presence of elevated levels of TL1A and/or TNF–α .

[00307] Accordingly, in one embodiment, the present invention provides a method of inhibiting one or more of proinflammatory cytokines, e.g., TL1A and TNF–α , in a mammal in need of such treatment comprising administering a therapeutically effective amount of a bispecific or monospecific antibody of the invention to a mammal in need of such treatment. In a preferred embodiment, the mammal is a human. The method may be used to treat a disorder characterized by elevated expression or activity of TL1A or TNF–α . The monospecific or bispecific antigen binding protein may be administered with another pharmaceutical agent, either in the same formulation or separately.

[00308] In another embodiment, the present invention provides a composition comprising a monospecific or bispecific antigen binding protein as described herein and a pharmaceutically acceptable carrier. A pharmaceutical composition comprising an antibody, e.g., a bispecific antigen binding protein, of the invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the therapeutic antibodies are combined in a mixture with a pharmaceutically acceptable carrier. A composition is said to comprise a "pharmaceutically acceptable carrier" if its administration can be tolerated by a recipient patient. Sterile phosphate-buffered saline is one example of a pharmaceutically acceptable carrier. Other suitable carriers are well-known to those in the art. See, for example, Getman), ed., Remington's Pharmaceutical Sciences, 19th Edition, Mack Publishing Company (1995).

[00309] For pharmaceutical use, an antibody, e.g., a bispecific antigen binding protein, of the present invention is formulated for parenteral, particularly intravenous or subcutaneous, delivery according to conventional methods. Intravenous administration may be by bolus injection, controlled release, e.g., using mini-pumps or other appropriate technology, or by infusion over a typical period of one to several hours. In general, pharmaceutical formulations will include an antibody, e.g., a bispecific antigen binding protein, of the invention in combination with a pharmaceutically acceptable carrier, such as saline, buffered saline, 5%

dextrose in water or the like. Formulations may further include one or more excipients, preservatives, solubilizers, buffering agents, albumin to prevent protein loss on vial surfaces, etc. When utilizing such a combination therapy, the antibodies, which include bispecific antibodies, may be combined in a single formulation or may be administered in separate formulations. Methods of formulation are well known in the art and are disclosed, for example, in Gennaro, ed., Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton Pa. (1990), which is incorporated herein by reference. Therapeutic doses will generally be in the range of 0.1 to 100 mg/kg of patient weight per day, preferably 0.5-20 mg/kg per day, with the exact dose determined by the clinician according to accepted standards, taking into account the nature and severity of the condition to be treated, patient traits, etc. Determination of dose is within the level of ordinary skill in the art. More commonly, the antibodies will be administered over one week or less, often over a period of one to three days. Generally, the dosage of administered antibodies will vary depending upon such factors as the patient's age, weight, height, sex, general medical condition and previous medical history. Typically, it is desirable to provide the recipient with a dosage of antibodies which is in the range of from about 1 pg/kg to 10 mg/kg (amount of agent/body weight of patient), although a lower or higher dosage also may be administered as circumstances dictate.

[00310] Administration of an antibody, e.g., bispecific antigen binding protein, of the invention to a subject can be intravenous, intraarterial, intraperitoneal, intramuscular, subcutaneous, intrapleural, intrathecal, by perfusion through a regional catheter, or by direct intralesional injection. When administering therapeutic antibodies by injection, the administration may be by continuous infusion or by single or multiple boluses.

[00311] Additional routes of administration include oral, mucosal-membrane, pulmonary, and transcutaneous. Oral delivery is suitable for polyester microspheres, zein microspheres, proteinoid microspheres, polycyanoacrylate microspheres, and lipid-based systems (see, for example, DiBase et al., "Oral Delivery of Microencapsulated Proteins", in Sanders et al., eds., Protein Delivery: Physical Systems, pp. 255-288, Plenum Press (1997)). The feasibility of an intranasal delivery is exemplified by such a mode of insulin administration (see, for example, Hinchcliffe et al., Adv. Drug Deliv. Rev., 35:199 (1999)). Dry or liquid particles comprising antibodies of the invention can be prepared and inhaled with the aid of dry-powder dispersers, liquid aerosol generators, or nebulizers (e.g., Pettit et al., TIBTECH, 16:343 (1998); Patton et al., Adv. Drug Deliv. Rev., 35:235 (1999)). This approach is illustrated by the AERX® diabetes management system, which is a hand-held electronic inhaler that delivers aerosolized insulin into the lungs. Studies have shown that proteins as large as

48,000 kDa have been delivered across skin at therapeutic concentrations with the aid of low-frequency ultrasound, which illustrates the feasibility of transcutaneous administration (Mitragotri et al., Science, 269:850 (1995)). Transdermal delivery using electroporation provides another means to administer a molecule having IL-17 and TNF–α /p19 binding activity (Potts et al., Pharm. Biotechnol., 10:213 (1997)).

[00312] For purposes of therapy, compositions comprising an antibody, e.g., a bispecific antigen binding protein, of the invention and a pharmaceutically acceptable carrier are administered to a patient in a therapeutically effective amount. A combination of an antibody, e.g., a bispecific antigen binding protein, of the present invention and a pharmaceutically acceptable carrier is said to be administered in a "therapeutically effective amount" if the amount administered is physiologically significant. An agent is physiologically significant if its presence results in a detectable change in the physiology of a recipient patient. For example, an agent used to treat inflammation is physiologically significant if its presence alleviates the inflammatory response. Effective treatment may be assessed in a variety of ways. In one embodiment, effective treatment is determined by reduced inflammation. In other embodiments, effective treatment is marked by inhibition of inflammation. In still other embodiments, effective therapy is measured by increased well-being of the patient including such signs as weight gain, regained strength, decreased pain, thriving, and subjective indications from the patient of better health.

[00313] A pharmaceutical composition comprising an antibody, e.g., a bispecific antigen binding protein, of the invention can be furnished in liquid form, in an aerosol, or in solid form. Liquid forms, are illustrated by injectable solutions and oral suspensions. Exemplary solid forms include capsules, tablets, and controlled-release forms. The latter form is illustrated by miniosmotic pumps and implants (Bremer et al., Pharm. Biotechnol., 10:239 (1997); Ranade, "Implants in Drug Delivery", in Ranade et al., eds., Drug Delivery Systems, pp. 95-123, CRC Press (1995); Bremer et al., "Protein Delivery with Infusion Pumps", in Sanders et al., eds., Protein Delivery: Physical Systems, pp. 239-254, Plenum Press (1997); Yewey et al., "Delivery of Proteins from a Controlled Release Injectable Implant", in Sanders et al., eds., Protein Delivery: Physical Systems, pp. 93-117, Plenum Press (1997).

[00314] Liposomes provide one means to deliver therapeutic polypeptides to a subject intravenously, intraperitoneally, intrathecally, intramuscularly, subcutaneously, or via oral administration, inhalation, or intranasal administration. Liposomes are microscopic vesicles that consist of one or more lipid bilayers surrounding aqueous compartments (see, generally, Bakker-Woudenberg et al., Eur. J. Clin. Microbiol. Infect. Dis., 12(Suppl. 1):561 (1993), Kim,

Drugs, 46:618 (1993), and Ranade, "Site-Specific Drug Delivery Using Liposomes as Carriers", in Ranade et al., eds., Drug Delivery Systems, pp. 3-24, CRC Press (1995)). Liposomes are similar in composition to cellular membranes and as a result, liposomes can be administered safely and are biodegradable. Depending on the method of preparation, liposomes may be unilamellar or multilamellar, and liposomes can vary in size with diameters ranging from 0.02 .mu.m to greater than 10 .mu.m. A variety of agents can be encapsulated in liposomes: hydrophobic agents partition in the bilayers and hydrophilic agents partition within the inner aqueous space(s) (see, for example, Machy et al., Liposomes in Cell Biology and Pharmacology, John Libbey (1987), and Ostro et al., American J. Hosp. Pharm., 46:1576 (1989)). Moreover, it is possible to control the therapeutic availability of the encapsulated agent by varying liposome size, the number of bilayers, lipid composition, as well as the charge and surface characteristics of the liposomes.

[00315] Liposomes can absorb to virtually any type of cell and then slowly release the encapsulated agent. Alternatively, an absorbed liposome may be endocytosed by cells that are phagocytic. Endocytosis is followed by intralysosomal degradation of liposomal lipids and release of the encapsulated agents (Scherphof et al., Ann. N.Y. Acad. Sci., 446:368 (1985)). After intravenous administration, small liposomes (0.1 to 1.0 .mu.m) are typically taken up by cells of the reticuloendothelial system, located principally in the liver and spleen, whereas liposomes larger than 3.0 .mu.m are deposited in the lung. This preferential uptake of smaller liposomes by the cells of the reticuloendothelial system has been used to deliver chemotherapeutic agents to macrophages and to tumors of the liver.

[00316] The reticuloendothelial system can be circumvented by several methods including saturation with large doses of liposome particles, or selective macrophage inactivation by pharmacological means (Claassen et al., Biochim. Biophys. Acta, 802:428 (1984)). In addition, incorporation of glycolipid- or polyethelene glycol-derivatized phospholipids into liposome membranes has been shown to result in a significantly reduced uptake by the reticuloendothelial system (Allen et al., Biochim. Biophys. Acta, 1068:133 (1991); Allen et al., Biochim. Biophys. Acta, 1150:9 (1993)).

[00317] Liposomes can also be prepared to target particular cells or organs by varying phospholipid composition or by inserting receptors or ligands into the liposomes. For example, liposomes, prepared with a high content of a nonionic surfactant, have been used to target the liver (Hayakawa et al., Japanese Patent No. 04-244,018; Kato et al., Biol. Pharm. Bull., 16:960 (1993)). These formulations were prepared by mixing soybean phospatidylcholine, .alpha.-tocopherol, and ethoxylated hydrogenated castor oil (HCO-60) in methanol, concentrating the

mixture under vacuum, and then reconstituting the mixture with water. A liposomal formulation of dipalmitoylphosphatidylcholine (DPPC) with a soybean-derived sterylglucoside mixture (SG) and cholesterol (Ch) has also been shown to target the liver (Shimizu et al., Biol. Pharm. Bull., 20:881 (1997)).

[00318] Alternatively, various targeting ligands can be bound to the surface of the liposome, such as antibodies, antibody fragments, carbohydrates, vitamins, and transport proteins. For example, liposomes can be modified with branched type galactosyllipid derivatives to target asialoglycoprotein (galactose) receptors, which are exclusively expressed on the surface of liver cells (Kato et al., Crit. Rev. Ther. Drug Carrier Syst., 14:287 (1997); Murahashi et al., Biol. Pharm. Bull., 20:259 (1997)). Similarly, Wu et al., Hepatology, 27:772 (1998), have shown that labeling liposomes with asialofetuin led to a shortened liposome plasma half-life and greatly enhanced uptake of asialofetuin-labeled liposome by hepatocytes. On the other hand, hepatic accumulation of liposomes comprising branched type galactosyllipid derivatives can be inhibited by preinjection of asialofetuin (Murahashi et al., Biol. Pharm. Bull., 20:259 (1997)). Polyaconitylated human serum albumin liposomes provide another approach for targeting liposomes to liver cells (Kamps et al., Proc. Nat'l Acad. Sci. USA, 94:11681 (1997)). Moreover, Geho et al. U.S. Pat. No. 4,603,044, describe a hepatocyte-directed liposome vesicle delivery system, which has specificity for hepatobiliary receptors associated with the specialized metabolic cells of the liver.

[00319] In a more general approach to tissue targeting, target cells are prelabeled with biotinylated antibodies specific for a ligand expressed by the target cell (Harasym et al., Adv. Drug Deliv. Rev., 32: 99 (1998)). After plasma elimination of free antibody, streptavidin-conjugated liposomes are administered. In another approach, targeting antibodies are directly attached to liposomes (Harasym et al., Adv. Drug Deliv. Rev., 32: 99 (1998)).

[00320] Antibodies can be encapsulated within liposomes using standard techniques of protein microencapsulation (see, for example, Anderson et al., Infect. Immun., 31:1099 (1981), Anderson et al., Cancer Res., 50:1853 (1990), and Cohen et al., Biochim. Biophys. Acta, 1063:95 (1991), Alving et al. "Preparation and Use of Liposomes in Immunological Studies", in Gregoriadis, ed., Liposome Technology, 2nd Edition, Vol. III, p. 317, CRC Press (1993), Wassef et al., Meth. Enzymol., 149:124 (1987)). As noted above, therapeutically useful liposomes may contain a variety of components. For example, liposomes may comprise lipid derivatives of poly(ethylene glycol) (Allen et al., Biochim. Biophys. Acta, 1150:9 (1993)).

[00321] Degradable polymer microspheres have been designed to maintain high systemic levels of therapeutic proteins. Microspheres are prepared from degradable polymers such as

poly(lactide-co-glycolide) (PLG), polyanhydrides, poly (ortho esters), nonbiodegradable ethylvinyl acetate polymers, in which proteins are entrapped in the polymer (Gombotz et al., Bioconjugate Chem., 6:332 (1995); Ranade, "Role of Polymers in Drug Delivery", in Ranade et al., eds., Drug Delivery Systems, pp. 51-93, CRC Press (1995); Roskos et al., "Degradable Controlled Release Systems Useful for Protein Delivery", in Sanders et al., eds., Protein Delivery: Physical Systems, pp. 45-92, Plenum Press (1997); Bartus et al., Science, 281:1161 (1998); Putney et al., Nature Biotechnology, 16:153 (1998); Putney, Curr. Opin. Chem. Biol., 2:548 (1998)). Polyethylene glycol (PEG)-coated nanospheres can also provide carriers for intravenous administration of therapeutic proteins (see, for example, Gref et al., Pharm. Biotechnol., 10:167 (1996).

[00322] The formulation can also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Alternatively, or in addition, the composition can comprise an agent that enhances its function, such as, for example, a cytotoxic agent, cytokine, chemotherapeutic agent, or growth-inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

[00323] In one embodiment, an antibody, e.g., a bispecific antigen binding protein, of the invention is administered in combination therapy, i.e., combined with other agents, e.g., therapeutic agents, that are useful for treating pathological conditions or disorders, such as autoimmune disorders and inflammatory diseases. The term "in combination" in this context means that the agents are given substantially contemporaneously, either simultaneously or sequentially. If given sequentially, at the onset of administration of the second compound, the first of the two compounds is preferably still detectable at effective concentrations at the site of treatment.

[00324] For example, the combination therapy can include one or more an antibodies, e.g., bispecific antibodies, of the invention coformulated with, and/or coadministered with, one or more additional therapeutic agents, e.g., one or more cytokine and growth factor inhibitors, immunosuppressants, anti-inflammatory agents, metabolic inhibitors, enzyme inhibitors, and/or cytotoxic or cytostatic agents, as described in more detail below. Furthermore, one or more antibodies, e.g., bispecific antibodies, described herein may be used in combination with two or more of the therapeutic agents described herein. Such combination therapies may advantageously utilize lower dosages of the administered therapeutic agents, thus avoiding possible toxicities or complications associated with the various monotherapies.

[00325] Preferred therapeutic agents used in combination with an antibody, e.g., bispecific antigen binding protein, of the invention are those agents that interfere at different stages in an inflammatory response. In one embodiment, one or more antibodies, e.g., bispecific antibodies, described herein may be co-formulated with, and/or co-administered with, one or more additional agents such as other cytokine or growth factor antagonists (e.g., soluble receptors, peptide inhibitors, small molecules, ligand fusions); or antibodies or antigen binding fragments thereof that bind to other targets (e.g., antibodies that bind to other cytokines or growth factors, their receptors, or other cell surface molecules); and anti-inflammatory cytokines or agonists thereof. Non-limiting examples of the agents that can be used in combination with the antibodies described herein, include, but are not limited to, antagonists of one or more interleukins (ILs) or their receptors, e.g., antagonists of IL-1, IL-2, IL-6, IL-7, IL-8, IL-12, IL-13, IL-15, IL-16, IL17A-F, IL-18, IL-20, IL-21, IL-22, IL-25 and IL-31; antagonists of cytokines or growth factors or their receptors, such as, LT, EMAP-II, GM-CSF, FGF and PDGF. Antibodies of the invention can also be combined with inhibitors of e.g., antibodies to, cell surface molecules such as CD2, CD3, CD4, CD8, CD20 (e.g., the CD20 inhibitor rituximab (RITUXAN®), CD25, CD28, CD30, CD40, CD45, CD69, CD80 (B7.1), CD86 (B7.2), CD90, or their ligands, including CD154 (gp39 or CD40L), or LFA-1/ICAM-1 and VLA-4/VCAM-1 (Yusuf-Makagiansar et al., Med. Res. Rev., 22:146-167 (2002)). Preferred antagonists that can be used in combination with one or more antibodies, e.g., bispecific antibodies, described herein include antagonists of IL-1, IL-6, IL-12, TNF–α, IL-15, IL-18, IL-20, IL-22 and IL-31.

[00326] Examples of those agents include IL-12 antagonists, such as chimeric, humanized, human or in vitro-generated antibodies (or antigen binding fragments thereof) that bind to IL-12 (preferably human IL-12), e.g., the antibody disclosed in WO 00/56772; IL-12 receptor inhibitors, e.g., antibodies to human IL-12 receptor; and soluble fragments of the IL-12 receptor, e.g., human IL-12 receptor. Examples of IL-15 antagonists include antibodies (or antigen binding fragments thereof) against IL-15 or its receptor, e.g., chimeric, humanized, human or in vitro-generated antibodies to human IL-15 or its receptor, soluble fragments of the IL-15 receptor, and IL-15-binding proteins. Examples of IL-17 antagonists include brodalumab, secukinumab, and ixekizumab. Examples of IL-18 antagonists include antibodies, e.g., chimeric, humanized, human or in vitro-generated antibodies (or antigen binding fragments thereof), to human IL-18, soluble fragments of the IL-18 receptor, and IL-18 binding proteins (IL-18BP). Examples of IL-1 antagonists include Interleukin-1-converting enzyme (ICE)

inhibitors, such as Vx740, IL-1 antagonists, e.g., IL-1 RA (anakinra, Kineret®), sIL1RII, and anti-IL-1 receptor antibodies (or antigen binding fragments thereof).

[00327] In other embodiments, one or more antibodies, e.g., bispecific antibodies, described herein may be administered in combination with one or more of the following: IL-13 antagonists, e.g., soluble IL-13 receptors (sIL-13) and/or antibodies against IL-13; IL-2 antagonists, e.g., DAB 486-IL-2 and/or DAB 389-IL-2 (IL-2 fusion proteins, Seragen), and/or antibodies to IL-2R, e.g., anti-Tac (humanized anti-IL-2R, Protein Design Labs). Yet another combination includes one or more antibodies, e.g., bispecific antibodies, of the invention, antagonistic small molecules, and/or inhibitory antibodies in combination with nondepleting anti-CD4 inhibitors (DEC-CE9.1/SB 210396; non-depleting primatized anti-CD4 antibody; IDEC/SmithKline). Yet other preferred combinations include antagonists of the costimulatory pathway CD80 (B7.1) or CD86 (B7.2), including antibodies, soluble receptors or antagonistic ligands; as well as p-selectin glycoprotein ligand (PSGL), anti-inflammatory cytokines, e.g., IL-4 (DNAX/Schering); IL-10 (SCH 52000; recombinant IL-10 DNAX/Schering); IL-13 and TGF-beta, and agonists thereof (e.g., agonist antibodies).

[00328] In other embodiments, one or more antibodies, e.g., bispecific antibodies, of the invention can be co-formulated with, and/or co-administered with, one or more anti-inflammatory drugs, immunosuppressants, or metabolic or enzymatic inhibitors. Non-limiting examples of the drugs or inhibitors that can be used in combination with the antibodies described herein, include, but are not limited to, one or more of: nonsteroidal anti-inflammatory drug(s) (NSAIDs), e.g., ibuprofen, tenidap, naproxen, meloxicam, piroxicam, diclofenac, and indomethacin; sulfasalazine; corticosteroids such. as prednisolone; cytokine suppressive anti-inflammatory drug(s) (CSAIDs); inhibitors of nucleotide biosynthesis, e.g., inhibitors of purine biosynthesis, folate antagonists (e.g., methotrexate (N-[4-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-glutamic acid); and inhibitors of pyrimidine biosynthesis, e.g., dihydroorotate dehydrogenase (DHODH) inhibitors. Preferred therapeutic agents for use in combination with one or more antibodies, e.g., bispecific antibodies, of the invention include NSAIDs, CSAIDs, (DHODH) inhibitors (e.g., leflunomide), and folate antagonists (e.g., methotrexate).

[00329] Examples of additional inhibitors include one or more of: corticosteroids (oral, inhaled and local injection); immunosuppresants, e.g., cyclosporin, tacrolimus (FK-506); and mTOR inhibitors, e.g., sirolimus (rapamycin--RAPAMUNE® or rapamycin derivatives, e.g., soluble rapamycin derivatives (e.g., ester rapamycin derivatives, e.g., CCI-779); agents which interfere with signaling by proinflammatory cytokines such as IL-1 (e.g., IRAK, NIK, IKK, p38

or MAP kinase inhibitors); COX2 inhibitors, e.g., celecoxib, rofecoxib, and variants thereof; phosphodiesterase inhibitors, e.g., R973401 (phosphodiesterase Type IV inhibitor); phospholipase inhibitors, e.g., inhibitors of cytosolic phospholipase 2 (cPLA2) (e.g., trifluoromethyl ketone analogs); inhibitors of vascular endothelial cell growth factor or growth factor receptor, e.g., VEGF inhibitor and/or VEGF-R inhibitor; and inhibitors of angiogenesis. Preferred therapeutic agents for use in combination with the antibodies of the invention are immunosuppresants, e.g., cyclosporin, tacrolimus (FK-506); mTOR inhibitors, e.g., sirolimus (rapamycin) or rapamycin derivatives, e.g., soluble rapamycin derivatives (e.g., ester rapamycin derivatives, e.g., CCI-779); COX2 inhibitors, e.g., celecoxib and variants thereof; and phospholipase inhibitors, e.g., inhibitors of cytosolic phospholipase 2 (cPLA2), e.g., trifluoromethyl ketone analogs.

[00330] Additional examples of therapeutic agents that can be combined with an antibody, e.g., bispecific antigen binding protein, of the invention include one or more of: 6-mercaptopurines (6-MP); azathioprine sulphasalazine; mesalazine; olsalazine; chloroquine/hydroxychloroquine (PLAQUENIL®); pencillamine; aurothiornalate (intramuscular and oral); azathioprine; coichicine; beta-2 adrenoreceptor agonists (salbutamol, terbutaline, salmeteral); xanthines (theophylline, aminophylline); cromoglycate; nedocromil; ketotifen; ipratropium and oxitropium; mycophenolate mofetil; adenosine agonists; antithrombotic agents; complement inhibitors; and adrenergic agents.

[00331] Anti-TL1A antibodies of the invention may be combined with TNF–α antagonists for treatment of the same conditions as noted herein for anti-TL1A/anti-TNF-α bispecific antigen binding proteins. Such TNF–α antagonists include, for example, etanercept, adalimumab, infliximab, golimumab, and certolizumab pegol.

[00332] Non-limiting examples of agents for treating or preventing arthritic disorders (e.g., rheumatoid arthritis, inflammatory arthritis, rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis and psoriatic arthritis), with which an antibody, e.g., bispecific antigen binding protein, of the invention may be combined include one or more of the following: IL-12 antagonists as described herein; NSAIDs; CSAIDs; nondepleting anti-CD4 antibodies as described herein; IL-2 antagonists as described herein; anti-inflammatory cytokines, e.g., IL-4, IL-10, IL-13 and TGF–α, or agonists thereof; IL-1 or IL-1 receptor antagonists as described herein; phosphodiesterase inhibitors as described herein; Cox-2 inhibitors as described herein; iloprost: methotrexate; thalidomide and thalidomide-related drugs (e.g., Celgen); leflunomide; inhibitor of plasminogen activation, e.g., tranexamic acid; cytokine inhibitor, e.g., T-614;

prostaglandin E1; azathioprine; an inhibitor of interleukin-1 converting enzyme (ICE); zap-70 and/or 1 ck inhibitor (inhibitor of the tyrosine kinase zap-70 or 1 ck); an inhibitor of vascular endothelial cell growth factor or vascular endothelial cell growth factor receptor as described herein; an inhibitor of angiogenesis as described herein; corticosteroid anti-inflammatory drugs (e.g., SB203580); TNF–α -convertase inhibitors; IL-1; IL-13; IL-17 inhibitors; gold; penicillamine; chloroquine; hydroxychloroquine; chlorambucil; cyclophosphamide; cyclosporine; total lymphoid irradiation; antithymocyte globulin; CD5-toxins; orally administered peptides and collagen; lobenzarit disodium; cytokine regulating agents (CRAs) HP228 and HP466 (Houghten Pharmaceuticals, Inc.); ICAM-1 antisense phosphorothioate oligodeoxynucleotides (ISIS 2302; Isis Pharmaceuticals, Inc.); soluble complement receptor 1 (TP 10; T Cell Sciences, Inc.); prednisone; orgotein; glycosaminoglycan polysulphate; minocycline (MINOCIN®); anti-IL2R antibodies; marine and botanical lipids (fish and plant seed fatty acids); auranofin; phenylbutazone; meclofenamic acid; flufenamic acid; intravenous immune globulin; zileuton; mycophenolic acid (RS-61443); tacrolimus (FK-506); sirolimus (rapamycin); amiprilose (therafectin); cladribine (2-chlorodeoxyadenosine); and azaribine. Preferred combinations include one or more antibodies, e.g., bispecific antibodies, of the invention in combination with methotrexate or leflunomide, and in moderate or severe rheumatoid arthritis cases, cyclosporine.

[00333] Preferred examples of inhibitors to use in combination with one or more antigen binding proteins, e.g., bispecific antigen binding proteins, of the invention to treat arthritic disorders include antagonists of IL-12, IL-15, IL-18, IL-22; T cell and B cell-depleting agents (e.g., anti-CD4 or anti-CD22 antibodies); small molecule inhibitors, e.g., methotrexate and leflunomide; sirolimus (rapamycin) and analogs thereof, e.g., CCI-779; cox-2 and cPLA2 inhibitors; NSAIDs; p38 inhibitors, TPL-2, Mk-2 and NFκB inhibitors; RAGE or soluble RAGE; P-selectin or PSGL-1 inhibitors (e.g., small molecule inhibitors, antibodies to PSGL-1, antibodies to P-selectin); estrogen receptor beta (ERB) agonists or ERB-NFκB antagonists. Most preferred additional therapeutic agents that can be co-administered and/or co-formulated with one or more antibodies, e.g., bispecific antibodies, of the invention include one or more of: methotrexate, leflunomide, or a sirolimus (rapamycin) or an analog thereof, e.g., CCI-779.

[00334] Non-limiting examples of agents for treating or preventing an inflammatory disease or disorder, (e.g., IBD, CD, UC, IBS) with which an antibody, e.g., bispecific antigen binding protein, of the invention can be combined include the following: budenoside; epidermal growth factor; corticosteroids; cyclosporine; sulfasalazine; aminosalicylates; 6-mercaptopurine;

azathioprine; metronidazole; lipoxygenase inhibitors; mesalamine; olsalazine; balsalazide; antioxidants; thromboxane inhibitors; IL-1 receptor antagonists; anti-IL-1 monoclonal antibodies; anti-IL-6 monoclonal antibodies (e.g., anti-IL-6 receptor antibodies and anti-IL-6 antibodies); growth factors; elastase inhibitors; pyridinyl-imidazole compounds; IL-4, IL-10, IL-13 and/or TGF.beta. cytokines or agonists thereof (e.g., agonist antibodies); IL-11; glucuronide- or dextran-conjugated prodrugs of prednisolone, dexamethasone or budesonide; ICAM-1 antisense phosphorothioate oligodeoxynucleotides (ISIS 2302; Isis Pharmaceuticals, Inc.); soluble complement receptor 1 (TP10; T Cell Sciences, Inc.); slow-release mesalazine; methotrexate; antagonists of platelet activating factor (PAF); ciprofloxacin; and lignocaine.

[00335] Non-limiting examples of agents for treating or preventing multiple sclerosis with one or more antibodies, e.g., bispecific antibodies, of the invention can be combined include the following: interferons, e.g., interferon–α (e.g., AVONEX®, Biogen) and interferon-1b (BETASERON®, Chiron/Berlex); Copolymer 1 (Cop-1; COPAXONE®, Teva Pharmaceutical Industries, Inc.); dimethyl fumarate (e.g., BG-12; Biogen); hyperbaric oxygen; intravenous immunoglobulin; cladribine; corticosteroids; prednisolone; methylprednisolone; azathioprine; cyclophosphamide; cyclosporine; cyclosporine A, methotrexate; 4-aminopyridine; and tizanidine. Additional antagonists that can be used in combination with antibodies of the invention include antibodies to or antagonists of other human cytokines or growth factors, for example, LT, IL-1, IL-2, IL-6, EL-7, IL-8, IL-12 IL-15, IL-16, IL-18, EMAP-11, GM-CSF, FGF, and PDGF. Antibodies as described herein can be combined with antibodies to cell surface molecules such as CD2, CD3, CD4, CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD80, CD86, CD90 or their ligands. One or more antibodies, e.g., bispecific antibodies, of the invention may also be combined with agents, such as methotrexate, cyclosporine, FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, for example, ibuprofen, corticosteroids such as prednisolone, phosphodiesterase inhibitors, adenosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, agents which interfere with signaling by proinflammatory cytokines as described herein, IL-1b converting enzyme inhibitors (e.g., Vx740), anti-P7s, PSGL, TACE inhibitors, T-cell signaling inhibitors such as kinase inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors and derivatives thereof, as described herein, and anti-inflammatory cytokines (e.g., IL-4, IL-10, IL-13 and TGF).

[00336] Preferred examples of therapeutic agents for multiple sclerosis with which the antibodies of the invention can be combined include dimethyl fumarate (e.g., BG-12; Biogen),

interferon-beta, for example, IFN-β-1a and IFN-β-1b; COPAXONE®, corticosteroids, IL-1 inhibitors, antibodies to CD40 ligand and CD80, IL-12 antagonists.

[00337] Non-limiting examples of agents for treating or preventing psoriasis with which an antibody, e.g., bispecific antigen binding protein, of the invention can be combined include the following: corticosteroids; vitamin. D_3 and analogs thereof; retinoiods (e.g., soriatane); methotrexate; cyclosporine, 6-thioguanine; Accutane; hydrea; hydroxyurea; sulfasalazine; mycophenolate mofetil; azathioprine; tacrolimus; fumaric acid esters; biologics such as AMEVIVE®, Raptiva ustekinumab, and XP-828L; phototherapy; and photochemotherapy (e.g., psoralen and ultraviolet phototherapy combined).

[00338] Non-limiting examples of agents for treating or preventing inflammatory airway/respiratory disease (e.g., chronic obstructive pulmonary disorder, asthma) with which an antibody, e.g., bispecific antigen binding protein, of the invention can be combined include the following: beta2-adrenoceptor agonists (e.g., salbutamol (albuterol), levalbuterol, terbutaline, bitolterol); long-acting beta2-adrenoceptor agonists (e.g., salmeterol, formoterol, bambuterol); adrenergic agonists (e.g., inhaled epinephrine and ephedrine tablets); anticholinergic medications (e.g., ipratropium bromide); combinations of inhaled steroids and long-acting bronchodilators (e.g., fluticasone/salmeterol (ADVAIR® in the United States, and Seretide in the United Kingdom)) or. budesonide/formoterol (SYMBICORT®)); inhaled glucocorticoids (e.g., ciclesonide, beclomethasone, budesonide, flunisolide, fluticasone, mometasone, triamcinolone); leukotriene modifiers (e.g., montelukast, zafirlukast, pranlukast, and zileuton); mast cell stabilizers (e.g., cromoglicate (cromolyn), and nedocromil); antimuscarinics/anticholinergics (e.g., ipratropium, oxitropium, tiotropium); methylxanthines (e.g., theophylline, aminophylline); antihistamines; IgE blockers (e.g., omalizumab); M_3 muscarinic antagonists (anticholinergics) (e.g., ipratropium, tiotropium); cromones (e.g., chromoglicate, nedocromil); zanthines (e.g., theophylline); IL-17 inhibitors (e.g., brodalumab, secukinumab, ixekizumab), IL-4 inhibitors; and IL-13 inhibitors.

[00339] In one embodiment, an antibody, e.g., bispecific antigen binding protein, of the invention can be used in combination with one or more antibodies directed at other targets involved in regulating immune responses, e.g., transplant rejection.

[00340] Non-limiting examples of agents for treating or preventing immune responses with which an antibody, e.g., bispecific antigen binding protein, of the invention can be combined include the following: antibodies against other cell surface molecules, including but not limited to CD25 (interleukin-2 receptor–α), CD11a (LFA-1), CD54 (ICAM-1), CD4, CD45, CD28/CTLA4 (CD80 (B7.1), e.g., CTLA4 Ig (abatacept , ORENCIA®), ICOSL, ICOS

and/or CD86 (B7.2). In yet another embodiment, an antibody of the invention is used in combination with one or more general immunosuppressive agents, such as cyclosporin A or FK506.

[00341] In other embodiments, antibodies are used as vaccine adjuvants against autoimmune disorders, inflammatory diseases, etc. The combination of adjuvants for treatment of these types of disorders are suitable for use in combination with a wide variety of antigens from targeted self-antigens, i.e., autoantigens, involved in autoimmunity, e.g., myelin basic protein; inflammatory self-antigens (e.g., amyloid peptide protein) or transplant antigens (e.g., alloantigens). The antigen may comprise peptides or polypeptides derived from proteins, as well as fragments of any of the following: saccharides, proteins, polynucleotides or oligonucleotides, autoantigens, amyloid peptide protein, transplant antigens, allergens, or other macromolecular components. In some instances, more than one antigen is included in the antigenic composition.

[00342] For example, desirable vaccines for moderating responses to allergens in a vertebrate host, which contain the adjuvant combinations of this invention, include those containing an allergen or fragment thereof. Examples of such allergens are described in U.S. Pat. No. 5,830,877 and PCT Publication No. WO 99/51259, which are hereby incorporated by reference in their entireties, and include pollen, insect venoms, animal dander, fungal spores and drugs (such as penicillin). The vaccines interfere with the production of IgE antibodies, a known cause of allergic reactions. In another example, desirable vaccines for preventing or treating disease characterized by amyloid deposition in a vertebrate host, which contain the adjuvant combinations of this invention, include those containing portions of amyloid peptide protein (APP). This disease is referred to variously as Alzheimer's disease, amyloidosis or amyloidogenic disease. Thus, the vaccines of this invention include the adjuvant combinations of this invention plus A.beta. peptide, as well as fragments of Aβ peptide and antibodies to Aβ peptide or fragments thereof.

[00343] In another embodiment, pharmaceutical compositions may be supplied as a kit comprising a container that comprises an antibody, bispecific antigen binding protein or antigen-binding fragment of the invention. Antibodies, e.g., bispecific antibodies, of the invention can be provided in the form of an injectable solution for single or multiple doses, or as a sterile powder that will be reconstituted before injection. Alternatively, such a kit can include a dry-powder disperser, liquid aerosol generator, or nebulizer for administration of the antigen binding protein (e.g., anti-TL1A/anti-TNF-α bispecific antigen binding protein). Such a kit may further comprise written information on indications and usage of the pharmaceutical

composition. Moreover, such information may include a statement that the antibody composition is contraindicated in patients with known hypersensitivity to TL1A and TNF–α .

[00344] In a further embodiment, the invention provides an article of manufacture, comprising: (a) a composition of matter comprising an antibody, bispecific antigen binding protein or antigen-binding fragment as described herein; (b) a container containing said composition; and (c) a label affixed to said container, or a package insert included in said container referring to the use of said antibody in the treatment of an immune related disease.

[00345] In another aspect, the composition comprises a further active ingredient, which may, for example, be a further antibody or an anti-inflammatory, cytotoxic or chemotherapeutic agent. Preferably, the composition is sterile.

[00346] The antibodies, e.g., bispecific antibodies, as described herein are also useful to prepare medicines and medicaments for the treatment of immune-related and inflammatory diseases, including for example, IBS, IBD, CD, UC. In a specific aspect, such medicines and medicaments comprise a therapeutically effective amount of a bispecific antigen binding protein, antibody or antigen-binding fragment of the invention with a pharmaceutically acceptable carrier. In an embodiment, the admixture is sterile.

[00347] The bispecific antigen binding proteins of the invention are useful for detecting TL1A and/or TNF–α in biological samples and identification of cells or tissues that express the TL1A receptor DR3 and/or TNF–α receptor. For instance, the bispecific antigen binding proteins can be used in diagnostic assays, *e.g.*, binding assays to detect and/or quantify TL1A and/or TNF–α binding and/or expression in a tissue or cell. In addition, the bispecific antigen binding proteins described herein can be used to inhibit TL1A from forming a complex with its receptor DR3, thereby modulating the biological activity of TL1A or DR3 in a cell or tissue. Likewise, the bispecific antigen binding proteins described herein can be used to inhibit TNF-α from forming a complex with its receptor, thereby modulating the biological activity of TNF-α or its receptor in a cell or tissue. Exemplary activity that can be modulated includes, but is not limited to, inhibiting and/or reducing inflammation.

[00348] The bispecific antigen binding proteins described herein can be used for diagnostic purposes to detect, diagnose, or monitor diseases and/or conditions associated with TL1A and/or TNF-α. Also provided are methods for the detection of the presence of TL1A and/or TNF-α in a sample using classical immunohistological methods known to those of skill in the art. See, for example, Tijssen (1993), Practice and Theory of Enzyme Immunoassays, Vol 15 (Eds R.H. Burdon and P.H. van Knippenberg, Elsevier, Amsterdam); Zola (1987),

Monoclonal Antibodies: A Manual of Techniques, pp. 147-158 (CRC Press, Inc.); Jalkanen et al. (1985), J. Cell. Biol. 101:976-985; Jalkanen et al. (1987), J. Cell Biol. 105:3087-3096. The detection of either TL1A and/or TNF-α can be performed *in vivo* or *in vitro*.

[00349] Diagnostic applications provided herein include use of the antigen binding proteins to detect expression of TL1A and/or TNF-α and binding of these ligands to their receptors. Examples of methods useful in the detection of the presence of the ligand include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA).

[00350] For diagnostic applications, the antigen binding protein typically will be labeled with a detectable labeling group. Suitable labeling groups include, but are not limited to, the following: radioisotopes or radionuclides (e.g., ^{3}H, ^{14}C, ^{15}N, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I), fluorescent groups (e.g., FITC, rhodamine, lanthanide phosphors), enzymatic groups (e.g., horseradish peroxidase, β-galactosidase, luciferase, alkaline phosphatase), chemiluminescent groups, biotinyl groups, or predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In some embodiments, the labeling group is coupled to the antigen binding protein via spacer arms of various lengths to reduce potential steric hindrance. Various methods for labeling proteins are known in the art and may be used.

[00351] In another embodiment, the bispecific antigen binding protein described herein can be used to identify a cell or cells that express TL1A and/or TNF-α. In a specific embodiment, the antigen binding protein is labeled with a labeling group and the binding of the labeled antigen binding protein to TL1A and/or TNF-α is detected. In a further specific embodiment, the binding of the antigen binding protein to TL1A and/or TNF-α is detected in vivo. In a further specific embodiment, the bispecific antigen binding protein is isolated and measured using techniques known in the art. See, for example, Harlow and Lane, 1988, Antibodies: A Laboratory Manual, New York: Cold Spring Harbor (ed. 1991 and periodic supplements); John E. Coligan, ed., 1993, Current Protocols In Immunology New York: John Wiley & Sons.

[00352] Another aspect of the invention provides for detecting the presence of a test molecule that competes for binding to TL1A and/or TNF-α with the antigen binding proteins described herein. An example of one such assay would involve detecting the amount of free antigen binding protein in a solution containing an amount of TL1A and/or TNF-α in the presence or absence of the test molecule. An increase in the amount of free antigen binding

protein (i.e., the antigen binding protein not bound to TL1A and/or TNF-α) would indicate that the test molecule is capable of competing for TL1A and/or TNF-α binding with the bispecific antigen binding protein. In one embodiment, the antigen binding protein is labeled with a labeling group. Alternatively, the test molecule is labeled and the amount of free test molecule is monitored in the presence and absence of the antigen binding protein.

WORKING EXAMPLES

[00353] The invention is further illuminated by the following working examples, which exemplify but do not limit the scope of the invention

EXAMPLE 1

Preparation of XenoMouse® anti-TL1A monoclonal antibodies

Mouse strains

[00354] Fully human antibodies to human TL1A were generated by immunizing XENOMOUSE® transgenic mice. U.S. Pat. Nos. 6,114,598; 6,162,963;6,833,268; 7,049,426; 7,064,244, which are incorporated herein by reference in their entirety; Green et al. (1994), Nature Genetics 7:13-21; Mendez et al. (1997), Nature Genetics 15:146-156; Green and Jakobovitis (1998), J. Ex. Med, 188:483-495; Kellerman and Green (2002), Current Opinion in Biotechnology 13, 593-597; each of which is incorporated by reference in its entirety. Animals from the XMG1-KL, XMG2-K, XMG2-K/Balbc, XMG2-KL, XMG4-K and XMG4-KL XENOMOUSE® strains were used for all immunizations.

Generation of TL1A Immunogen

[00355] TL1A polypeptides containing the N-terminal His tag (H6) of which the first 22 amino acids are the VK1 signal peptide) were generated by transiently transfecting 293HEK cells with the corresponding cDNAs. The commonly used polyHis tag was employed to facilitate detection and subsequent purification.

[00356] 293-6E cells at $9.48x10^5$ cells/ml were transfected with 0.5 mg/L DNA (0.1 mg/L His-TL1A in pTT5 vector with 0.4 mg/L empty pTT5 vector) (Durocher et al. (2002) NRCC, Nucleic Acids. Res. 30, e9) with 3 ml PEI/mg DNA in FreeStyle 293 media (Invitrogen). Tryptone N1 was added to cultures 1 hour after transfection. Cells were grown in suspension in

FreeStyle 293 expression medium supplemented with 0.1% Pluronic F68 and 50 μg/ml Geneticin for 7 days and harvested for purification.

EXAMPLE 2

Generation of TL1A

[00357] TL1A polypeptides containing the N-terminal His tag (H6) of which the first 22 amino acids are the VK1 signal peptide) were generated by transiently transfecting 293HEK cells with the corresponding cDNAs. The commonly used polyHis tag was employed to facilitate detection and subsequent purification.

[00358] 293-6E cells at 9.48×10^5 cells/ml were transfected with 0.5mg/L DNA (0.1mg/L His-TL1A in pTT5 vector with 0.4mg/L empty pTT5 vector) (Durocher et al. NRCC, Nucleic Acids. Res. (2002) 30, e9) with 3ml PEI/mg DNA in FreeStyle 293 media (Invitrogen). Tryptone N1 was added to cultures 1 hour after transfection. Cells were grown in suspension in FreeStyle 293 expression medium supplemented with 0.1% Pluronic F68 and 50 μg/ml Geneticin for 7 days and harvested for purification.

Immunizations

Immunizations are conducted using one or more suitable forms of TL1A antigen, including recombinant human TL1A expressed on cells and recombinant human TL1A soluble protein or combinations thereof.

[00359] A suitable amount of immunogen (i.e., 10 μg of protein delivered by injection to the abdomen) is used for initial immunization in XenoMouse®. Following the initial immunization, subsequent boost immunizations of immunogen (i.e., 2 x 10 e6 cells or 5 μg of protein) are administered on a schedule and for the duration necessary to induce a suitable titer of anti-TL1A antibody in the mice. Titers are determined by any suitable method, for example, enzyme immunoassay or fluorescence activated cell sorting (FACS).

[00360] Multiple immunogens and routes of immunization were used to generate anti-human TL1A immune responses. For genetic immunizations, mice were immunized 12-16 times over 6-8 weeks using the Helios Gene Gun system according to the manufacturer's instructions (BioRad, Hercules, California). Briefly, expression vectors encoding wild type human or cynomolgus TL1A were coated onto gold beads (BioRad, Hercules, California) and delivered to the epidermis of a shaved mouse abdomen. For cell-based immunizations, mice were immunized with a suspension-adapted CHO-K1 cell line (Invitrogen, Carlsbad, California), stably transfected with an expression vector encoding human TL1A. Animals were immunized with cells mixed with Alum prepared from aluminum potassium sulfate (EMD Chemicals Inc.,

Gibbstown, NJ) and CpG-ODN (Eurofins MWG Operon LLC, Huntsville, AL) 10-12 times over 6-8 weeks using a protocol that alternated between subcutaneous and intraperitoneal injections. The initial boost was comprised of $4x10^6$ cells while subsequent boosts contained $2x10^6$ cells. For soluble, recombinant protein immunizations, mice were immunized with a 6x His-tagged, trimeric form of the human and cynomolgus TL1A extracellular domain (amino acids 72-251 of the TL1A sequences). Animals were immunized with recombinant protein mixed with Alum and CpG-ODN, 8-12 times over 4-8 weeks using sub-cutaneous injections. The initial boost was comprised of 10 μg while subsequent boosts contained 5 μg. Human TL1A-specific serum titers were monitored by live-cell FACS analysis on an Accuri or FacsCalibur (BD Biosciences) flow cytometer. Animals with the highest antigen-specific serum titers directed against human and cynomolgus TL1A were sacrificed and used for hybridoma generation (Kohler and Milstein, 1975).

EXAMPLE 3

Preparation of Monoclonal Antibodies

Hybridoma Generation

[00361] Animals exhibiting suitable titers are identified, and lymphocytes are obtained from draining lymph nodes and, if necessary, pooled for each cohort. Pooled lymphocytes (from each immunization cohort) were dissociated from lymphoid tissue by grinding in a suitable medium (for example, Dulbecco's Modified Eagle Medium (DMEM); Invitrogen, Carlsbad, CA).. B cells may be selected and/or expanded using a suitable method, and fused with suitable fusion partner; for example, non-secretory myeloma P3X63Ag8.653 cells (American Type Culture Collection CRL 1580; Kearney et al, J. Immunol. 123, 1979, 1548-1550), using techniques that are known in the art. B cells were selected and/or expanded using standard methods, and fused with a suitable fusion partner using techniques that were known in the art.

[00362] In one suitable fusion method, lymphocytes are mixed with fusion partner cells at a ratio of 1:4. The cell mixture is gently pelleted by centrifugation at 400 x g for 4 minutes, the supernatant decanted, and the cell mixture gently mixed (for example, by using a 1 ml pipette). Fusion is induced with PEG/DMSO (polyethylene glycol/dimethyl sulfoxide; obtainable from Sigma-Aldrich, St. Louis MO; 1 ml per million lymphocytes). PEG/DMSO is slowly added with gentle agitation over one minute followed, by one minute of mixing. IDMEM (DMEM without glutamine; 2 ml per million B cells), is then added over 2 minutes with gentle agitation, followed by additional IDMEM (8 ml per million B-cells) which is added over 3 minutes.

[00363] The fused cells are gently pelleted (400 x g, 6 minutes) and resuspended in 20 ml Selection media (for example, DMEM containing Azaserine and Hypoxanthine [HA] and other supplemental materials as necessary) per million B-cells. Cells are incubated for 20-30 minutes at 37 C and then resuspended in 200 ml Selection media and cultured for three to four days in T175 flasks prior to 96-well plating.

[00364] Cells are distributed into 96-well plates using standard techniques to maximize clonality of the resulting colonies. After several days of culture, supernatants are collected and subjected to screening assays as detailed in the examples below, including confirmation of binding to human TL1A, evaluation of cross-reactivity with other species' TL1A (for example, cynomologous monkey TL1A), and ability to inhibit the activity of TL1A. Positive cells are further selected and subjected to standard cloning and subcloning techniques. Clonal lines may be expanded in vitro, and the secreted human antibodies obtained for analysis.

[00365] In this manner, mice were immunized with recombinant human TL1A soluble protein for a total of 15 immunizations over a period of approximately 2 months; several hybridoma cell lines secreting TL1A-specific antibodies were obtained, and the antibodies were further characterized. The sequences thereof are presented in the Sequence Listing and in Tables A, C and D and results of various tests using these antibodies are shown herein.

[00366] Tables A to E herein show the sequences of anti-TL1A antibodies prepared in accordance with this working example.

EXAMPLE 4

Antigen Enrichment of Hybridoma Pools

[00367] Fused hybridoma pools from select immune tissue harvest were used as a source of material for FACS-based enrichments. To enrich for hybridomas expressing antibodies specific to native (full length, on-cell) human TL1A membranes were prepared from 293T cells transiently expressing the TL1A cDNA construct. 24 hours after transfection using 293Fectin™ (ThermoFisher Scientific Inc.) cells were biotinylated with E-Z link NHS-LC-LC- Biotin according to the manufacturer's recommendation (ThermoFisher Scientific Inc.). After biotinylation, cells were homogenized with a needle and syringe to form membrane fragments and referred to as "membrane preps". The biotinylated membrane preps were then used to detect hybridomas expressing surface antibodies specific to the target of interest via standard biotin-streptavidin chemistry.

[00368] To enrich hybridoma pools for the antigen of interest, they were first incubated with the membrane prep probe. Unbound probe was then washed away and the antigen-specific hybridomas were identified by simultaneous detection of surface IgG (with an Alexa 488 conjugated Gt anti-human Fc secondary antibody; Jackson ImmunoResearch) and the biotinylated membrane prep TL1A probe (Alexa Fluor 647 conjugated streptavidin; Jackson ImmunoResearch). Hybridomas expressing surface IgG and binding antigen were detected by FACS analysis on an Accuri flow cytometer. Dual positive events were sorted as single cells into 384-well plates on a FACS Aria cell sorter (BD Biosciences). After several days of culture, the hybridoma supernatants containing monoclonal antibodies were collected and used in the screening assays described in the examples below.

EXAMPLE 5

Initial Selection of TL1A-Specific Binding Antibodies

[00369] Human TL1A was expressed on host Human Embryonic Kidney 293 cells by transfection using an expression vector expressing huTL1A cDNA, Gibco™ Opti-MEM® media (Gibco, Cat. No. 31985088) and 293Fectin™ reagent (Invitrogen, Cat. No. 12347019) following the protocol set out by the manufacturer. Hybridoma supernatants were screened for the presence of huTL1A-specific monoclonal antibodies using the FMAT 8200 Screening System (Molecular Devices) and the CellInsight™ High Content Imaging Platform (ThermoFisher Scientific). The number of huTL1A positive wells (i.e., those that have signal over irrelevant hybridoma supernatant) is presented in Table 5.1. For CellInsight screens, 15 µl/well of hybridoma supernatant (and positive and negative controls) were added to black, 384-well, clear bottom plates (Corning Can. No. 3712) followed by the addition of 30 µl/well of a mixture of TL1A/293T cells, nuclear Hoescht stain (Pierce, Cat. No. 62249) and Alexa 488-goat anti-human IgG (H+L) (Jackson, Cat. No. 109-545-088). After 3 hours of incubation at room temperature, plates were washed 2 times on an AquaMax 4000 plate washer (fitted with a 384-well cell wash head) and read on the CellInsight instrument according to the manufacturer's recommendations. For FMAT-based screens, 20 µl/well of hybridoma supernatant (and positive and negative controls) were added to black, 384-well, clear bottom plates followed by addition of 40 µl/well of a mixture of TL1A/293T cells, 293T parental cells and Cy5-goat anti-human IgG (Fc) (Jackson, Cat. No. 109-075). After 3 hours of incubation at room temperature, plates were read on the FMAT 8200 system according to the manufacturer's recommendations.

[00370] Table 5.1: TL1A-specific antibodies selected

Harvest #	Platform	Total # Positive
1	FMAT 8200	1488
2	FMAT 8200	900
5	Cellinsight	1830
6	FMAT 8200	438
7&8	Cellinsight	1541

EXAMPLE 6

Identification of TL1A Receptor-Ligand Blocking Antibodies

[00371] Biotinylated huTL1A was prepared by reacting 100 μg/ml of NHS LC LC biotin (Pierce, Cat. No. 21338) and 100 μg huTL1A (prepared as described in Example 2) in 1 ml of PBS pH 8.5 for 1hr at room temperature. Un-reacted biotin was removed by ultra-filtration using a 5 kDa Amicon Ultra spin column (Millipore, Cat. No. UFC8 005). Hybridoma supernatants containing huTL1A-binding antibodies were assayed for their ability to block huTL1A binding to human Death Receptor 3 (huDR3) via an ELISA-based receptor-ligand assay. ELISA plates (Corning Cat. No. 3702) were coated with 40 μl/well of human DR3-Fc chimera (1 μg/ml) (R&D Systems, Cat. No. 943-D3) in coating buffer (1x PBS/0.05% azide), then incubated overnight at 4°C. Plates were then washed with water 3 times and blocked with 90 μl diluent (1x PBS/1% milk) for 30 min at room temperature. 15 μl of anti-TL1A hybridoma supernatant was preincubated with 45 μl of biotinylated TL1A (30 ng/ml final) in 96-well storage plates (Sigma, Cat. No. P6866) in assay diluent for 2 hr at room temperature prior to adding to the pre-blocked DR3 ELISA plates. Assay plates were then incubated for 1 hr at room temperature. Sample plates were subsequently washed 3 times followed by the addition of 40 μl/well of streptavidin-HRP (Pierce, Cat. No. 21126) and another 1 hr incubation at room temperature. Plates were washed an additional three times and 40 μl TMB (Neogen, Cat. No. 308177) was added. The TMB reaction was incubated for 30 min at room temperature and then quenched with 40 μl/well of 1 N hydrochloric acid. Finally, plates were read on an ELISA plate reader at a wavelength of 450 nm. Cutoffs were set at < 38% of signal of negative control, irrelevant ESNs (exhausted supernatants). The numbers of samples able to block the huTL1A-DR3 interaction (as defined by this cutoff) are indicated in Table 6.1.

[00372] Table 6.1: Selected TL1A/DR3 blockers

Harvest #	Total # TL1A/DR3 blockers
1	208
2	39

EXAMPLE 7

TL1A functional blocking assays

[00373] In order to screen for hybridomas capable of blocking TL1A functional activity, IFNγ release from primary T cells or a NF-κB reporter assay in TF1 cells were employed. For the IFNγ release assay, purified primary human T cells (Biological Speciality Corp.,Cat. # 215-01-10) were stimulated with soluble human TL1A in the presence or absence of hybridoma supernatants specific to TL1A. 2 x 10^5 primary human T cells were stimulated with 8 – 16 ng/mL human TL1A (Amgen), 1 – 2 ng/mL IL-12 (Peprotech) and 0.5 – 1 ng/mL IL-18 (R&D Systems) in the presence of hybridoma supernatants containing anti-TL1A antibodies in 96-well round bottom plate at 37 °C for 72 hours. Culture supernatants were then tested for IFNγ level by ELISA according to the manufacturer's instructions (R&D Systems). For the TL1A responsive reporter assay, a TF-1 NF-κB reporter cell line (Amgen) was stimulated with soluble or membrane-bound human TL1A or soluble cynomolgus monkey TL1A. 0.2 – 3 nM or 2 – 20 nM of soluble human or cynomolgus monkey TL1A (respectively) was incubated with 10^4 – 10^5 TF-1 NF-κB reporter cells in the presence of serially diluted hybridoma supernatants (or controls) in 96 or 384-well plates at 37 °C overnight. For testing samples against membrane-bound TL1A, activity assays were performed by co-culturing the TF-1 NF-κB reporter cell line with human TL1A-expressing AM1D cells. 10^5 TF-1 NF-κB reporter cells and 10^3 AM1D cells were co-cultured in the presence of 5 ug/mL of anti-TL1A antibody (or controls) in a 384-well plate at 37 °C overnight. Reporter signal in each well was determine using the Steady-Glo Luciferase Assay System according to the manufacturer's recommendation (Promega).

EXAMPLE 8

Molecular Rescue and Sequencing of TL1A Receptor-Ligand Blocking Antibodies

[00374] RNA (total or mRNA) was purified from wells containing the TL1A-neutralizing antibody-producing hybridoma cells using a Qiagen RNeasy mini or the Invitrogen mRNA catcher plus kit. Purified RNA was used to amplify the antibody heavy and light chain variable region (V) genes using cDNA synthesis via reverse transcription, followed by a polymerase chain reaction (RT-PCR). The fully human antibody gamma heavy chain was obtained using the Qiagen One Step Reverse Transcriptase PCR kit (Qiagen). This method was used to generate the first strand cDNA from the RNA template and then to amplify the variable region of the gamma heavy chain using multiplex PCR (see Table 8.1 for the complete primer

list, SEQ ID NOS: 1191 to 1252, respectively). The 5' gamma chain-specific primer annealed to the signal sequence of the antibody heavy chain, while the 3' primer annealed to a region of the gamma constant domain. The fully human kappa light chain was obtained using the Qiagen One Step Reverse Transcriptase PCR kit (Qiagen). This method was used to generate the first strand cDNA from the RNA template and then to amplify the variable region of the kappa light chain using multiplex PCR. The 5' kappa light chain-specific primer annealed to the signal sequence of the antibody light chain while the 3' primer annealed to a region of the kappa constant domain. The fully human lambda light chain was obtained using the Qiagen One Step Reverse Transcriptase PCR kit (Qiagen). This method was used to generate the first strand cDNA from the RNA template and then to amplify the variable region of the lambda light chain using multiplex PCR. The 5' lambda light chain-specific primer annealed to the signal sequence of light chain while the 3' primer annealed to a region of the lambda constant domain.

[00375] The amplified cDNA was purified enzymatically using exonuclease I and alkaline phosphatase and the purified PCR product was sequenced directly. Amino acid sequences were deduced from the corresponding nucleic acid sequences bioinformatically. Two additional, independent RT-PCR amplification and sequencing cycles were completed for each hybridoma sample in order to confirm that any mutations observed were not a consequence of the PCR. The derived amino acid sequences were then analyzed to determine the germline sequence origin of the antibodies and to identify deviations from the germline sequence. The amino acid sequences corresponding to CDRs of the sequenced antibodies were aligned and these alignments were used to group the clones by similarity.

[00376] Table 8.1: Multiplex primers used to amplify antibody V genes.

Heavy chain	Sequence 5' to 3'	SEQ ID NO.
5' primer	C ACC ATG GAC TGG ACC TGG AGG ATC	1191
	C ACC ATG GAC TGG ACC TGG AGC ATC	1192
	C ACC ATG GAC TGC ACC TGG AGG ATC	1193
	C ACC ATG GAC TGG ACC TGG AGA ATC	1194
	C ACC ATG GAC TGG ACC TGG AGG G	1195
	C ACC ATG GAC TGG ATT TGG AGG ATC C	1196
	C ACC ATG GAC ACA CTT TGC TCC ACG	1197
	C ACC ATG GAC ACA CTT TGC TAC ACA CTC C	1198
	C ACC ATG GAG TTT GGG CTG AGC TG	1199
	C ACC ATG GAA TTG GGG CTG AGC TG	1200
	C ACC ATG GAG TTG GGG CTG AGC TG	1201
	C ACC ATG GAA CTG GGG CTC CGC	1202
	C ACC ATG GAA TTT GGG CTG AGC TGG	1203
	C ACC ATG GAG TTG GGG CTG TGC TG	1204
	C ACC ATG GAG TTT GGG CTT AGC TGG	1205
	C ACC ATG GAG TTT TGG CTG AGC TGG	1206
	C ACC ATG AAA CAC CTG TGG TTC TTC CTC	1207
	C ACC ATG AAG CAC CTG TGG TTC TTC C	1208
	C ACC ATG AAA CAT CTG TGG TTC TTC CTT CTC	1209
	C ACC ATG GGG TCA ACC GCC ATC C	1210
	C ACC ATG TCT GTC TCC TTC CTC ATC TTC	1211
3' primer	GCTGAGGGAGTAGAGTCCTGAGGACTGT	1212

Kappa chain	Sequence 5' to 3'	SEQ ID NO.
5' primer	C ACC ATG GAC ATG AGG GTC CCC G	1213
	C ACC ATG GAC ATG AGG GTC CCT GC	1214
	C ACC ATG GAC ATG AGG GTC CTC GC	1215
	C ACC ATG AGG CTC CCT GCT CAG C	1216
	C ACC ATG AGG CTC CTT GCT CAG CTT C	1217
	C ACC ATG GAA ACC CCA GCG CAG C	1218
	C ACC ATG GAA GCC CCA GCG CAG	1219
	C ACC ATG GAA GCC CCA GCT CAG	1220
	C ACC ATG GAA CCA TGG AAG CCC CAG	1221
	C ACC ATG GTG TTG CAG ACC CAG GTC	1222
	C ACC ATG GGG TCC CAG GTT CAC C	1223
	C ACC ATG TTG CCA TCA CAA CTC ATT GGG	1224
	C ACC ATG GTG TCC CCG TTG CAA TTC	1225
3' primer	ACCCGATTGGAGGGCGTTATCCACC	1226

Lambda chain	Sequence 5' to 3'	SEQ ID NO.
5' primer	C ACC ATG GCC TGG TCC CCT CTC	1227
	C ACC ATG GCC TGG TCT CCT CTC C	1228
	C ACC ATG GCC AGC TTC CCT CTC C	1229
	C ACC ATG GCC GGC TTC CCT CTC	1230
	C ACC ATG ACC TGC TCC CCT CTC C	1231
	C ACC ATG GCC TGG GCT CTG CTC	1232
	C ACC ATG GCC TGG GCT CTG CTG	1233
	C ACC ATG GCA TGG ATC CCT CTC TTC	1234
	C ACC ATG GCC TGG ACC GCT CTC	1235
	C ACC ATG GCC TGG ACC CCT CTC	1236
	C ACC ATG GCC TGG ATC CCT CTC C	1237
	C ACC ATG GCC TGG ACC GTT CTC C	1238
	C ACC ATG GCA TGG GCC ACA CTC C	1239
	C ACC ATG GCC TGG ATC CCT CTA C	1240
	C ACC ATG GCC TGG GTC TCC TTC TAC	1241
	C ACC ATG GCC TGG ACC CAA CTC C	1242
	C ACC ATG GCT TGG ACC CCA CTC C	1243
	C ACC ATG GCC TGG ACT CCT CTC C	1244
	C ACC ATG GCC TGG ACT CCT CTT CTT C	1245
	C ACC ATG GCC TGG ACT CTT CTC CTT C	1246
	C ACC ATG GCC TGG GCT CCA CTA C	1247
	C ACC ATG GCC TGG ACT CCT CTC TTT C	1248
	C ACC ATG GCC TGG ATG ATG CTT CTC C	1249
	C ACC ATG GCC TGG GCT CCT CTG	1250
	C ACC ATG CCC TGG GCT CTG CTC	1251
3' primer	GGA GGG TKT GGT GGT CTC CAC TCC C	1252

(where K = G + T)

EXAMPLE 9

Preparation of hetero Ig constructs

[00377] Generation of a bispecific antibody through co-expression of two different antibodies leads to contaminants primarily consisting of mispaired heavy and light chains. The preferred bispecific, heterotetramer molecule with two different heavy chains associated with correctly paired light chains is only a minority of the total amount of combinations that can assemble. The contaminants occur mainly due to two different reasons. The first reason is that the heavy chain that comes together at the Fc region of the antibody can homodimerize, leading to conventional monospecific antibody, or heterodimerize, leading to a potential bispecific antibody. The second reason is that light chain is promiscuous and can pair with either of the

heavy chains, leading to mispaired light-heavy chain Fab assembly that may not retain binding to the desired target. For these reasons, the bispecific engineering is a two-step process. The first goal is to prevent the homodimerization of the heavy chains and encourage heterodimerization. This can be achieved through engineering the Fc region of the antibodies, using, for example, the knobs-into-holes or charge pair mutations strategies. The second goal is to engineer the light-heavy chain interface in such a way that the light chain is specifically associated only with its cognate heavy chain.

[00378] The "Hetero-Ig" platform technology (see, e.g., WO2009089004 and WO2014081955, both of which are hereby incorporated by reference in their entireties) takes advantage of the electrostatic steering mechanism to overcome the pairing problems mentioned above. Specifically, charged residues are introduced or exploited to drive heavy chain heterodimerization and correct light-heavy chain association. The charge pair mutations (CPMs) in the CH3 domain of the Fc region drive the heterodimerization of the two different heavy chains through opposite charges that cause electrostatic attraction (see, e.g., WO2009089004 and U.S. Patent No. 8,592,562); the two identical heavy chain combinations have identical juxtaposed charges and are therefore repelled.

[00379] The correct heavy chain-light chain pairing is facilitated by CPMs at the HC/LC binding interface or the HC1/HC2 binding interface (see Figure 1). The correct heavy chain-light chain combinations will have opposite charges and therefore be attracted to each other, whereas the incorrect heavy chain-light chain combinations will have the same charges juxtaposed, resulting in repulsion. In Figure 1, correctly assembled hetero-Ig molecules have two or three HC1/HC2 CPMs and two to four HC/LC CPMs that drive the assembly of the preferred heterotetramer comprising two different heavy chains and two different light chains so that the heterotetramer will be the majority component generated by the expression system. The DNAs encoding anti-TL1A/anti-TNF-α hetero Ig-s contain fragments coding for anti-TL1A (or anti-TNF-α) heavy chain and anti-TNF-α (or anti-TL1A) Fab. The DNAs were cloned into pTT5.1 vector. These expression vectors were then used to transfect and express anti-TL1A/anti-TNF-α bi-specifics in human 293 6E cells.

[00380] Anti-TNF-α antibodies 3.2, 234 and certolizumab were used with anti-TL1A antibodies 3B3, 2G11, 23B3 VH3, 23B3 VH4, and 3C6 to engineer hetero Ig molecules as described in Table J using high throughput cloning, expression and purification. Each of the bispecific hetero Ig molecules had one of the four formats shown in Figure 1 using the IgG1 effector functionless scaffold or an IgG2 scaffold. Preferred IgG molecules incorporate the

charge mutations shown in Figure 1 (v2), which are shown in Table M. The IgG1 effector functionless scaffold comprises substitutions R292C and V302C and may also comprise substitution N297G (also known as the SEFL2 scaffold).

EXAMPLE 10

Method for Expression and Purification of anti-TL1A/anti-TNFα Hetero-Ig Molecules

[00381] Hetero-Ig expression was performed via transient transfection of 293-6E cells. One day prior to transfection N-1 culture was set up in a 20 L Wave bag, at 36 °C-37 °C, 5% CO_2, 0.2 LPM overlay with a total volume of 9 L of culture at 8.5 E5 vc/mL in Freestyle F-17 media (Thermo Fisher). The transfection complex was then prepared by mixing FreeStyle F-17 media, pre-warmed with 0.5 mg/L transfection DNA, with a 1:1:1:1 plasmid DNA chain ratio. Transfection complex (media+DNA+PEI reagent) volume was 10% of final culture volume. Four hours post-transfection , a feed of yeastolate and glucose was added. Culture was harvested on day six at 2.11 E6 cells/mL and 78.2% viability. Expression titer was measured using the ForteBio Octet Q System at 84.0 mg/L. The conditioned media was harvested by centrifugation and filtered using 0.2 μm cellulose acetate filter using a peristaltic pump.

[00382] For purification, the hetero-Ig molecules were affinity captured by MabSelect SuRe chromatography (GE Life Sciences, Piscataway, NJ), using Dulbecco's PBS without divalent cations (Invitrogen, Carlsbad, CA) as the wash buffer and 100 mM acetic acid, pH 3.6 as the elution buffer (Figure 3). All separations were carried out at ambient temperature. The elution peak was pooled based on the chromatogram, neutralized to pH 7.0 using 2 M tris base, diluted with 5-volumes water, and filtered through a 0.22 μm cellulose acetate filter. To remove half antibody species, the sample was then loaded on to an SP-HP sepharose column (GE Life Sciences, Piscataway, NJ) and washed with 8 column volumes of SP-Buffer A (20 mM sodium phosphate, pH 7.0) followed by elution using a 20 column volume gradient to 60% SP-Buffer B (20 mM sodium phosphate, 1 M NaCl, pH 7.0) (Figure 4). A pool was made based on the chromatogram and Caliper LabChip (Perkin Elmer, Waltham, MA) analysis of fractions. The pool was conditioned with an equal volume of 2X HIC Buffer (200 mM sodium phosphate, 1.5 M ammonium sulfate, pH 7.0) and filtered through a 0.22 μm cellulose acetate filter. To remove mispaired species, the sample was loaded on to a Butyl-HP sepharose column (GE Life Sciences, Piscataway, NJ) and washed with 8 column volumes of Butyl-Buffer A (50 mM sodium phosphate, 0.75 M ammonium sulfate, pH 7.0) followed by elution using a 20 column volume gradient to 100% Butyl-Buffer B (50 mM sodium phosphate, pH 7.0) (Figure 5). A pool was made based on the chromatogram and Caliper LabChip (Perkin Elmer, Waltham,

MA) analysis of fractions under non-reducing and reducing conditions. The pool was diafiltered against approximately 30 volumes of 10 mM sodium acetate, 9% sucrose, pH 5.2 using Slide-A-Lyzer dialysis cassettes with a 10 kDa cutoff membrane (Pierce, Rockford, IL) and further concentrated using a Vivaspin-20 centrifugal concentrator with a 10 kDa cutoff membrane (Sartorius Stedim Biotech, Goettingen, Germany). The concentrated material was then filtered through a 0.8/0.2 μm cellulose acetate filter and the concentration was determined by the absorbance at 280 nm using an extinction coefficient of approximately 212,000. Sample purity was determined by Caliper LabChip analysis under reducing (with 2% 2-mercaptoethanol) and non-reducing (with 25 mM iodoacetamide) conditions (Figure 6). Analytical SEC was carried out using a Zenix-C SEC-300 column (Sepax Technologies, Newark, DE) with an isocratic elution in 50 mM sodium phosphate, 250 mM NaCl, pH 6.9 over 18' (Figure 7).

[00383] In order to assess both hetero-Ig integrity and confirm heavy chain-light chain pairing, LC-MS was performed on non-reduced hetero-Ig, as well as hetero-Ig after limited lysyl endoproteinase C (Wako, Richmond, VA) digestion to produce the hetero-Ig Fab's (fragment antigen binding) regions of the hetero-Ig. Analysis of non-reduced hetero-Ig was performed by simple dilution of 30 μg native hetero-Ig sample, 1:1 in 0.1% TFA, and injecting 20 μg. Analysis of Fab's was achieved by incubation of hetero-Ig in the presence of lysyl endoproteinase C using a 1:400 enzyme/substrate ratio in the presence of 100 mM Tris, adjusted to pH8, for 30 minutes at 37 °C. The reaction was then stopped by dilution in an equal volume of 0.1% TFA. Initial cleavage of antibody by Lysyl Endoproteinase C is just above the hinge disulfides after the lysine in the sequence motif SCDK/THTCPPC yielding Fab and Fc fragments.

[00384] Mass analysis was performed using an Agilent 6230 ESI-TOF Mass Spectrometer and 1260 quaternary HPLC system equipped with a Zorbax 300SB-C8, 2.1 x 50 mm 3.5 μm column (Agilent, Santa Clara, CA). Mobile phase A consisted of 0.1% TFA, and mobile phase B consisted of 90% n-propanol, 0.1% TFA in water. Chromatographic gradient conditions for analysis of non-reduced hetero-Ig were as follows: 20% mobile phase B for 1 minute; 1-9 min, 20-70% B; 9-10 min, 70-100% B; 10-11 min, 100% B. Column temperature was kept at 75 °C and post column equilibration at 20% mobile phase B was performed for 7 minutes prior to injection of the next sample. The ESI-TOF settings were as follows: capillary voltage, 5900 V; gas temperature, 340 °C; dry gas, 13 L/min; nebulizer pressure, 25 psig; fragmentor voltage 460 V and skimmer voltage, 95 V (figure 8).

[00385] Analysis of hetero-Ig digested with lysl endoproteinase C was conducted by injecting 20μg onto the reverse-phase HPLC column and eluting using the following LC gradient: 2% mobile phase held for 2 minutes; 2-12 min, 2-45% B; 12-16 min, 45-90% B; 16-17 min 90% B. Column temperature was kept at 75 °C and post column equilibration at 20% mobile phase B was performed for 7 minutes prior to injection of the next sample (figure 9).

EXAMPLE 11

Preparation of IgG-ScFv constructs

[00386] Each anti-TL1A / anti-TNF-α IgG-scFv antigen binding protein consists of two antigen binding domains, one directed against TL1A and the other against TNF-α. The DNAs encoding anti-TL1A / anti-TNF-α IgG-scFv contain fragments coding for anti-TL1A (or ant-TNF-α) heavy chain (HC) in which the C-terminus is fused to anti-TNF-α (or anti-TL1A) antibody single chain Fv (scFv) (see Figure 2) with or without cysteine clamp for the purpose of improving biophysical properties. In order to introduce the cysteine clamp, positions 44 (Kabat numbering) VH and 100 (Kabat numbering) in VL were mutated to cysteine. The DNAs were cloned into pTT5.1 vector. These expression vectors were then used to transfect and express anti-TL1A / anti-TNF-α bi-specifics in human 293-6E cells. Full sequences for anti-TL1A / anti-TNF-α IgG-scFv antigen binding proteins produced are shown in Tables L and M.

EXAMPLE 12

Method for Purifying anti-TL1A/anti-TNFα IgG-scFv Molecules

[00387] IgG-ScFv expression was performed via transient 293 productions. One day prior to transfection, cultures were set up in eight 5 L Thompson Ultra Yield flasks, with a total volume of 2.250 L of culture at 8.5E5 vc/mL each in Freestyle F-17 media (Thermo Fisher). The cultures were kept at 36 °C-37 °C, 5% CO_2 at and shaking at 120 RPM. The transfection complex was prepared by mixing FreeStyle F-17 media with 0.5 mg/L DNA, using a 20% of coding plasmid and 80% empty pTT5 vector and PEI at 10% final culture volume Four hours later a yeast lysate and glucose feed was added to each flask. Six days post transfection the cells were harvested through centrifugation, pooled and filtered. Average titer was measured by Forte Bio Octet at 25 mg/L.

[00388] The IgG-scFv molecules were affinity captured by MabSelect SuRe chromatography (GE Life Sciences, Piscataway, NJ), using Dulbecco's PBS without divalent cations (Invitrogen, Carlsbad, CA) as the wash buffer and 100 mM acetic acid, pH 3.6 as the elution buffer (Figure 10). Affinity separations were carried out at ambient temperature. The

elution peak was pooled based on the chromatogram, neutralized to pH 7.0 using 2 M tris base, diluted with one volume 10 mM citrate, 75 mM lysine, 4% trehalose, pH 7.0, and concentrated to approximately 20 mg/mL using a Vivacell-100 centrifugal concentrator with a 30 kDa cutoff membrane (Sartorius Stedim Biotech, Goettingen, Germany). To remove high molecular weight aggregates, the sample was filtered through a 0.8/0.2-μm cellulose acetate filter then loaded on to a Superdex 200 Prep Grade column (GE Life Sciences, Piscataway, NJ) equilibrated with 10 mM citrate, 75 mM lysine, 4% trehalose, pH 7.0 and eluted with an isocratic gradient with the same buffer (Figure 11). Gel filtration separations were carried out at approximately 7 °C. A pool was made based on the chromatogram and analytical SEC of the fractions using a Zenix-C SEC-300 column (Sepax Technologies, Newark, DE). The pool was further concentrated using a Vivaspin-20 centrifugal concentrator with a 30 kDa cutoff membrane (Sartorius Stedim Biotech, Goettingen, Germany) and filtered through a 0.8/0.2-μm cellulose acetate filter. The concentration was determined by the absorbance at 280 nm using an extinction coefficient of approximately 341,000. Sample purity was determined by Caliper LabChip analysis under reducing (with 2% 2-mercaptoethanol) and non-reducing (with 25 mM iodoacetamide) conditions (Figure 12). Analytical SEC was carried out using a Zenix-C SEC-300 column (Sepax Technologies, Newark, DE) with an isocratic elution in 50 mM sodium phosphate, 250 mM NaCl, pH 6.9 over 18' (Figure 13).

[00389] Due to the large size of the Ig-scFvs, 200 kDa or greater, accurate mass was not achievable by ESI-TOF mass analysis. In order to verify mass and assess the quality, Ig-scFv's were analyzed after digestion with IdeS protease (Promega, Madison WI), which cleaves just below the IgG hinge disulfides, between glycines in the sequence motif, CPPCPAPELLG/GP yielding $(Fab)_2$ and Fc with C-terminally fused scFv. The Ig-scFv's were incubated in the presence of IdeS protease using a 1:10 enzyme substrate ratio for 1 hour at 37 °C. The sample was then diluted 1:1 in 0.1% TFA, and 20 μg was injected onto LC-MS.

[00390] Mass analysis was performed using an Agilent 6230 ESI-TOF Mass Spectrometer and 1260 quaternary HPLC system equipped with a Zorbax 300SB-C8, 2.1 x 50 mm 3.5 μm column (Santa Clara, CA). Mobile phase A consisted of 0.1% TFA, and mobile phase B consisted of 90% n-propanol, 0.1% TFA in water. Analysis of Ig-scFv digested with IdeS Protease was conducted by injecting 20 μg and eluting using the following LC gradient: 2% mobile phase held for 2 minutes; 2-12 min, 2-45% B; 12-16 min, 45-90% B; 16-17 min 90% B. Column temperature was kept at 75 °C and post column equilibration at 20% mobile phase B was performed for 7 minutes prior to injection of the next sample (figure 14).

EXAMPLE 13

TL1A binding assay

[00391] Goat anti-human Fc (Jackson Lab, Cat#109-005-098) was first immobilized on an SCM5 sensor chip using amine coupling. Anti-TL1A monoclonal antibodies, Fab fragment (using anti-huFab antibody, GE Healthcare cat # 28-9583-25) or anti-TL1A/anti-TNF-α bispecific molecules were injected to the chip at 10 µl/min for 1 minute. Various concentrations of human or cynomolgus TL1A protein from 0.78 to 25 nM in sample buffer (PBS, 0.005% P20, 0.1 mg/ml BSA) were injected at flow rate of 50 µl/min for 3 min association, 10 min dissociation. On rate, off rate and equilibrium dissociation constant were calculated using 1:1 binding model on BIAevaluation software.

[00392] Tables 13.1-13.3 show anti-TL1A binding data for anti-TL1A antibodies, hetero Ig bispecific antibodies, and IgG-scFv bispecific antibodies.

[00393] Table 13.1: TL1A binding of anti-TL1A antibodies

Anti-TL1A mAb	Protein ID	SEQ ID NOS:	human TL1A			cyno TL1A		
			ka (1/Ms)	kd (1/s)	KD (M)	ka(1/Ms)	kd (1/s)	KD (M)
3C6	PL-39112	50, 52	NA	<5.0E-05	<1.0E-10	5.7E+05	1.4E-03	2.4E-09
3B3 parental	PL-39114	66, 68	4.0E+05	9.5E-05	2.4E-10	2.5E+05	1.4E-04	5.7E-10
5G4	PL-39115	455, 457	5.0E+05	1.8E-04	3.6E-10	2.9E+05	1.9E-04	6.6E-10
17E9	PL-39113	459, 461	2.5E+05	5.3E-05	2.2E-10	7.3E+05	2.0E-03	2.8E-09
9C8	PL-39116	58, 60	1.6E+05	5.3E-05	3.4E-10	9.4E+04	6.9E-05	7.3E-10
23B3	PL-36543	62, 64	4.7E+05	4.2E-04	8.9E-10	2.3E+05	3.2E-04	1.4E-09
2G11	PL-36544	54, 56	1.7E+05	1.8E-04	1.1E-09	6.6E+04	4.9E-05	7.3E-10
3B3 variant	PL-36552	130, 134	4.3E+05	2.2E-04	5.0E-10	2.8E+05	1.4E-04	5.0E-10

[00394] Table 13.2: TL1A binding of anti-TL1A Fab fragment

Anti-TL1A Fab	Protein ID	human TL1A			cyno TL1A		
		ka (1/Ms)	kd (1/s)	KD (M)	ka (1/Ms)	kd (1/s)	KD (M)
3C6	PL-39193	NA	<5.0E-05	<1.0E-10	NB up to 100 nM		
3B3 parental	PL-39195	4.1E+05	1.6E-04	4.0E-10	3.5E+05	1.8E-04	5.1E-10
5G4	PL-39196	4.7E+05	3.3E-04	7.0E-10	4.4E+05	1.6E-04	3.7E-10
17E9	PL-39194	NA	<5.0E-05	<1.0E-10	NB up to 100 nM		
9C8	PL-39197	NA	<5.0E-05	<1.0E-10	NA	<5.0E-05	<1.0E-10
23B3	PL-38024	7.6E+05	6.4E-04	8.5E-10	5.3E+05	1.6E-04	3.1E-10
2G11	PL-38025	NA	<5.0E-05	<3.0E-10	NA	<5.0E-05	<1.0E-10

3B3 variant	PL-38026	3.8E+05	4.1E-04	1.1E-09	4.5E+05	5.6E-05	1.3E-10

[00395] Table 13.3: TL1A binding of hetero Ig bispecific antibodies

iPS	anti-TNF	anti-TL1A	ka (1/Ms)	kd (1/s)	KD (M)
376541	Certolizumab parent	3B3 variant (322520)	7.1E+05	2.1E-04	3.0E-10
376542	C234	3B3 variant (322520)	7.1E+05	2.1E-04	3.0E-10
376543	Certolizumab variant	3B3 variant (322520)	7.2E+05	2.3E-04	3.2E-10
349461	Certolizumab Variant	2G11 Variant	5.4E+05	1.3E-04	2.5E-10
349463	Certolizumab Variant	23B3 VH4	6.3E+05	4.0E-04	6.4E-10
371222	3.2/CC	3B3 variant (322520)	6.6E+05	1.4E-04	2.1E-10

EXAMPLE 14

TL1A activity assay

[00396] TL1A activity assay was performed using TF-1 NF-κB reporter cell line. In brief, 30 ng/ml (EC90) of human or cynomolgus monkey TL1A was incubated with 10^4 TF-1 NF-κB reporter cells in the presence of serially diluted anti-TL1A antibodies or anti-TL1A/anti-TNF–α bispecific molecules in 96-well plate at 37 °C overnight. Each well was supplemented with 50 µl of Steady-glo Luciferase testing solution (Promega). Plate was covered and incubated while shaking for 10 minutes. Luciferase activity was analyzed by microbeta reader.

[00397] Tables 14.1-14.3 show anti-TL1A quality control (QC) and activity data for anti-TL1A antibodies, hetero Ig bispecific antibodies, IgG-scFv bispecific antibodies, and Fab's with both human and cynomolgus monkey TL1A.

[00398] Table 14.1: anti-TL1A activity of anti-TL1A antibodies and Fab fragments

mAb	mAb/Fab	huTL1A (0.2 nM) NF-kB IC50 nM	cyno TL1A (2 nM) NF-kB IC50 nM
3C6	mAb	0.153	>100
	Fab	0.162	>100
17E9	mAb	0.168	>100
	Fab	70.83	>100
3B3S65A	mAb	0.083	0.595
	Fab	0.178	0.434
3B3/7B3	mAb	0.037	0.390

	Fab	0.112	0.647
5G4	mAb	0.058	0.622
	Fab	2.838	10.06
2G11	mAb	0.223	0.938
	Fab	0.551	1.637
9C8	mAb	0.131	0.721
	Fab	2.434	2.748
23B3	mAb	0.089	0.619
	Fab	2.992	2.629

[00399] Table 14.2: QC and activity of hetero Ig bispecific antibodies

					SE-HPLC		Caliper						Activity	
iPS No.	**TL1A**	**TNF-α**	**Final Conc.**	**Final Yield**	**HMW**	**Main**	**NR 1**	**NR 2**	**HC1**	**H2**	**LC1**	**LC2**	**TL1A (pM)**	**TNF-α (pM)**
376541	Certolizumab (parent)	3B3 (variant)	12.05	295	0.7	99.3	72	28	42	58	51	49	187	51
349461	Certolizumab (variant)	2G11 (variant)	10.49	188	0.7	99.3	45	55	100	0	51	49	1284	60
349463	Certolizumab (variant)	23B3 (VH4)	10.61	450	0.6	99.4	63	37	50	50	33	67	3464	64
376542	C234	3B3 (variant)	9.76	174	0.0	100.0	71	29	44	56	50	50	147	544
376543	Certolizumab (variant)	3B3 (variant)	13.60	320	0.4	99.6	75	25	43	57	51	49	158	107

[00400] Table 14.3: QC and activity of IgG-scFv bispecific antibodies

					SE-HPLC			Caliper				Activity	
iPS	**IgG**	**scFv**	**Final Conc.**	**Final Yield**	**HMW**	**Main**	**Shoulder**	**NR 1**	**NR 2**	**HC1**	**LC1**	**TL1A (pM)**	**TNF-α (pM)**
371213	TNF-α (adalimumab)	TL1A (3B3 Var2)	12.40	310	.05	98.3	1.4	11	89	81	19	61	64
371217	TNF-α (C234)	TL1A (3B3 Var2/CC)	13.06	290	0.4	98.5	1.2	39	61	81	18	69	40
369989	TNF-α (adalimumab)	TL1A (9C8)	13.83	199	0.3	98.1	1.1	27	73	76	18	140	59
369995	TNF-α (3.2)	TL1A (9C8)	15.81	217	1.0	97.9	1.6		100	80	17	199	53
370001	TNF-α (C234)	TL1A (9C8)	12.41	289	0.2	98.2	1.1	42	58	84	16	119	52
369992	TNF-α (adalimumab)	TL1A (9C8/CC)	11.48	169	0.3	98.2	1.6	16	84	84	16	151	72
370004	TNF-α (C234)	TL1A (9C8/CC)	11.34	111	0.3	98.2	1.5	45	55	84	16	163	70
370013	TL1A (9C8)	TNF-α (3.2)	16.53	464	0.3	98.3	1.5	41	59	79	18	171	25

371219	TL1A (3B3 Var2)	TNF-α (3.2)	10.9	197	0.4	98.1	1.4	100		70	20	53	22
371222	TL1A (3B3 Var2)	TNF-α (3.2/CC)	20.07	157	0.2	98.3	1.5	100		78	18	86	26
370018	TL1A (23B3)	TNF-α (adalimumab)	11.59	112	2.2	96.5	1.5	100		86	14	157	25
370021	TL1A (23B3)	TNF-α (adalimumab/CC)	15.29	321	0.0	100.0	1.3	7	93	86	14	139	24

EXAMPLE 15

TNF–α binding assay

[00401] Goat anti-human Fc was first immobilized on SCM5 sensor chip using amine coupling. Anti-TNF monoclonal antibodies, Fab fragment or TL1A/TNF bispecifics were injected to the chip at 10 µl/min for 1 min. Various concentration of human or cynomologus TNF protein from 0.78 to 25 nM in sample buffer (PBS, 0.005% P20, 0.1 mg/ml BSA) were injected at flow rate of 50 µl/min for 3 min association, 10 min dissociation. On rate, off rate and equilibrium dissociation constant were calculated using 1:1 binding model on BIAevaluation software.

[00402] Tables 15.1-15.3 show TNF–α binding activity data for anti-TNF–α antibodies, hetero Ig bispecific antibodies, IgG-scFv bispecific antibodies, and IgG-Fab bispecific antibodies.

[00403] Table 15.1: TNF–α binding activity of anti-TNF–α antibodies and Fab fragment

		Human TNF-α			Cyno TNF-α		
		k_a (1/Ms)	k_d (1/s)	KD (M)	k_a (1/Ms)	k_d (1/s)	KD(M)
3.2	mAb	1.5E+06	7.3E-05	4.8E-11	9.0E+05	9.9E-05	1.1E-10
	Fab		<5.0E-5			<5.0E-5	
4.14	mAb	5.3E+05	6.1E-05	1.2E-10	3.5E+05	1.0E-04	2.9E-10
	Fab		<5.0E-5			<5.0E-5	
234	mAb	1.5E+06	7.9E-05	5.2E-11	1.2E+06	1.0E-04	8.7E-11
	Fab		<5.0E-5			<5.0E-5	
Adalimumab	mAb	7.1E+05	6.1E-05	8.6E-11	6.4E+05	5.5E-05	8.7E-11
	Fab	4.5E+05	1.4E-04	3.2E-10	3.6E+05	8.1E-05	2.3E-10
Certolizumab pegol	Peg-Fab	3.9E+06	6.2E-05	1.6E-11	6.4E+06	2.2E-03	3.5E-10

[00404] Table 15.2: TNF–α binding activity of hetero Ig bispecific antibodies

iPS	Lot	anti-TNF-α	anti-TL1A	ka (1/Ms)	kd (1/s)	KD (M)

376541	PL-42786	Certolizumab parent	3B3 variant (322520)	3.2E+06	4.4E-05*	1.4E-11
376542	PL-42789	C234	3B3 variant (322520)	2.3E+06	1.1E-04	4.8E-11
376543	PL-42790	Certolizumab variant	3B3 variant (322520)	3.6E+06	5.5E-05	1.6E-11
349461	PL-42787	Certolizumab variant	2G11 Variant	3.4E+06	6.4E-05	1.9E-11
349463	PL-42788	Certolizumab variant	23B3 VH4	3.4E+06	5.5E-05	1.6E-11

[00405] Table 15.3: TNF–α binding activity of IgG-scFv bispecific antibodies

iPS	Lot	IgG	scFV	ka (1/Ms)	kd (1/s)	KD (M)
369989	PL-42765	TNF–α (Adalimumab)	TL1A (9C8)	1.2E+06	7.6E-05	6.6E-11
369992	PL-42770	TNF–α (Adalimumab)	TL1A (9C8/CC)	1.2E+06	8.4E-05	7.3E-11
369995	PL-42779	TNF–α (3.2)	TL1A (9C8)	1.7E+06	7.7E-05	4.4E-11
370001	PL-42767	TNF–α (C234)	TL1A (9C8)	1.7E+06	9.9E-05	5.9E-11
370004	PL-42785	TNF–α (C234)	TL1A (9C8/CC)	1.7E+06	1.0E-04	5.9E-11
370013	PL-42781	TL1A (9C8)	TNF–α (3.2)	1.3E+06	8.8E-05	6.5E-11
370018	PL-42763	TL1A (23B3)	TNF–α (Adalimumab)	1.1E+06	5.3E-05	5.0E-11
370021	PL-42778	TL1A (23B3)	TNF–α (Adalimumab/CC)	1.1E+06	4.9E-05*	4.5E-11
371213	PL-42771	TNF–α (Adalimumab)	TL1A (3B3.322520/CC)	1.3E+06	8.2E-05	6.5E-11
371217	PL-42782	TNF–α (C234)	TL1A (3B3.322520/CC)	1.9E+06	8.8E-05	4.8E-11
371219	PL-42774	TL1A (3B3.322520)	TNF–α (3.2)	1.3E+06	5.5E-05	4.2E-11
371222	PL-42783	TL1A (3B3.322520)	TNF–α (3.2/CC)	1.3E+06	6.3E-05	4.7E-11

EXAMPLE 16

TNF–α activity assays

[00406] TNF–α activity assay was performed using TF-1 NF-kB reporter cell line. In brief, 1 ng/ml (EC90) of human or cynomolgus monkey TNF–α was incubated with 10^4 TF-1 NF-κB reporter cells in the presence of a serially diluted anti-TNF–α antibodies or TL1A/TNF–α bispecific molecules in 96-well plate at 37 °C overnight. 50 µl of Steady-glo

Luciferase testing solution (Promega) was added to each well. Plate was covered and incubated while shaking for 10 minutes. Luciferase activity was analyzed by microbeta reader.

[00407] Table 16.1 shows anti-TNF–α QC and activity data for anti-TNF antibodies. Tables 14.2 and 14.3 show anti-TNF-α binding activity for hetero Ig bispecific antigen binding proteins, and IgG-scFv bispecific antigen binding proteins, respectively.

[00408] Table 16.1: Anti-TNF–α activity of TNF–α antibodies and Fab fragments

Antibody designation	Modality	Human TNF (20 pM) IC50 (pM) NFkB	Cyno TNF (20 pM) IC50 (pM) NFkB
3.2	mAb	75	334
	Fab	367	2950
4.14	mAb	127	739
	Fab	526	3105
234	mAb	74	138
	Fab	825	2027
adalimumab	mAb	119	180
	Fab	670	1800
certolizumab pegol	Peg-Fab	91	1.38 μM

EXAMPLE 17

Bispecific molecule activity

[00409] The human and cynomolgus monkey TL1A and TNF-α binding activities of bispecific antigen binding proteins were determined as described in Examples 13 and 15. Data are shown in Table 17.1

[00410] Table 17.1: human and cyno TL1A and TNF binding activities

leads	TL1A warhead	TNF-α warhead	iPS no.	TL1A binding (Kd pM)		TNF binding (Kd pM)	
				Hu TL1A	Cyno TL1A	Hu TNF	Cyno TNF
Hetero-Ig	3B3 V2	certolizumab	376543	11	26	12	210
IgG-scFv	9C8/CC	adalimumab	381505	120	150	21	33
IgG-scFv	9C8/CC	C234	381489	160	94	10	19
IgG-scFv	3B3 V2/CC	adalimumab	381513	1.8	6.6	19	23

[00411] The human and cynomolgus monkey TL1A and TNF-α blocking activities of bispecific antigen binding proteins were determined as described in Examples 14 and 16. Data are shown in Table 17.2.

[00412] Table 17.2: human and cyno TL1A and TNF-α blocking activites

leads	Anti-TL1A warhead	Anti-TNF-α warhead	iPS No.	TL1A assay IC50 pM			TNF assay IC50 pM		
				soluble hu	mem hu	soluble cyno	soluble hu	mem hu	soluble cyno
Hetero-Ig	3B3 V2	certolizumab	376543	45.5	50.1	49.7	101.1	942.6	0.9uM
IgG-scFv	9C8/CC	adalimumab	381505	52.2	46.5	45.3	72.9	719.5	96.4
IgG-scFv	9C8/CC	C234	381489	66.7	46.3	62.6	95.8	639.2	157.2
IgG-scFv	3B3 V2/CC	adalimumab	381513	35.6	19.2	23.7	69.7	906.3	91.3

EXAMPLE 18

SNP genotyping

[00413] To evaluate potential TL1A genotype association with expression, genomic DNA (gDNA) was isolated from healthy PBMC donors using Gentra Puregene Tissue kit from Qiagen. Genomic DNA were genotyped using TaqMan SNP genotyping assays for rs7848647, rs6478109, rs6478108, and rs3810936 assays from LifeTech and standard protocols on the Bio-Rad droplet digital PCR platform. Briefly, 10 ng of gDNA from each donor was mixed with ddPCR™ Supermix for Probes (Bio-Rad) and TaqMan SNP genotyping assay (LifeTech) for each SNP of interest. Droplets were generated for each reaction using the QX100™ droplet generator (Bio-Rad) and subjected to thermal cycling on a C1000 Touch™ thermal cycler (Bio-Rad). Following amplification, droplet fluorescence was read on a QX100™ droplet reader (Bio-Rad) and data were analyzed using QuantaSoft software (Bio-Rad). Donors were considered homozygous risk haplotype if only risk alleles were present at all 4 genotyped SNPs (rs7848647, rs6478109, rs6478108, and rs3810936). Donors were considered homozygous non-risk haplotype if only non-risk alleles were present at all 4 genotyped SNPs. Donors were considered heterozygous haplotype if both risk and non-risk alleles were present at all 4 genotyped SNPs. Donors that were considered "recombinant" had only homozygous risk alleles

at rs7848647, rs6478109, and rs6478108, but were heterozygous (risk and non-risk alleles present) at rs3810936.

[00414] The presence of synonymous SNP (rs3810936) in the exon 4 of the TL1A gene enabled us to track risk allele vs non-risk allele expression by droplet digit PCR (ddPCR) using allelic specific fluorescent probes in the allelic expression imbalance (AEI) study. The frequency of risk vs non-risk allele usage in heterozygous PBMC at basal level or after immune complex stimulation was evaluated at various time points. In brief, PBMCs heterozygous for TL1A genotype were treated with immune complex for various lengths of time. RNA was then isolated from PBMC using an RNeasy mini kit with on-column DNase I digestion from Qiagen. RNA from each sample was converted to cDNA using the High Capacity cDNA Reverse Transcription Kit according to manufacturer's instructions (LifeTech). Allele-specific expression at rs3810936 was determined using TaqMan SNP genotyping assay specific for rs3810936 (LifeTech) and standard protocols on the Bio-Rad droplet digital PCR platform. In brief, the amount of input cDNA was first optimized for TNFSF15 expression at each time point in order to increase precision by maximizing the number of positive droplets counted for each sample by QuantaSoft software. The optimal amount of cDNA per sample was mixed with ddPCR™ Supermix for Probes (Bio-Rad) and TaqMan SNP genotyping assay (LifeTech) for rs3810936. Droplets were generated for each reaction using the QX100™ droplet generator (Bio-Rad) and subjected to thermal cycling on a C1000 Touch™ thermal cycler (Bio-Rad). Following amplification, droplet fluorescence was read on a QX100™ droplet reader (Bio-Rad). Data were analyzed using QuantaSoft software (Bio-Rad) and copies/ml of each allele at rs3810936 was determined. The allelic expression ratio was calculated by dividing the copies/ml of the risk allele by the copies/ml of the non-risk allele. Total copy number of each allele was normalized by the input amount of cDNA (copies/ng), and the fold-induction for each allele at each time point was calculated by dividing the copies/ng at the time point of interest by the copies/ng at baseline (0 hr). Higher induction of TL1A risk allele was observed in PBMC after immune complex treatment compared to non-risk allele in heterozygous PBMC in allelic expression imbalance study.

[00415] The inventor(s) identified a small number of donors homozygous for risk alleles at rs7848647, rs6478109, and rs6478108, but heterozygous at rs3810936, likely due to recombination between rs6478109 and synonymous SNP rs3810936. Since these donors are heterozygous at synonymous SNP rs381093, the inventor(s) could track the risk allele and non-risk allele usage and evaluate if the allelic expression imbalance was still retained in these donors. Interestingly, compared to heterozygous donors, no allelic expression imbalance was

detected in these recombinant individuals before or after immune complex stimulation. This demonstrates that synonymous SNP rs381093 itself is not responsible for allelic imbalance regulation. The regulatory SNPs likely derive from rs7848647, rs6478109, rs6478108 and/or other SNPs 5' to the synonymous SNP rs3810936.

[00416] To evaluate if TL1A allelic expression imbalance data correlate with expression quantitative trait loci (eQTL), PBMC donors with homozygous TL1A risk allele, homozygous non-risk alleles or heterozygous alleles were treated with immune complex. RNA from each sample was isolated and then converted to cDNA as described previously. Total expression of TL1A (TNFSF15) in each donor was determined by digital PCR. In brief, cDNA from each sample was mixed with ddPCR™ Supermix for Probes (Bio-Rad) and PrimeTime® qPCR assay ID Hs.PT.56a.41003970. Droplets were generated for each reaction using the QX100™ droplet generator (Bio-Rad) and subjected to thermal cycling on a C1000 Touch™ thermal cycler (Bio-Rad). Following amplification, droplet fluorescence was read on a QX100™ droplet reader (Bio-Rad). Data were analyzed using QuantaSoft software (Bio-Rad) and copies/μl of TL1A was determined and normalized for the amount of input cDNA (copies/ng). P values for differences in TL1A expression levels between different TNFSF15 haplotypes were determined using the student t test. TL1A is expressed at a very low basal level in PBMCs, but strongly induced after immune complex stimulation. At basal level, PBMCs from donors homozygous for TL1A risk SNPs have lower TL1A mRNA compared to non-risk homozygous donors, albeit at very low copy number, consistent with the allelic expression imbalance study which demonstrated lower ratio of risk allele versus non-risk allele without stimulation. However, after immune complex stimulation, PBMCs from donors homozygous for TL1A risk SNPs have higher TL1A expression compared to non-risk homozygous donors. These data, together, demonstrated TL1A risk SNPs regulate higher TL1A induction. The inventor(s) speculate that in inflammatory conditions such as IBD, donors carrying TL1A risk SNPs likely encounter higher TL1A induction upon encounter of stimuli such as cytokines, opsonized or non-opsonized microbes. Therefore, TL1A risk SNPs could be used for patient stratification for TL1A inhibitor or anti-TL1A/anti-TNF-α bispecific inhibitors.

[00417] To evaluate if TL1A genotype-mediated expression regulation is tissue-specific, HUVEC cells from various donors were genotyped as described previously. Genotyped HUVEC cells from various donors were treated with IL-1 at various time points. Total copy number of each allele was measured as described previously. The allelic expression ratio was calculated by dividing the copies/ml of the risk allele by the copies/ml of the non-risk allele. No allelic expression imbalance was observed in HUVEC cells with or without IL-1 treatment.

EXAMPLE 19

Mouse IBD models

[00418] To evaluate role of TL1A in IBD, a series of preclinical experiments were performed in mouse IBD models. The effect of TL1A inhibition was evaluated in mouse mdr1a-/- spontaneous colitis model. Mdr1a gene encodes multiple drug resistance gene for P-glycoprotein 170. Mice with knockout of mdr1a gene are prone to develop spontaneous colitis from 12 weeks of age due to weakened intestinal barrier. Disease course is affected by gut microbes. In our experiment, female $Mdr1a^{-/-}$ or wild type FVB controls were obtained from Taconic at 4-6 weeks of age. Animal weight and clinical disease activity was monitored regularly. The clinical disease activity score was arrived at using a summation of the scores obtained evaluating anal inflammation (0=none, 1=mild, 2=moderate, 3=severe, 4=rectal prolapse) and stool consistency (0=normal, 1=moist/sticky, 2=soft, 3=diarrhea, 4=bloody). Mice (n=10, 6-7 weeks age) were randomized to different groups based upon baseline clinical disease activity measurements, and treated intra-peritoneally once a week with 500 μg of anti-mouse TL1A antibody, anti-IL23p19 antibody, anti-mouse IL17RA antibody, or mouse isotype control or no treatment once per week for 8 weeks. Mice were then sacrificed, sections of the intestine were taken and processed for H&E staining prior to scoring for disease by a pathologist. The following score was assigned based upon the histopathology: 0 = normal; 1 = minimal, mononuclear infiltrate and/or epithelial hypertrophy/hyperplasia; 2 = mild, mononuclear infiltrate and/or epithelial hypertrophy/hyperplasia; 3 = moderate, mononuclear infiltrate and/or epithelial hypertrophy/hyperplasia with rare crypt abscesses; 4 = marked, same as for 3 plus abundant crypt abscesses and crypt dropout and/or focal ulceration; 5 = severe, same as for 4 but with large areas of crypt dropout and/or extensive areas of ulceration. Statistical analysis was performed using one-way ANOVA with Dunett's compared to mIgG1 group. In conclusion, prophylactic treatment with anti-TL1A mAb inhibited spontaneous colitis development in $mdr1a^{-/-}$ Mice.

[00419] The inventor(s) also evaluated if challenge with TL1A protein will exacerbate colitis development in $mdr1a^{-/-}$ mice, which are prone to develop spontaneous colitis from 12 weeks of age due to a weakened intestinal barrier. $Mdr1a^{-/-}$ mice or wild-type control mice at 6-8 weeks of age were treated intra-peritoneally once a week with 150 μg of recombinant mFc-TL1A fusion protein or isotype control three times each week for 4 weeks. Clinical disease activity was monitored by evaluating anal inflammation and stool consistency as described previously. After 4 weeks of treatment, mice were sacrificed, sections of the intestine were taken and processed for H&E staining prior to scoring for disease as described previously.

Statistical analysis was performed using one-way ANOVA with Dunett's compared to mIgG1 group. Challenge of TL1A protein severely exacerbated colitis development in mdr1a$^{-/-}$ mice but not in wild-type mice.

[00420] The inventor(s) further examined the impact of TL1A challenge in lamina propria lymphocytes in mdr1a$^{-/-}$ mice versus wild-type mice. In brief, lamina propria lymphocytes were isolated as previously described (D'Souza WN et al., JI, V168:5566-5572, 2002). In brief, intestines were isolated, cut open longitudinally, rinsed with buffer and cut into 0.5 cm pieces. They were then washed twice in EDTA and the supernatants were discarded. The pieces were washed with RPMI and incubated in collagenase/DNase for 30 minutes. The isolated cells were run through a percoll gradient and the cells collected at the interphase were stained for surface antigens and analyzed by FACS. TL1A challenge in mdr1a$^{-/-}$ mice resulted in increased inflammatory cells in lamina propria.

EXAMPLE 20

Pharmacodynamics studies

[00421] To evaluate if TL1A and TNF challenges result in similar or different pharmacodynamics effects, C57Bl/6 mice (8 week, female) were first intraperitonially injected with 500 μg/mice of anti-mouse TL1A, anti-mouse TNF-α or PBS. After 4 hours, the mice were then challenged with 100 μg/mice of TL1A or 10 μg/mice of TNF-α or without challenging. The sera were collected after 24 hours. The cytokines were measured by MSD (IL-22 was measured by ELISA). Challenge with TL1A or TNF protein in mice resulted in distinct cytokine induction. TL1A challenge mainly induced IFN-γ and IL-5, whereas TNF challenge induced IL-6, IL-8 and IL-10.

[00422] The inventor(s) evaluated cytokine induction by TL1A or TNF treatment in human PBMC. In brief, PBMC freshly isolated from human blood were cultured in media (RPMI1640 supplemented with 10% FBS, 2 mM glutamine, 1 mM sodium pyruvate, 5 X 10^{-5} M 2-ME, and antibiotics) in the presence of 100 ng/ml of human TL1A or TNF-α. Supernatant was collected after 72 hours. The cytokines in the supernatant were measured by MSD (IL-22 was measured by ELISA). Similar to the mice challenge experiment, treatment of TL1A with human PBMC resulted in induction of IFN-γ, IL-5 and IL-22, whereas TNF treatment resulted in increase of IL-8, IL-10 and MCP-1. Therefore, TL1A and TNF induce distinct cytokine profiles in human PBMC.

EXAMPLE 21

IgG-Fab molecules

[00423] Bispecific antigen binding proteins were prepared with a subset of the anti-TNFα and anti-TL1A antibodies. In some embodiments of this IgG-Fab format, a polypeptide comprising a VH-CH1 domain from a second antibody is fused through a peptide linker to the carboxyl- terminus of the heavy chain of a first antibody to form a modified heavy chain. A polypeptide comprising the remaining domains of the Fab fragment from the first antibody (i.e. a VL-CL domain) is co-expressed with the light chain of the first antibody and the modified heavy chain to produce the complete molecule. Assembly of the full molecule creates a tetravalent binding protein having two antigen binding domains against a first antigen located on the amino terminal side of a dimerized immunoglobulin Fc region and two antigen binding domains against a second antigen located on the carboxyl terminal side of the dimerized Fc region.

[00424] The TNFα/TL1A IgG-Fab consists of two antigen binding domains, one directed against TNFα and the other against TL1A. The DNA molecules encoding TNFα/TL1A IgG-Fab molecules contain fragments encoding an anti- TNFα (or anti- TL1A) antibody light chain, an anti- TNFα (or anti-TL1A) antibody heavy chain in which the C-terminus is fused to (i) an anti-TL1A (or anti- TNFα) antibody light chain or (ii) an anti-TL1A (or anti- TNFα) Fd (VH-CH1), and a third polypeptide comprising the other half of the Fab fragment to complete the carboxy-terminal binding domain; for example, (i) an anti-TL1A (or anti- TNFα) Fd or (ii) an anti-TL1A (or anti- TNFα) antibody light chain. The IgG-Fab bispecific molecules contain charge pair mutations introduced into CH1 and CL domains of each Fab region (Fab 1 and Fab 2 as illustrated in Figure 3). The charge pairs are designed to allow preferential assembly of anti-TNFAR light chain/VHCH1(Fd) pair and anti-TL1A light chain/VHCH1 (Fd) pair. As an additional approach to promote correct pairing of the light chain/VHCH1 (Fd) pair, for a subset of the IgG-Fab molecules generated, the CL and CH1 regions in the carboxyl-terminal Fab (i.e. Fab 2) were swapped such that the polypeptide fused to the carboxyl-terminal region of the heavy chain of the second antibody comprised VL and CH1 regions from the first antibody and the second polypeptide comprised VH and CL regions from the first antibody. See molecules listed in Tables 21.1 and 21.3, with the VL and VH CDRs listed in Table 21.2A and 21.2B and purity listed in Table 21.4. The DNA molecules were generated by synthesized gBlocks and cloned into the pTT5.1 vector. These expression vectors were used to transfect and express the TNFα/TL1A bispecific molecules in human 293-6E cells. 144 different IgG-Fab bispecific molecules were generated. The full sequences for each molecule are set forth in Table 21.1 and the Sequence Listing.

[00425] The IgG-Fab molecules were purified using affinity captured by MabSelect SuRe chromatography (GE Life Sciences, Piscataway, NJ) using a Large Format Autosampler (LFAS, Amgen, Inc., Thousand Oaks, CA). Clarified, conditioned media was loaded onto a 1 mL HiTrap MabSelect SuRe column (GE Life Sciences, Piscataway, NJ) equilibrated with Dulbecco's phosphate buffered saline without divalent cations (D-PBS, Life Technologies, Grand Island, NY). MabSelect columns were washed with 8 column volumes of D-PBS and eluted with 100 mM acetic acid, pH 3.6. When protein A eluates had an absorbance above 5 mAU at 280 nm, the eluent was directly loaded onto a HiTrap Desalting column (GE Life Sciences, Piscataway, NJ) and developed with 1.2 column volumes of 10 mM sodium acetate, 150 mM NaCl, pH 5.0. When desalting eluates had an absorbance above 3 mAU at 280 nm, sample collection was triggered and fractions were collected in 96-well deepwell blocks to a maximum of 2 mL each. Sample purity was determined by Caliper LabChip analysis under reducing (with 2% 2-mercaptoethanol) and non-reducing (with 25 mM iodoacetamide) conditions. Analytical SEC was carried out using a Zenix-C SEC-300 column (Sepax Technologies, Newark, DE) with an isocratic elution in 50 mM sodium phosphate, 250 mM NaCl, pH 6.9 over 8'.

[00426] The IgG-Fab molecules were tested for their expressability (titer and recovery) and activity. The results are shown in Figures 27 to 33 and in Table 21.3. In Table 21.3 and throughout, "ada" refers to adalimumab.

[00427] TL1A activity assay was performed using TF-1 NF-κB reporter cell line. In brief, 30 ng/ml (EC90) of human or cynomolgus monkey TL1A was incubated with 10^4 TF-1 NF-κB reporter cells in the presence of serially diluted anti-TL1A antibodies or TL1A/TNF–α bispecific molecules in 96-well plate at 37 °C overnight. Each well was supplemented with 50 µl of Steady-glo Luciferase testing solution (Promega). Plate was covered and incubated while shaking for 10 minutes. Luciferase activity was analyzed by microbeta reader.

[00428] TNFα activity assay was performed using TF-1 NF-kB reporter cell line. In brief, 1 ng/ml (EC90) of human or cynomolgus monkey TNF–α was incubated with 10^4 TF-1 NF-κB reporter cells in the presence of a serially diluted anti-TNF–α antibodies or TL1A/TNFα bispecific molecules in 96-well plate at 37 °C overnight. 50 µl of Steady-glo Luciferase testing solution (Promega) was added to each well. Plate was covered and incubated while shaking for 10 minutes. Luciferase activity was analyzed by microbeta reader.

[00429] Table 21.1: anti-TL1A anti-TNF-α IgG-Fab molecule amino acid sequences

Molecule Designation (iPS no.)	IgG source, Fab source, aa substitutions	Light chain 1 SEQ ID NO	Light chain 2 SEQ ID NO	Heavy chain SEQ ID NO
376597	001_Adalimumab_IgG.001_3B3v2_VH_CH1 (S183E)	1254	1256	1258
376601	002_Adalimumab_IgG.001_9C8_VH_CH1(S 183E)	1260	1262	1264
376605	003_Adalimumab_IgG.001_23B3_VH4_VH_ CH1(S183E)	1266	1268	1270
376609	004_3.2_IgG.001_3B3v2_VH_CH1(S183E)	1272	1274	1276
376613	005_3.2_IgG.001_9C8_VH_CH1(S183E)	1278	1280	1282
376617	006_3.2_IgG.001_23B3_VH4_VH_CH1(S18 3E)	1284	1286	1288
376621	007_3B3v2_IgG.001_Adalimumab_VH_CH1 (S183E)	1290	1292	1294
376625	008_3B3v2_IgG.001_3.2_VH_CH1(S183E)	1296	1298	1300
376629	009_9C8_IgG.001_Adalimumab_VH_CH1(S 183E)	1302	1304	1306
376633	010_9C8_IgG.001_3.2_VH_CH1(S183E)	1308	1310	1312
376637	011_23B3_VH4_IgG.001_Adalimumab_VH_ CH1(S183E)	1314	1316	1318
376641	012_23B3_VH4_IgG.001_3.2_VH_CH1(S18 3E)	1320	1322	1324
376645	013_Adalimumab_CK(S176K)_Fc_3B3v2_V H_CH1(S183E)	1326	1328	1330
376651	014_Adalimumab_CK(S176K)_Fc_9C8_VH_ CH1(S183E)	1332	1334	1336
376655	015_Adalimumab_CK(S176K)_Fc_23B3_VH 4_VH_CH1(S183E)	1338	1340	1342
376659	016_3.2_CL(S176K)_Fc_3B3v2_VH_CH1(S 183E)	1344	1346	1348
376665	017_3.2_CL(S176K)_Fc_9C8_VH_CH1(S18 3E)	1350	1352	1354
376669	018_3.2_CL(S176K)_Fc_23B3_VH4_VH_C H1(S183E)	1356	1358	1360
376673	019_3B3v2_CK(S176K)_Fc_Adalimumab_V H_CH1(S183E)	1362	1364	1366
376679	020_3B3v2_CK(S176K)_Fc_3.2_VH_CH1(S 183E)	1368	1370	1372

376683	021_9C8_CK(S176K)_Fc_Adalimumab_VH_CH1(S183E)	1374	1376	1378
376689	022_9C8_CK(S176K)_Fc_3.2_VH_CH1(S183E)	1380	1382	1384
376693	023_23B3_VH4_CK(S176K)_Fc_Adalimumab_VH_CH1(S183E)	1386	1388	1390
376699	024_23B3_VH4_CK(S176K)_Fc_3.2_VH_CH1(S183E)	1392	1394	1396
376703	025_Adalimumab_IgG.001_3B3v2_VH_CK(S176E) NA	1398	1400	1402
376709	026_Adalimumab_IgG.001_9C8_VH_CK(S176E)	1404	1406	1408
376715	027_Adalimumab_IgG.001_23B3_VH4_VH_CK(S176E)	1410	1412	1414
376721	028_3.2_IgG.001_3B3v2_VH_CK(S176E)	1416	1418	1420
376725	029_3.2_IgG.001_9C8_VH_CK(S176E)	1422	1424	1426
376729	030_3.2_IgG.001_23B3_VH4_VH_CK(S176E)	1428	1430	1432
376733	031_3B3v2_IgG.001_Adalimumab_VH_CK(S176E)	1434	1436	1438
376739	032_3B3v2_IgG.001_3.2_VH_CL(S176E)	1440	1442	1444
376745	033_9C8_IgG.001_Adalimumab_VH_CK(S176E)	1446	1448	1450
376750	034_9C8_IgG.001_3.2_VH_CL(S176E)	1452	1454	1456
376755	035_23B3_VH4_IgG.001_Adalimumab_VH_CK(S176E)	1458	1460	1462
376760	036_23B3_VH4_IgG.001_3.2_VH_CL(S176E)	1464	1466	1468
376765	037_Adalimumab_CK(S176K)_Fc_3B3v2_VH_CK(S176E)	1470	1472	1474
376770	038_Adalimumab_CK(S176K)_Fc_9C8_VH_CK(S176E)	1476	1478	1480
376775	039_Adalimumab_CK(S176K)_Fc_23B3_VH4_VH_CK(S176E)	1482	1484	1486
376779	040_3.2_CL(S176K)_Fc_3B3v2_VH_CK(S176E)	1488	1490	1492
376784	041_3.2_CL(S176K)_Fc_9C8_VH_CK(S176E)	1494	1496	1498

376789	042_3.2_CL(S176K)_Fc_23B3_VH4_VH_CK(S176E)	1500	1502	1504
376793	043_3B3v2_CK(S176K)_Fc_Adalimumab_VH_CK(S176E)	1506	1508	1510
376797	044_3B3v2_CK(S176K)_Fc_3.2_VH_CL(S176E)	1512	1514	1516
376801	045_9C8_CK(S176K)_Fc_Adalimumab_VH_CK(S176E)	1518	1520	1522
376806	046_9C8_CK(S176K)_Fc_3.2_VH_CL(S176E)	1524	1526	1528
376811	047_23B3_VH4_CK(S176K)_Fc_Adalimumab_VH_CK(S176E)	1530	1532	1534
376816	048_23B3_VH4_CK(S176K)_Fc_3.2_VH_CL(S176E)	1536	1538	1540
376821	059_Adalimumab_IgG.002_3B3v2_VH_CH1(S183E).001	1542	1544	1546
376826	060_Adalimumab_IgG.002_9C8_VH_CH1(S183E).001	1548	1550	1552
376830	061_Adalimumab_IgG.002_23B3_VH4_VH_CH1(S183E).001	1554	1556	1558
376834	062_3.2_IgG.002_3B3v2_VH_CH1(S183E).001	1560	1562	1564
376868	063_3.2_IgG.002_9C8_VH_CH1(S183E).001	1566	1568	1570
376842	064_3.2_IgG.002_23B3_VH4_VH_CH1(S183E).001	1572	1574	1576
376846	065_3B3v2_IgG.002_Adalimumab_VH_CH1(S183E).001	1578	1580	1582
376850	066_3B3v2_IgG.002_3.2_VH_CH1(S183E).001	1584	1586	1588
376854	067_9C8_IgG.002_Adalimumab_VH_CH1(S183E).001	1590	1592	1594
376858	068_9C8_IgG.002_3.2_VH_CH1(S183E).001	1596	1598	1600
376862	069_23B3_VH4_IgG.002_Adalimumab_VH_CH1(S183E).001	1602	1604	1606
376867	070_23B3_VH4_IgG.002_3.2_VH_CH1(S183E).001	1608	1610	1612
376872	071_Adalimumab_CK(S176K)_Fc.001_3B3v2_VH_CH1(S183E).001	1614	1616	1618

376879	072_Adalimumab_CK(S176K)_Fc.001_9C8_VH_CH1(S183E).001	1620	1622	1624
376884	073_Adalimumab_CK(S176K)_Fc.001_23B3_VH4_VH_CH1(S183E).001	1626	1628	1630
376889	074_3.2_CL(S176K)_Fc.001_3B3v2_VH_CH1(S183E).001	1632	1634	1636
376896	075_3.2_CL(S176K)_Fc.001_9C8_VH_CH1(S183E).001	1638	1640	1642
376901	076_3.2_CL(S176K)_Fc.001_23B3_VH4_VH_CH1(S183E).001	1644	1646	1648
376906	077_3B3v2_CK(S176K)_Fc.001_Adalimumab_VH_CH1(S183E).001	1650	1652	1654
376913	078_3B3v2_CK(S176K)_Fc.001_3.2_VH_CH1(S183E).001	1656	1658	1660
376918	079_9C8_CK(S176K)_Fc.001_Adalimumab_VH_CH1(S183E).001	1662	1664	1666
376925	080_9C8_CK(S176K)_Fc.001_3.2_VH_CH1(S183E).001	1668	1670	1672
376930	081_23B3_VH4_CK(S176K)_Fc.001_Adalimumab_VH_CH1(S183E).001	1674	1676	1678
376937	082_23B3_VH4_CK(S176K)_Fc.001_3.2_VH_CH1(S183E).001	1680	1682	1684
376941	083_Adalimumab_IgG.002_3B3v2_VH_CK(S176E).001	1686	1688	1690
376948	084_Adalimumab_IgG.002_9C8_VH_CK(S176E).001	1692	1694	1696
376955	085_Adalimumab_IgG.002_23B3_VH4_VH_CK(S176E).001	1698	1700	1702
376962	086_3.2_IgG.002_3B3v2_VH_CK(S176E).001	1704	1706	1708
376967	087_3.2_IgG.002_9C8_VH_CK(S176E).001	1710	1712	1714
376972	088_3.2_IgG.002_23B3_VH4_VH_CK(S176E).001	1716	1718	1720
376976	089_3B3v2_IgG.002_Adalimumab_VH_CK(S176E).001	1722	1724	1726
376983	090_3B3v2_IgG.002_3.2_VH_CL(S176E).001	1728	1730	1732
376990	091_9C8_IgG.002_Adalimumab_VH_CK(S176E).001	1734	1736	1738

376995	092_9C8_IgG.002_3.2_VH_CL(S176E).001	1740	1742	1744
377000	093_23B3_VH4_IgG.002_Adalimumab_VH_CK(S176E).001	1746	1748	1750
377005	094_23B3_VH4_IgG.002_3.2_VH_CL(S176E).001	1752	1754	1756
377010	095_Adalimumab_CK(S176K)_Fc.001_3B3v2_VH_CK(S176E).001	1758	1760	1762
377015	096_Adalimumab_CK(S176K)_Fc.001_9C8_VH_CK(S176E).001	1764	1766	1768
377020	097_Adalimumab_CK(S176K)_Fc.001_23B3_VH4_VH_CK(S176E).001	1770	1772	1774
377025	098_3.2_CL(S176K)_Fc.001_3B3v2_VH_CK(S176E).001	1776	1778	1780
377030	099_3.2_CL(S176K)_Fc.001_9C8_VH_CK(S176E).001	1782	1784	1786
377035	100_3.2_CL(S176K)_Fc.001_23B3_VH4_VH_CK(S176E).001	1788	1790	1792
377040	101_3B3v2_CK(S176K)_Fc.001_Adalimumab_VH_CK(S176E).001	1794	1796	1798
377045	102_3B3v2_CK(S176K)_Fc.001_3.2_VH_CL(S176E).001	1800	1802	1804
377050	103_9C8_CK(S176K)_Fc.001_Adalimumab_VH_CK(S176E).001	1806	1808	1810
377055	104_9C8_CK(S176K)_Fc.001_3.2_VH_CL(S176E).001	1812	1814	1816
377060	105_23B3_VH4_CK(S176K)_Fc.001_Adalimumab_VH_CK(S176E).001	1818	1820	1822
377064	106_23B3_VH4_CK(S176K)_Fc.001_3.2_VH_CL(S176E).001	1824	1826	1828
377068	117_Adalimumab_IgG.003_3B3v2_VH_CH1(S183E).002	1830	1832	1834
377072	118_Adalimumab_IgG.003_9C8_VH_CH1(S183E).002	1836	1838	1840
377077	119_Adalimumab_IgG.003_23B3_VH4_VH_CH1(S183E).002	1842	1844	1846
377082	120_3.2_IgG.003_3B3v2_VH_CH1(S183E).002	1848	1850	1852
377087	121_3.2_IgG.003_9C8_VH_CH1(S183E).002	1854	1856	1858

377092	122_3.2_IgG.003_23B3_VH4_VH_CH1(S183E).002	1860	1862	1864
377097	123_3B3v2_IgG.003_Adalimumab_VH_CH1(S183E).002	1866	1868	1870
377102	124_3B3v2_IgG.003_3.2_VH_CH1(S183E).002	1872	1874	1876
377107	125_9C8_IgG.003_Adalimumab_VH_CH1(S183E).002	1878	1880	1882
3771112	126_9C8_IgG.003_3.2_VH_CH1(S183E).002	1884	1886	1888
377116	127_23B3_VH4_IgG.003_Adalimumab_VH_CH1(S183E).002	1890	1892	1894
377121	128_23B3_VH4_IgG.003_3.2_VH_CH1(S183E).002	1896	1898	1900
377126	129_Adalimumab_CK(S176K)_Fc.002_3B3v2_VH_CH1(S183E).002	1902	1904	1906
377133	130_Adalimumab_CK(S176K)_Fc.002_9C8_VH_CH1(S183E).002	1908	1910	1912
377138	131_Adalimumab_CK(S176K)_Fc.002_23B3_VH4_VH_CH1(S183E).002	1914	1916	1918
377142	132_3.2_CL(S176K)_Fc.002_3B3v2_VH_CH1(S183E).002	1920	1922	1924
377148	133_3.2_CL(S176K)_Fc.002_9C8_VH_CH1(S183E).002	1926	1928	1930
377152	134_3.2_CL(S176K)_Fc.002_23B3_VH4_VH_CH1(S183E).002	1932	1934	1936
377156	135_3B3v2_CK(S176K)_Fc.002_Adalimumab_VH_CH1(S183E).002	1938	1940	1942
377162	136_3B3v2_CK(S176K)_Fc.002_3.2_VH_CH1(S183E).002	1944	1946	1948
377166	137_9C8_CK(S176K)_Fc.002_Adalimumab_VH_CH1(S183E).002	1950	1952	1954
377172	138_9C8_CK(S176K)_Fc.002_3.2_VH_CH1(S183E).002	1956	1958	1960
377176	139_23B3_VH4_CK(S176K)_Fc.002_Adalimumab_VH_CH1(S183E).002	1962	1964	1966
377182	140_23B3_VH4_CK(S176K)_Fc.002_3.2_VH_CH1(S183E).002	1968	1970	1972
377186	141_Adalimumab_IgG.003_3B3v2_VH_CK(S176E).002	1974	1976	1978

377193	142_Adalimumab_IgG.003_9C8_VH_CK(S176E).002	1980	1982	1984
377200	143_Adalimumab_IgG.003_23B3_VH4_VH_CK(S176E).002	1986	1988	1990
377207	144_3.2_IgG.003_3B3v2_VH_CK(S176E).002	1992	1994	1996
377212	145_3.2_IgG.003_9C8_VH_CK(S176E).002	1998	2000	2002
377217	146_3.2_IgG.003_23B3_VH4_VH_CK(S176E).002	2004	2006	2008
377222	147_3B3v2_IgG.003_Adalimumab_VH_CK(S176E).002	2010	2012	2014
377229	148_3B3v2_IgG.003_3.2_VH_CL(S176E).002	2016	2018	2020
377236	149_9C8_IgG.003_Adalimumab_VH_CK(S176E).002	2022	2024	2026
377241	150_9C8_IgG.003_3.2_VH_CL(S176E).002	2028	2030	2032
377246	151_23B3_VH4_IgG.003_Adalimumab_VH_CK(S176E).002	2034	2036	2038
377251	152_23B3_VH4_IgG.003_3.2_VH_CL(S176E).002	2040	2042	2044
377256	153_Adalimumab_CK(S176K)_Fc.002_3B3v2_VH_CK(S176E).002	2046	2048	2050
377261	154_Adalimumab_CK(S176K)_Fc.002_9C8_VH_CK(S176E).002	2052	2054	2056
377266	155_Adalimumab_CK(S176K)_Fc.002_23B3_VH4_VH_CK(S176E).002	2058	2060	2062
377271	156_3.2_CL(S176K)_Fc.002_3B3v2_VH_CK(S176E).002	2064	2066	2068
377276	157_3.2_CL(S176K)_Fc.002_9C8_VH_CK(S176E).002	2070	2072	2074
377281	158_3.2_CL(S176K)_Fc.002_23B3_VH4_VH_CK(S176E).002	2076	2078	2080
377286	159_3B3v2_CK(S176K)_Fc.002_Adalimumab_VH_CK(S176E).002	2082	2084	2086
377290	160_3B3v2_CK(S176K)_Fc.002_3.2_VH_CL(S176E).002	2088	2090	2092
377295	161_9C8_CK(S176K)_Fc.002_Adalimumab_VH_CK(S176E).002	2094	2096	2098

377300	162_9C8_CK(S176K)_Fc.002_3.2_VH_CL(S176E).002	2100	2102	2104
377305	163_23B3_VH4_CK(S176K)_Fc.002_Adalimumab_VH_CK(S176E).002	2106	2108	2110
377310	164_23B3_VH4_CK(S176K)_Fc.002_3.2_VH_CL(S176E).002	2112	2114	2116

[00430] The foregoing amino acid sequences are encoded by the nucleic acid sequences immediately preceding them in the Sequence Listing.

[00431] The CDR sequences of the VL and VH domains of the foregoing are as shown in Tables 21.2A and 21.2B below.

[00432] Table 21.2A: anti-TL1A/anti-TNF-α IgG-Fab VL CDR sequences

Ab	Type	CDRL1	CDRL2	CDRL3
Anti-TNF-alpha (Adalimumab)	NA	CGCGCGTCCCAGGGAATCCGGAATTACCTCGCA	GCCGCCTCGACTCTTCAGAGT	CAGAGATACAACCGAGCGCCTTACACA
		SEQ ID NO: 957	SEQ ID NO: 959	SEQ ID NO: 960
	AA	RASQGIRNYLA	AASTLQS	QRYNRAPYT
		SEQ ID NO: 92	SEQ ID NO: 242	SEQ ID NO: 96
Anti-TNF-alpha (3.2)	NA	ACTGGGAGCAGTTCCAACATCGGGGCAGGTTATGATGTACAC	GGTAACAGCAATCGGCCCTCA	CAGTCCTATGACAGCAGCCTGAGTGGTTCGGTG
		SEQ ID NO: 962	SEQ ID NO: 965	SEQ ID NO: 1189
	AA	TGSSSNIGAGYDVH	GNSNRPS	QSYDSSLSGSV
		SEQ ID NO: 146	SEQ ID NO: 148	SEQ ID NO: 150
Anti-TL1A (3B3v2)	NA	AGGGCCAGTCAGAGTGTTAGAAGCAGTTACTTAGCC	GGTGCATCCAGCAGGGCCACT	CAGCAGTATGGTAGCTCACCTACC
		SEQ ID NO: 94	SEQ ID NO: 467	SEQ ID NO: 104
	AA	RASQSVRSSYLA	GASSRAT	QQYGSSPT
		SEQ ID NO: 122	SEQ ID NO: 124	SEQ ID NO: 126
Anti-TL1A (9C8)	NA	CGGGCAAGTCAGAGCATTAACAACTATTTAAAT	GCTGCATCCAGTTTGCAAAGT	CAACAGAGTTACAGTACCCCTCGGACG
		SEQ ID NO: 263	SEQ ID NO: 2117	SEQ ID NO: 2118
	AA	RASQSINNYLN	AASSLQS	QQSYSTPRT
		SEQ ID NO: 110	SEQ ID NO: 112	SEQ ID NO: 108
Anti-TL1A (23B3)	NA	AGGTCCAGCCAGAGTGTGTTATACAGCTCCAACAATAAGAACTACTTAGTT	TGGGCATCTACCCGGGAATCC	CAGCAATATTATAAGACTCCTCTCACT
		SEQ ID NO: 2119	SEQ ID NO: 2120	SEQ ID NO: 2121
	AA	RSSQSVLYSSNNKNYLV	WASTRES	QQYYKTPLT
		SEQ ID NO: 128	SEQ ID NO: 118	SEQ ID NO: 120

[00433] Table 21.2B: anti-TL1A/anti-TNF-α IgG-Fab VH CDR sequences

Ab	Type	CDRH1	CDRH2	CDRH3
Anti-TNF-alpha (Adalimumab)	NA	GATTATGCGATGCAT	GCCATTACGTGGAATAGCGGACACATCGATTATGCAGACAGTGTGGAGGGC	GTCTCCTACTTGTCTACAGCTTCGTCGCTCGACTAT
		SEQ ID NO: 2122	SEQ ID NO: 2123	SEQ ID NO: 2124
	AA	DYAMH	AITWNSGHIDYADSVEG	VSYLSTASSLDY
		SEQ ID NO: 158	SEQ ID NO: 160	SEQ ID NO: 162
Anti-TNF-alpha (3.2)	NA	AGCTACTGGATCGGC	ATCATCTATCTTGGTGACTCAGATACCAGATACAGCCCGTCCTTCCAAGGC	AGTAACTGGGGTCTTGACTAC
		SEQ ID NO: 2125	SEQ ID NO: 2126	SEQ ID NO: 2127

	AA	SYWIG	IIYLGDSDTRYSPSFQG	SNWGLDY
		SEQ ID NO: 212	SEQ ID NO: 214	SEQ ID NO: 216
Anti-TL1A (3B3v2)	NA	GGTTACTACTGGAAC	GAAATCAATCATGCTGGAAACA CCAACTACAACCCGTCCCTCAA GAGT	GGATATTGTAGAAGTACCACC TGCTACTTTGACTAC
		SEQ ID NO: 2128	SEQ ID NO: 2129	SEQ ID NO: 2130
	AA	GYYWN	EINHAGNTNYNPSLKS	GYCRSTTCYFDY
		SEQ ID NO: 188	SEQ ID NO: 190	SEQ ID NO: 192
Anti-TL1A (9C8)	NA	AGTTACTTCTGGAGC	TATATCTATTACAGTGGGCAGA CCAAATACAACCCCTCCCTCAA GAGT	GAAACTGGGAGCTACTACGGC TTTGACTAC
		SEQ ID NO: 2131	SEQ ID NO: 2132	SEQ ID NO: 2133
	AA	SYFWS	YIYYSGQTKYNPSLKS	ETGSYYGFDY
		SEQ ID NO: 170	SEQ ID NO: 633	SEQ ID NO: 180
Anti-TL1A (23B3)	NA	ACCAACAGTGTTGCT TGGAAC	AGGACATACTACAGGTCCAAGT GGTATAATGATTATGCAGTTTC TCTGAAAAGT	GAGGATGGGGATAGCTACTAC CGCTACGGTATGGACGTC
		SEQ ID NO: 2134	SEQ ID NO: 2135	SEQ ID NO: 2136
	AA	TNSVAWN	RTYYRSKWYNDYAVSLKS	EDGDSYYRYGMDV
		SEQ ID NO: 182	SEQ ID NO: 196	SEQ ID NO: 186

[00434] Table 21.3: Titer, Recovery, and Activity of IgG-Fabs

iPS No.	IgG	Fab	CL/CH1 Swap	CPM	Titer (mg/L)	Recovery (mg/L)	hu TL1A (pM)	hu TL1A Fold Change	hu TNFa (pM)	hu TNFa Fold Change
376597	TNFa (ada)	TL1A (3B3v2)	No swap	v1	63.5	56.26698	197.3	3.653704	87.5	0.603448
376601	TNFa (ada)	TL1A (9C8)	No swap	v1	49.4	44.46469	136.5	0.903974	96.5	0.665517
376605	TNFa (ada)	TL1A (23B3)	No swap	v1	46	37.35632	689.6	5.56129	941.7	6.494483
376609	TNFa (3.2)	TL1A (3B3v2)	No swap	v1	28.9	37.88288	4408	81.62963	94.2	1.345714
376613	TNFa (3.2)	TL1A (9C8)	No swap	v1	34.5	41.14599	260.3	1.723841	75.3	1.075714
376617	TNFa (3.2)	TL1A (23B3)	No swap	v1	30.3	37.13529	1025	8.266129	79.6	1.137143
376621	TL1A (3B3v2)	TNFa (ada)	No swap	v1	53.1	55.23171	66.3	1.227778	33.3	0.229655
376625	TL1A (3B3v2)	TNFa (3.2)	No swap	v1	51.2	62.13592	63.8	1.181481	37.3	0.532857
376629	TL1A (9C8)	TNFa (ada)	No swap	v1	46.1	43.10706	142.1	0.94106	22.4	0.154483
376633	TL1A (9C8)	TNFa (3.2)	No swap	v1	44.3	53.37693	215.7	1.428477	44.6	0.637143
376637	TL1A (23B3)	TNFa (ada)	No swap	v1	45.9	43.74483	348.8	2.812903	27.5	0.189655
376641	TL1A (23B3)	TNFa (3.2)	No swap	v1	49.7	56.93	208.8	1.683871	43.9	0.627143
376645	TNFa (ada)	TL1A (3B3v2)	N-term swap	v1	5.82	4.959868	139.1	2.575926	131	0.903448
376651	TNFa (ada)	TL1A (9C8)	N-term swap	v1	5.73	7.33602	337.8	2.237086	157.5	1.086207
376655	TNFa (ada)	TL1A (23B3)	N-term swap	v1	6.54	6.945786	200.2	1.614516	198.5	1.368966
376659	TNFa (3.2)	TL1A (3B3v2)	N-term swap	v1	7.33	7.576692	73.6	1.362963	92.4	1.32
376665	TNFa (3.2)	TL1A (9C8)	N-term swap	v1	18.3	20.86174	91	0.602649	41.7	0.595714
376669	TNFa (3.2)	TL1A (23B3)	N-term swap	v1	21.8	21.92793	77.6	0.625806	73.5	1.05
376673	TL1A (3B3v2)	TNFa (ada)	N-term swap	v1	27.3	27.46576	4825	89.35185	41.7	0.287586
376679	TL1A (3B3v2)	TNFa (3.2)	N-term swap	v1	28.8	40.93307	6250	115.7407	39.6	0.565714

376683	TL1A (9C8)	TNFa (ada)	N-term swap	v1	33.4	28.83716	414	2.741722	35.2	0.242759
376689	TL1A (9C8)	TNFa (3.2)	N-term swap	v1	32.4	39.77615	787.1	5.212583	45	0.642857
376693	TL1A (23B3)	TNFa (ada)	N-term swap	v1	25.4	19.95322			29.5	0.203448
376699	TL1A (23B3)	TNFa (3.2)	N-term swap	v1	22.9	25.85043			40.7	0.581429
376703	TNFa (ada)	TL1A (3B3v2)	C-term swap	v1	39.2	30.58153	150.4	2.785185	78.6	0.542069
376709	TNFa (ada)	TL1A (9C8)	C-term swap	v1	50	41.99633	199.5	1.321192	80.4	0.554483
376715	TNFa (ada)	TL1A (23B3)	C-term swap	v1	40.7	29.97569	640.4	5.164516	64.2	0.442759
376721	TNFa (3.2)	TL1A (3B3v2)	C-term swap	v1	23.2	24.91097	213.2	3.948148	92.8	1.325714
376725	TNFa (3.2)	TL1A (9C8)	C-term swap	v1	26.2	31.24415	185.8	1.230464	48.3	0.69
376729	TNFa (3.2)	TL1A (23B3)	C-term swap	v1	31.7	28.69498	330.8	2.667742	79	1.128571
376733	TL1A (3B3v2)	TNFa (ada)	C-term swap	v1	36.9	31.95548	66.1	1.224074	263	1.813793
376739	TL1A (3B3v2)	TNFa (3.2)	C-term swap	v1	2.95	1.921946				
376745	TL1A (9C8)	TNFa (ada)	C-term swap	v1	24.9	22.1839	108.7	0.719868	135.3	0.933103
376750	TL1A (9C8)	TNFa (3.2)	C-term swap	v1	7.71	8.223697	223	1.476821	71.4	1.02
376755	TL1A (23B3)	TNFa (ada)	C-term swap	v1	9.11	7.361222	271.5	2.189516	433.3	2.988276
376760	TL1A (23B3)	TNFa (3.2)	C-term swap	v1	7.17	5.960717	305.6	2.464516	129.8	1.854286
376765	TNFa (ada)	TL1A (3B3v2)	Both swap	v1	3.66	4.304958	343.6	6.362963	182	1.255172
376770	TNFa (ada)	TL1A (9C8)	Both swap	v1	2.68	1.712581				0
376775	TNFa (ada)	TL1A (23B3)	Both swap	v1	4.99	2.967115	389.7	3.142742	90.4	0.623448
376779	TNFa (3.2)	TL1A (3B3v2)	Both swap	v1	4.92	3.717088	707.1	13.09444	226.8	3.24
376785	TNFa (3.2)	TL1A (9C8)	Both swap	v1	9.36	9.546385	149.5	0.990066	42	0.6
376789	TNFa (3.2)	TL1A (23B3)	Both swap	v1	12.2	11.32345	554.2	4.469355	74	1.057143
376793	TL1A (3B3v2)	TNFa (ada)	Both swap	v1	19.3	15.35021	1937.5	35.87963	347.9	2.39931
376797	TL1A (3B3v2)	TNFa (3.2)	Both swap	v1	1	0				
376801	TL1A (9C8)	TNFa (ada)	Both swap	v1	13.6	11.14213	286.8	1.899338	310	2.137931
376806	TL1A (9C8)	TNFa (3.2)	Both swap	v1	1	0.961822				
376811	TL1A (23B3)	TNFa (ada)	Both swap	v1	3.22	1.671524				
376817	TL1A (23B3)	TNFa (3.2)	Both swap	v1	1	0				
376822	TNFa (ada)	TL1A (3B3v2)	No swap	v2	53.6	43.05822	128.5	2.37963	84.7	0.584138
376826	TNFa (ada)	TL1A (9C8)	No swap	v2	27.4	22.92398	106	0.701987	80.8	0.557241
376830	TNFa (ada)	TL1A (23B3)	No swap	v2	34.2	25.0415	262	2.112903	73.3	0.505517
376834	TNFa (3.2)	TL1A (3B3v2)	No swap	v2	18	17.62724	246.8	4.57037	97	1.385714
376838	TNFa (3.2)	TL1A (9C8)	No swap	v2	17.3	17.06775	548.8	3.634437	31.6	0.451429

376842	TNFa (3.2)	TL1A (23B3)	No swap	v2	14.9	12.78592	469.85	3.789113	82.9	1.184286
376846	TL1A (3B3v2)	TNFa (ada)	No swap	v2	46.7	45.57382	71.8	1.32963	31.5	0.217241
376850	TL1A (3B3v2)	TNFa (3.2)	No swap	v2	24.4	25.47009	83.3	1.542593	84.8	1.211429
376854	TL1A (9C8)	TNFa (ada)	No swap	v2	39.5	36.01855	229.3	1.518543	30.8	0.212414
376858	TL1A (9C8)	TNFa (3.2)	No swap	v2	17.5	17.37068	190.8	1.263576	35	0.5
376862	TL1A (23B3)	TNFa (ada)	No swap	v2	23.5	19.86708	436.3	3.518548	34.4	0.237241
376867	TL1A (23B3)	TNFa (3.2)	No swap	v2	11.3	10.11778	109	0.879032	29	0.414286
376872	TNFa (ada)	TL1A (3B3v2)	N-term swap	v2	5.26	5.362855	27.725	0.513426	519.5	3.582759
376879	TNFa (ada)	TL1A (9C8)	N-term swap	v2	4.24	2.812933	253.2	1.676821	173.2	1.194483
376885	TNFa (ada)	TL1A (23B3)	N-term swap	v2	3.01	2.592265	310.3	2.502419	940.8	6.488276
376889	TNFa (3.2)	TL1A (3B3v2)	N-term swap	v2	1	0.817101				
376897	TNFa (3.2)	TL1A (9C8)	N-term swap	v2	2.76	5.494626	760	5.033113	472.9	6.755714
376902	TNFa (3.2)	TL1A (23B3)	N-term swap	v2	1	0.985458				
376907	TL1A (3B3v2)	TNFa (ada)	N-term swap	v2	36.7	29.31227	3213	59.5	35.8	0.246897
376913	TL1A (3B3v2)	TNFa (3.2)	N-term swap	v2	19.4	25.22426	813	15.05556	52.55	0.750714
376918	TL1A (9C8)	TNFa (ada)	N-term swap	v2	39.6	31.18441	203.6	1.348344	17.855	0.123138
376925	TL1A (9C8)	TNFa (3.2)	N-term swap	v2	22.4	27.25854	189.65	1.25596	29.35	0.419286
376931	TL1A (23B3)	TNFa (ada)	N-term swap	v2	22.9	20.43729	7195	58.02419	26.77	0.184621
376937	TL1A (23B3)	TNFa (3.2)	N-term swap	v2	12.2	9.722544			176.95	2.527857
376941	TNFa (ada)	TL1A (3B3v2)	C-term swap	v2	40.6	31.79866	22.73	0.420926	50.4	0.347586
376949	TNFa (ada)	TL1A (9C8)	C-term swap	v2	38.7	35.043	48.165	0.318974	49.095	0.338586
376955	TNFa (ada)	TL1A (23B3)	C-term swap	v2	30	24.1244	333.95	2.693145	57.15	0.394138
376962	TNFa (3.2)	TL1A (3B3v2)	C-term swap	v2	9.96	13.79268	540	10	1957	27.95714
376967	TNFa (3.2)	TL1A (9C8)	C-term swap	v2	8.73	12.01796				
376972	TNFa (3.2)	TL1A (23B3)	C-term swap	v2	2.58	4.419175	12490	100.7258	7870	112.4286
376976	TL1A (3B3v2)	TNFa (ada)	C-term swap	v2	26.3	16.32049	31.31	0.579815	172.2	1.187586
376983	TL1A (3B3v2)	TNFa (3.2)	C-term swap	v2	1	0.993437				
376991	TL1A (9C8)	TNFa (ada)	C-term swap	v2	40.5	38.42849	59.9	0.396689	148	1.02069
376995	TL1A (9C8)	TNFa (3.2)	C-term swap	v2	1	0.339488				
377001	TL1A (23B3)	TNFa (ada)	C-term swap	v2	25.8	25.49058	41.675	0.336089	158.45	1.092759
377006	TL1A (23B3)	TNFa (3.2)	C-term swap	v2	1	0.124147				
377011	TNFa (ada)	TL1A (3B3v2)	Both swap	v2	1	3.586728	2702	50.03704	3065.5	21.14138
377015	TNFa (ada)	TL1A (9C8)	Both swap	v2	3.2	3.312442			141050	972.7586

377020	TNFa (ada)	TL1A (23B3)	Both swap	v2	1	1.15137				
377025	TNFa (3.2)	TL1A (3B3v2)	Both swap	v2	1	0.778064				
377031	TNFa (3.2)	TL1A (9C8)	Both swap	v2	1	2.940239	17640	116.8212	12965	185.2143
377036	TNFa (3.2)	TL1A (23B3)	Both swap	v2	1	0.595615				
377041	TL1A (3B3v2)	TNFa (ada)	Both swap	v2	10.8	10.82989	1689.5	31.28704	2014.5	13.8931
377045	TL1A (3B3v2)	TNFa (3.2)	Both swap	v2	1	0				
377050	TL1A (9C8)	TNFa (ada)	Both swap	v2	22.1	16.88003	269.05	1.781788	67.2	0.463448
377056	TL1A (9C8)	TNFa (3.2)	Both swap	v2	1	0				
377060	TL1A (23B3)	TNFa (ada)	Both swap	v2	1	3.78306	2371	19.12097	171.5	1.182759
377064	TL1A (23B3)	TNFa (3.2)	Both swap	v2	1	0				
377068	TNFa (ada)	TL1A (3B3v2)	No swap	v3	69.2	61.35414	23.5	0.435185	56.1	0.386897
377073	TNFa (ada)	TL1A (9C8)	No swap	v3	64.9	57.53099	47.08	0.311788	36.795	0.253759
377077	TNFa (ada)	TL1A (23B3)	No swap	v3	60	50.44568	67.9	0.547581	49.66	0.342483
377082	TNFa (3.2)	TL1A (3B3v2)	No swap	v3	35.6	50.01275	194.9	3.609259	1213.5	17.33571
377087	TNFa (3.2)	TL1A (9C8)	No swap	v3	44.7	33.18984	148.8	0.98543	41.555	0.593643
377092	TNFa (3.2)	TL1A (23B3)	No swap	v3	35	43.58861	598.5	4.826613	38.845	0.554929
377097	TL1A (3B3v2)	TNFa (ada)	No swap	v3	70.5	74.25303	37.29	0.690556	20.85	0.143793
377102	TL1A (3B3v2)	TNFa (3.2)	No swap	v3	68.1	84.23872	31.565	0.584537	29.98	0.428286
377107	TL1A (9C8)	TNFa (ada)	No swap	v3	57	55.22568	78.35	0.518874	16.775	0.11569
377112	TL1A (9C8)	TNFa (3.2)	No swap	v3	37.9	45.76692	53.5	0.354305	26.315	0.375929
377116	TL1A (23B3)	TNFa (ada)	No swap	v3	44.3	44.02405	73.9	0.595968	15.23	0.105034
377121	TL1A (23B3)	TNFa (3.2)	No swap	v3	40.7	28.59812	62.55	0.504435	22.85	0.326429
377126	TNFa (ada)	TL1A (3B3v2)	N-term swap	v3	1	1.981845				
377133	TNFa (ada)	TL1A (9C8)	N-term swap	v3	2.65	2.948434	806.5	5.34106	552	3.806897
377138	TNFa (ada)	TL1A (23B3)	N-term swap	v3	1	1.33942				
377142	TNFa (3.2)	TL1A (3B3v2)	N-term swap	v3	10.2	11.75934	32.78	0.607037	45.34	0.647714
377148	TNFa (3.2)	TL1A (9C8)	N-term swap	v3	11.1	13.07635	99.65	0.659934	48.495	0.692786
377152	TNFa (3.2)	TL1A (23B3)	N-term swap	v3	13	12.69725	46.51	0.375081	39.74	0.567714
377156	TL1A (3B3v2)	TNFa (ada)	N-term swap	v3	30.8	34.83478	1584.5	29.34259	19.9	0.137241
377162	TL1A (3B3v2)	TNFa (3.2)	N-term swap	v3	26.2	34.40556	2504	46.37037	24.875	0.355357
377166	TL1A (9C8)	TNFa (ada)	N-term swap	v3	31.9	26.76967	83	0.549669	16.955	0.116931
377172	TL1A (9C8)	TNFa (3.2)	N-term swap	v3	31.3	38.70441	81.5	0.539735	27.26	0.389429
377176	TL1A (23B3)	TNFa (ada)	N-term swap	v3	11.3	11.48623	5305	42.78226	28.73	0.198138
377182	TL1A (23B3)	TNFa (3.2)	N-term swap	v3	14	15.5418	28185	227.2984	48.17	0.688143

377186	TNFa (ada)	TL1A (3B3v2)	C-term swap	v3	46.4	36.6753	37.57	0.695741	35.75	0.246552
377193	TNFa (ada)	TL1A (9C8)	C-term swap	v3	32	28.52527	128.05	0.848013	30.93	0.21331
377200	TNFa (ada)	TL1A (23B3)	C-term swap	v3	56.6	43.6029	217.35	1.752823	38.425	0.265
377208	TNFa (3.2)	TL1A (3B3v2)	C-term swap	v3	22.1	26.10432	212.35	3.932407	170.15	2.430714
377212	TNFa (3.2)	TL1A (9C8)	C-term swap	v3	17.6	18.67336	874	5.788079	306.8	4.382857
377217	TNFa (3.2)	TL1A (23B3)	C-term swap	v3	27.4	25.63219	271.4	2.18871	114.8	1.64
377223	TL1A (3B3v2)	TNFa (ada)	C-term swap	v3	40.5	36.07182	30.175	0.558796	167	1.151724
377229	TL1A (3B3v2)	TNFa (3.2)	C-term swap	v3	3.05	3.031093	79.5	1.472222	1711.5	24.45
377236	TL1A (9C8)	TNFa (ada)	C-term swap	v3	48.3	43.315	54.25	0.359272	61.7	0.425517
377241	TL1A (9C8)	TNFa (3.2)	C-term swap	v3	8.95	8.490845	606	4.013245	268.4	3.834286
377246	TL1A (23B3)	TNFa (ada)	C-term swap	v3	22.4	20.52445	68.7	0.554032	118.5	0.817241
377252	TL1A (23B3)	TNFa (3.2)	C-term swap	v3	5.05	5.690246	219.8	1.772581	1381	19.72857
377256	TNFa (ada)	TL1A (3B3v2)	Both swap	v3	1	3.203042				
377261	TNFa (ada)	TL1A (9C8)	Both swap	v3	1	3.032322				
377266	TNFa (ada)	TL1A (23B3)	Both swap	v3	2.57	3.395799				
377272	TNFa (3.2)	TL1A (3B3v2)	Both swap	v3	1	3.071647	13300	246.2963		
377276	TNFa (3.2)	TL1A (9C8)	Both swap	v3	14	16.05846	1237.5	8.195364	362.4	5.177143
377281	TNFa (3.2)	TL1A (23B3)	Both swap	v3	12.6	13.70576	1937.5	15.625	282.35	4.033571
377286	TL1A (3B3v2)	TNFa (ada)	Both swap	v3	25.5	29.40929	486.7	9.012963	521.5	3.596552
377290	TL1A (3B3v2)	TNFa (3.2)	Both swap	v3	1	0.304074				
377295	TL1A (9C8)	TNFa (ada)	Both swap	v3	26.3	29.74724	177.55	1.175828	132.35	0.912759
377300	TL1A (9C8)	TNFa (3.2)	Both swap	v3	3.58	4.355968	17575	116.3907	69750	996.4286
377305	TL1A (23B3)	TNFa (ada)	Both swap	v3	11	13.60209			595.5	4.106897
377310	TL1A (23B3)	TNFa (3.2)	Both swap	v3	1	0.64267				

[00435] Table 21.4: anti-TL1A/anti-TNF-α IgG-Fab purity

	SE-HPLC			Caliper								
				Non-reduced				Reduced				
PS No.	pre-MP HMW	MP	post-MP	MP 1	MP 2	MP 3	LMW	HC 1	HC 2	HC 3	LC 1	R-LC 2
376597	3.832	96		1			0	1			0.206897	0.793103
376601	2.894	97		1			0	1			0.35316	0.64684
376605	3.362	96.634		1			0	1			1	
376609	1.241	99		1			0	0.153846	0.846154		1	
376613	1.838	98		1			0	1			0.298658	0.701342
376617	1.162	99		1			0	1			1	
376621	1.827	98.172		1			0	1			0	1
376625	2.552	97		1			0	0.136503	0.863497		0.511494	0.488506
376629	1.868	98		1			0	1			0.521429	0.478571
376633	5.854	94		1			0	1			0.454225	0.545775
376637	1.099	98.901		1			0	1			0.683333	0.316667

376641	3.255	96.745		1			0	1			0.44898	0.55102
376645	8.844	63.754		1			0	1			0.336066	0.663934
376651	39.874	60		0.46	0.54		0	1			0.364929	0.635071
376655	8.191	91.809						1			0.335052	0.664948
376659	3.515	96.485		0.91			0.09	0.07438	0.92562		0.514599	0.485401
376665	2.981	97		1			0	1			0.498168	0.501832
376669	2.213	97.787		1			0	1			1	
376673	4.635	60.842		0.95			0.05	0.110115	0.889885		1	
376679	1.774	98.226		0.7			0.3	0.123494	0.203008	0.673499	1	
376683	5.787	94		0.53	0.22		0.25	1			0.330189	0.669811
376689	3.896	96.104		0.7			0.3	1			0.209386	0.790614
376693	2.32	96.131	1.548	0.8			0.1	1			1	
376699	1.27	99		0.715			0.285	1			1	
376703	7.46	93		1			0	0.164756	0.835244		0.437086	0.562914
376709	8.439	92		1			0	1			0.43985	0.56015
376715	2.451	98		0.4	0.6		0	1			0.285124	0.714876
376721	5.544	94.456		0.85			0.15	0.197595	0.802405		0.327751	0.672249
376725	7.766	71.445		1			0	1			0.413333	0.586667
376729	4.728	95		1			0	1			0.414343	0.585657
376733	9.277	91		1			0	1			0.474359	0.525641
376739	11.358	70.083						1			0.719481	0.280519
376745	18.482	82		1			0	1			0.479675	0.520325
376750	5.36	94.64		1			0	0.153509	0.846491		1	
376755	4.451	96		1			0	1			0.509225	0.490775
376760	6.609	93.391		1			0	1			1	
376765	4.751	57.868		1			0	1			0.305136	0.694864
376770	7.176	50.33						1			1	
376775	36	63.705						1			0.527027	0.472973
376779	5.25	94.75		1			0	1			0.416107	0.583893
376785	19.124	80.876		1			0	1			0.443333	0.556667
376789	2.556	97		1			0	1			0.385294	0.614706
376793	9.161	79.135		1			0	0.120112	0.879888		1	
376797												
376801	16.037	83.963		1			0	1			1	
376806	3.911	96										
376811	35.57	41.522						1			1	
376817												
376822	3.67	96		1			0	1			0.304688	0.695313
376826	1.521	98.479		1			0	0.097792	0.902208		0.487738	0.512262
376830	3.377	14.015		1			0	1			1	
376834	3.397	96.613		1			0	0.495667	0.504333		0.229858	0.770142
376838	2.644	97.356		1			0	1			0.25463	0.74537
376842	2.839	97		1			0	1			0.149877	0.850123
376846	2.28	97.72		1			0	1			1	
376850	2.28	97.72		1			0	0.332192	0.667808		0.735577	0.264423
376854	0.963	99.037		1			0	1			0.533951	0.466049
376858	2.903	97.097		1			0	1			0.513889	0.486111
376862	2.567	97.433		1			0	1			0.666667	0.333333
376867	2.062	97.938		1			0	1			0.495702	0.504298
376872	50.192	50						1			0.171254	0.828746
376879	10.705	65.812						1			0.303207	0.696793
376885	1.202	77.072						1			0.232628	0.767372
376889	9.579	83.982									0.546	0.454
376897	6.197	93.803						1			0.540146	0.459854
376902	7.841	92.1259						1			1	
376907	2.508	97.492		0.81			0.19	0.213946	0.786054		0.176152	0.823848
376913	1.59	98.41		0.63763			0.3621	0.589091	0.410909		0.142539	0.857461
376918	2.689	97		0.607			0.393	1			0.310078	0.689922
376925	2.701	97.479					1	1			0.245283	0.754717
376931	3.825	17	23.971	0.845			0.15	1			1	
376937	6.425	73	6.777	1			0	1			1	
376941	6.09	93.91		0.995			0	0.104452	0.895548		0.456731	0.543269

376949	4.915	95.085		0.136	0.805		0.059	1			0.432161	0.567839
376955	3.589	96.411		0.197	0.287	0.51	0	1			0.222841	0.777159
376962	5.594	86.48	7.926	0.636074	0.636074		0.363926	1			0.321678	0.678322
376967	6.547	93.453		1			0	1			0.486869	0.513131
376972	3.031	96.969						1			0.42549	0.57451
376976	3.387	96.613		1			0	1			0.487965	0.512035
376983	34.082	66										
376991	9.467	90.533		1			0	1			0.501219	0.498781
376995	0											
377001	2.545	97.455		1			0	1			0.519288	0.480712
377006	0	66.136	33.864									
377011	2.172	79						0.544944	0.455056		0.247312	0.752688
377015	9.233	57						1			1	0
377020	7.127	85	51.551									
377025	8.132	85	15.948									
377031	3.924	91						0.473149	0.526851		0.577419	0.422581
377036	3.788	96										
377041	16.2361	83.982		1			0	0.315871	0.684129		1	
377045												
377050	11.164	77	10.496	1			0	1			1	
377056												
377060	7.075	77	33.064					1			1	
377064												
377068	3.292	97		1			0	1			0.299145	0.700855
377073	1.927	98.074						1			0.376947	0.623053
377077	2.583	97						1			0.110749	0.889251
377082	1.135	99		1			0	0.225086	0.774914		0.071429	0.928571
377087	2.006	97.929		1			0	1			0.274933	0.725067
377092	1.994	98.006		1			0	1			0.094395	0.905605
377097	1.261	98.739		1			0	1			1	
377102	2.11	97.767		1			0	0.164251	0.835749		0.501319	0.498681
377107	1.501	98		1			0	1			0.485893	0.514107
377112	3.322	97		1			0	1			0.468657	0.531343
377116	2.65	97		1			0	1			0.557003	0.442997
377121	3.284	97		1			0	1			0.459701	0.540299
377126	12.404	66						1			1	
377133	6.553	57						1			0.436508	0.563492
377138	3.988	73						1			0.400932	0.599068
377142	2.077	98		1			0	1			0.52861	0.47139
377148	3.772	96		1			0	1			0.529745	0.470255
377152	1.436	99		1			0	1			0.50289	0.49711
377156	2.614	77		1			0	0.118734	0.886544		1	
377162	1.394	98.536		0.64			0.33	0.065466	0.145663	0.485	0.172237	0.827763
377166	5.575	94	2.313	0.295	0.45		0.254	1			0.359116	0.640884
377172	3.898	96		0.261	0.325		0.414	1			0.349333	0.650667
377176	19.399	77		1			0	1			1	
377182	32.588	50					1	1			1	
377186	6.813	93		1			0	0.171975	0.828025		0.454054	0.545946
377193	1.394	98.605		1			0	1			0.446108	0.553892
377200	5.103	95		0.15	0.165	0.688	0	1			0.334448	0.665552
377208	3.092	97		0.84			0.16	0.229787	0.770213		0.338983	0.661017
377212	20.005	80		0.867			0.133	1			0.412888	0.587112
377217	5.089	95		1			0	1			0.44709	0.55291
377223	7.45	93		1			0	0.065574	0.934426		0.497423	0.502577
377229	20.138	62						1			1	
377236	18.44	82		1			0	1			0.488439	0.511561

377241	10.022	90						1			1	
377246	7	92.694		1			0	1			0.519878	0.480122
377252	16	83.804	6.111					1			1	
377256	0	100									1	
377261	0	100		1			0				1	
377266	0	100						1			1	
377272	8.675	84						1			0.387841	0.612159
377276	11.006	88.626		1			0	1			0.486486	0.513514
377281	4.722	95.279		1			0	1			0.485488	0.514512
377286	5.497	64		1			0	0.134328	0.865672		1	
377290	0	100										
377295	13.807	10.899		1			0	1			1	
377300	6.932	87						1			0.566553	0.433447
377305	11.808	83		1			0	0.331418	0.668582		0.8125	0.1875
377310	0	100										

[00436] All publications, patents, and patent applications discussed and cited herein are hereby incorporated by reference in their entireties. It is understood that the disclosed invention is not limited to the particular methodology, protocols and materials described as these can vary. It is also understood that the terminology used herein is for the purposes of describing particular embodiments only and is not intended to limit the scope of the appended claims.

[00437] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the claims that follow.

ABBREVIATIONS

[00438] Abbreviated terms used throughout this specification are defined as follows.

aa, AA	amino acid
AEI	allelic expression imbalance
ANOVA	analysis of variance
BSA	bovine serum albumin
CDR	complementarity determining regions
CHO	Chinese hamster ovary cells
CPM	charge pair mutation
DMEM	Dulbecco's Modified Eagle Medium
DMSO	dimethyl sulfoxide
ELISA	enzyme-linked immunosorbent assay
eQTL	expression quantitative trait loci
ESI-TOF	electrospray ionization time of flight

ESN	exhausted supernatant
FACS	fluorescence-activated cell sorting
FBS	fetal bovine serum
FPLC	fast protein liquid chromatography
FVB	a strain of mice inbred for the Friend leukemia virus 1b (Fv1b) allele
H&E	Hematoxylin and eosin
HA	hypoxanthine
HIC	hydrophobic interaction chromatography
HPLC	high performance liquid chromatography
HRP	horse radish peroxidase
HUVEC	human umbilical vein epithelial cell
IBD	inflammatory bowel disease
IDMEM	DMEM without glutamine
IFN	interferon
IL	interleukin
MCP	monocyte chemotactic protein
MSD	macromolecular structure database
NA	nucleic acid
PBMC	peripheral blood mononuclear cell
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PEG	polyethylene glycol
PEI	polyethylenimine
QTL	quantitative trait loci
RPMI	media developed at Roswell Park Memorial Institute
RT-PCR	polymerase chain reaction at room temperature
SNP	single nucleotide polymorphism
TFA	trifluoroacetic acid
TL1A	TNF-like ligand 1A (TNFSF15)
TMB	tetramethylbenzene
TNF	tumor necrosis factor-α

CLAIMS

What is claimed is:

1. An antigen binding protein comprising a TL1A binding entity and a second binding entity that is not a TL1A binding entity, wherein:
   a. the TL1A binding entity has one or two light chain variable domains and one or two heavy chain variable domains;
   b. the TL1A binding entity light chain variable domain comprises a LCDR1, LCDR2, and LCDR3;
   c. the TL1A binding entity heavy chain variable domain comprises a HCDR1, HCDR2, and HCDR3; and
   d. the HCDR1 has a sequence selected from Tables A, B, and C (SEQ ID NOS: 164, 170, 182, 188, 777, 783, 792, 798, 804, 809, 815, 819, 265, 836, 847, 857, 639, 654, 655, 632, 664, 955, 979, 994, 1010, 1017, 1022, 1027, 1028, 1029, 1037, 1045, 1087, 253, 256, 259, 262, 268, 271, 265, and 950);
   e. the HCDR2 has a sequence selected from Tables A, B, and C (SEQ ID NOS: 166, 172, 178, 184, 190, 196, 202, 483, 485, 779, 785, 794, 800, 811, 817, 821, 827, 832, 838, 843, 849, 853, 859, 864, 640, 204, 678, 679, 644, 648, 649, 633, 656, 659, 660, 665, 666, 677, 114, 227, 230, 691, 715, 669, 711, 971, 972, 973, 974, 975, 980, 981, 982, 983, 984, 995, 996, 997, 998, 0999, 1011, 1023, 1030, 1038, 1039, 1040, 1041, 1042, 1046, 1047, 1048, 1058, 1059, 1060, 1061, 1062, 1066, 1067, 1068, 1069, 1070, 1074, 1088, 1089, 1090, 1095, 1096, 1097, 1102, 1103, 1104, 1108, 1109, 1110, 1111, 1112, 254, 260, 184, 269, 272, 266, 951, 953, 956, and 963); and
   f. the HCDR3 sequence is selected from Tables A, B, and C (SEQ ID NOS: 168, 174, 180, 186, 192, 489, 641, 645, 650, 657, 676, 657, 667, 671, 255, 258, 261, 264, 267, 270, 273, 672, and 675).
2. The antigen binding protein of claim 1, wherein the HCDR3 comprises a sequence selected from Table A (SEQ ID NOS: 168, 174, 180, 186, 192, 489, 781, 787, 796, 802, 807, 813, 823, 829, 834, 840, 845, 851, 855, and 861).
3. The antigen binding protein of claim 2, wherein the HCDR3 has a sequence selected from antibodies and 9C8 and 3B3 (SEQ ID NO: 180 and 192).

4. The antigen binding protein of claim 2, wherein:
   a. the HCDR1 has a sequence selected from Table A (SEQ ID NOS: 164, 170, 182, 188, 777, 783, 792, 798, 804, 809, 815, 819, 265, 836, 847, and 857) and
   b. the HCDR2 has a sequence selected from Table A (SEQ ID NOS: 166, 172, 178, 184, 190, 196, 202, 483, 485, 779, 785, 794, 800, 811, 817, 821, 827, 832, 838, 843, 849, 853, 859, and 864).
5. The antigen binding protein of claim 3 wherein;
   a. the HCDR1 has a sequence selected from antibodies 9C8 and 3B3 (SEQ ID NO: 170 and 188) and
   b. the HCDR2 has a sequence selected from antibodies 9C8 and 3B3 (SEQ ID NO: 178 and 190).
6. The antigen binding protein of claim 1 wherein:
   a. the LCDR1 has a sequence selected from Tables A, B, and C (SEQ ID NOS: 98, 104, 110, 116, 122, 128, 467, 469, 683, 689, 235, 697, 146, 709, 713, 719, 723, 229, 226, 741, 747, 753, 773, 635, 642, 646, 651, 658, 661, 668, 759, 743, 765, 977, 986, 987, 1001, 1016, 1021, 1025, 1031, 1032, 1033, 1044, 1053, 1064, 1072, 1078, 1079, 1080, 1106, 226, 229, 232, 235, 241, 238, , 1113, , 146, and 947);
   b. the LCDR2 has a sequence selected from Tables A, B, and C (SEQ ID NOS: 100, 106, 112, 118, 124, 235, 699, 705, 721, 725, 731, 737, 749, 755, 761, 769, 636, 643, 647, 652, 662, 789, 988, 1001, 1002, 1004, 1026, 1054, 1073, 1081, 1082, 1083, 1084, 1085, 1092, 1098, 242, 935, 148, 948, and 943); and
   c. the LCDR3 has a sequence selected from Tables A, B, and C (SEQ ID NOS:102, 108, 263, 120, 126, 687, 693, 701, 707, 717, 727, 733, 739, 745, 751, 757, 763, 767, 771, 775, 637, 638, 631, 653, 663, 637, 233, 940, 978, 989, 990, 991, 992, 993, 1005, 1006, 1007, 1008, 1009, 1034, 1035, 1036, 1055, 1056, 1057, 1065, 1086, 1093, 1094, 1099, 1100, 1101, 1107, 228, 231, 234, 237, 243, 240, 674, 936, 938, 701, 707, , 949, , 942, and 944).
7. The antigen binding protein of claim 2 wherein the LCDR3 has a sequence selected from Table A (SEQ ID NOS: 102, 108, 263, 120, 126, 687, 693, 701, 707, 717, 727, 733, 739, 745, 751, 757, 763, 767, 771, and 775).

8. The antigen binding protein of claim 3 wherein the LCDR3 has a sequence selected from antibodies 9C8 and 3B3 (SEQ ID NOS: 263 and 126).

9. The antigen binding protein of claim 7, wherein:

   a. the LCDR1 has a sequence selected from Table A (SEQ ID NOS: 98, 104, 110, 116, 122, 128, 467, 469, 683, 689, 235, 697, 146, 709, 713, 719, 723, 229, 226, 741, 747, 753, and 773) and

   b. the LCDR2 has a sequence selected from Table A (SEQ ID NOS: 100, 106, 112, 118, 124, 685, 699, 705, 721, 725, 731, 737, 749, 755, 761, and 769).

10. The antigen binding protein of claim 8 wherein:

   a. the LCDR1 has a sequence selected from antibodies 9C8 and 3B3 (SEQ ID NOS: 110 and 122) and

   b. the LCDR2 has a sequence selected from antibodies 9C8 and 3B3 (SEQ ID NOS: 112 and 124).

11. The antigen binding protein of claim 1 wherein the heavy chain variable domain comprises a sequence at least about 90% identical to a heavy chain variable domain sequence selected from Table D (SEQ ID NOS: 8, 12, 16, 20, 24, 28, 32, 495, 497, 868, 872, 876, 880, 884, 888, 892, 896, 900, 904, 908, 912, 916, 920, 926, 930, and 934).

12. The antigen binding protein of claim 1 wherein the heavy chain variable domain comprises a heavy chain variable domain sequence selected from Table D (SEQ ID NOS: 8, 12, 16, 20, 24, 28, 32, 495, 497, 868, 872, 876, 880, 884, 888, 892, 896, 900, 904, 908, 912, 916, 920, 926, 930, and 934).

13. The antigen binding protein of claim 1 wherein the heavy chain variable domain comprises a heavy chain variable domain sequence selected from antibodies 9C8 and 3B3 (SEQ ID NOS: 16 and 24).

14. The antigen binding protein of claim 1, wherein the light chain variable domain comprises a sequence at least about 90% identical to a light chain variable domain sequence selected from Table D (SEQ ID NOS: 6, 10, 14, 18, 22, 26, 30, 491, 493, 866, 870, 874, 878, 882, 886, 890, 894, 898, 902, 906, 910, 914, 918, 922, 924, 928, and 932).

15. The antigen binding protein of claim 1, wherein the light chain variable domain comprises a light chain variable domain sequence selected from Table D (SEQ ID NOS:

6, 10, 14, 18, 22, 26, 30, 491, 493, 866, 870, 874, 878, 882, 886, 890, 894, 898, 902, 906, 910, 914, 918, 922, 924, 928, and 932).

16. The antigen binding protein of claim 11 wherein the light chain variable domain comprises a sequence at least about 90% identical to a light chain variable domain comprises a sequence at least about 90% identical to a light chain variable domain sequence selected from Table D (SEQ ID NOS: 6, 10, 14, 18, 22, 26, 30, 491, 493, 866, 870, 874, 878, 882, 886, 890, 894, 898, 902, 906, 910, 914, 918, 922, 924, 928, and 932).

17. The antigen binding protein of claim 12 wherein the light chain variable domain comprises a light chain variable domain sequence selected from Table D (SEQ ID NOS: 6, 10, 14, 18, 22, 26, 30, 491, 493, 866, 870, 874, 878, 882, 886, 890, 894, 898, 902, 906, 910, 914, 918, 922, 924, 928, and 932).

18. The antigen binding protein of claim 13 wherein the light chain variable domain sequence comprises a light chain variable domain sequence selected from antibodies 9C8 and 3B3 (SEQ ID NOS: 14 and 22).

19. The antigen binding protein of claim 1 wherein the protein comprises a heavy chain having a heavy chain sequence selected from Table E (SEQ ID NOS: 52, 56, 60, 64, 68, 72, 76, 457, 461, 1118, 1122, 1126, 1130, 1134, 1138, 1142, 1146, 1150, 1154, 1158, 1162, 1166, 1170, 1174, 1178, 1182, 1186 and 1190).

20. The antigen binding protein of claim 1 wherein the protein comprises a light chain having a light chain sequence selected from Table E (SEQ ID NOS: 66, 54, 58, 62, 66, 70, 74, 455, 459, 1116, 1120, 1124, 1128, 1132, 1136, 1140, 1144, 1148, 1152, 1156, 1160, 1164, 1168, 1172, 1176, 1180, 1184 and 1188).

21. The antigen binding protein of claim 1 comprising the light and heavy chain sequences of an antibody selected from Table E, SEQ ID NOS:

    a. 50 and 52,
    b. 54 and 56,
    c. 58 and 60,
    d. 62 and 64,
    e. 66 and 68,
    f. 70 and 72,
    g. 74 and 76,

h. 455 and 457,

i. 459 and 461

j. 1116 and 1118,

k. 1120 and 1122,

l. 1124 and 1126,

m. 1128 and 1130,

n. 1132 and 1134,

o. 1136 and 1138,

p. 1140 and 1142,

q. 1144 and 1146,

r. 1148 and 1150,

s. 1152 and 1154,

t. 1156 and 1158,

u. 1160 and 1162,

v. 1164 and 1166,

w. 1168 and 1170,

x. 1172 and 1174,

y. 1176 and 1178,

z. 1180 and 1182,

aa. 1184 and 1186, and

bb. 1188 and 1190.

22. The antigen binding protein of claims 1 to 21, wherein the second binding entity is a TNF–α binding entity.

23. The antigen binding protein of claim 22, wherein:

a. the TNF–α binding entity has one or two light chain variable domains and one or two heavy chain variable domains;

b. the TNF–α binding entity light chain variable domain comprises an LCDR1, LCDR2, and LCDR3; and

c. the TNF–α binding entity heavy chain variable domain comprises an HCDR1, HCDR2, and HCDR3.

24. The antigen binding protein of claim 23, wherein the TNF–α binding entity HCDR3 comprises a sequence selected from Table G (SEQ ID NOS: 162, 222, 210, and 216).

25. The antigen binding protein of claim 23, wherein the TNF–α binding entity HCDR3 comprises a sequence selected from certolizumab (SEQ ID NO: 222).

26. The antigen binding protein of claim 24, wherein:

a. the TNF–α binding entity HCDR1 comprises a sequence selected from Table G (SEQ ID NOS: 158, 218, 206, and 212); and

b. the TNF–α binding entity HCDR2 comprises a sequence selected from Table G (SEQ ID NOS: 160, 220, 208, and 214).

27. The antigen binding protein of claim 26 wherein the TNF binding entity light chain sequence comprises:

a. an LCDR1 sequence selected from Table G (SEQ ID NOS:92, 152, 140, and 146),

b. an LCDR2 sequence selected from Table G (SEQ ID NOS: 94, 154, 112, and 148), and

c. an LCDR3 sequence selected from Table G (SEQ ID NOS: 96, 156, 144, and 150).

28. The antigen binding protein of claim 23, wherein the TNF binding entity heavy chain variable domain comprises a sequence at least about 90% identical to a heavy chain variable domain sequence selected from Table H (SEQ ID NOS: 4, 44, 318, 40, and 36).

29. The antigen binding protein of claim 23, wherein the TNF binding entity heavy chain variable domain sequence is a heavy chain variable domain sequence selected from Table H (SEQ ID NOS: 4, 44, 318, 40, and 36).

30. The antigen binding protein of claim 23, wherein the TNF–α binding entity light chain variable domain comprises a sequence at least about 90% identical to a light chain variable domain sequence selected from Table H (SEQ ID NOS: 2, 42, 38, and 34).

31. The antigen binding protein of claim 23, wherein the TNF binding entity comprises light chain variable domain sequence is a light chain variable domain sequence selected from Table H (SEQ ID NOS: 2, 42, 38, and 34).

32. The antigen binding protein of claim 28, wherein the TNF–α binding entity light chain variable domain comprises a sequence at least about 90% identical to a light chain variable domain sequence selected from Table H (SEQ ID NOS: 2, 42, 38, and 34).

33. The antigen binding protein of claim 29, wherein the TNF binding entity light chain variable domain sequence is a light chain variable domain sequence selected from Table H (SEQ ID NOS: 2, 42, 38, and 34).

34. The antigen binding protein of claim 23, wherein the antigen binding protein comprises variable region sequences selected from an antigen binding protein in Table I, SEQ ID NOS:

a. 42, 286, 288, and 290;
b. 42, 44, 292, and 294;
c. 42, 44, 296, and 298;
d. 42, 44, 300 and 302;
e. 42, 44, 304, and 306;
f. 42, 44, 308, and 310;
g. 312, 314, 288, and 290;
h. 312, 314, 292, and 294;
i. 312, 314, 300 and 302;
j. 312, 314, 304, and 306;
k. 312, 314, 308, and 310;
l. 42, 318, 288, and 290;
m. 42, 318, 292, and 294;
n. 42, 318, 296, and 298;
o. 42, 318, 304, and 306;
p. 42, 318, 308, and 310;
q. 320, 322, 288, and 290;
r. 320; 322, 292, and 294;
s. 320, 322, 31, and 298;
t. 320, 322, 300, and 302;
u. 320, 322, 304, and 306; or
v. 320, 322, 308, and 310.

35. The antigen binding protein of claim 23, wherein:

a. the heavy and light chains are IgG1, IgG2, or IgG4;

b. the antigen binding protein comprises one TL1A binding entity heavy chain variable domain, one TL1A binding entity light chain variable domain, one TNF–α binding entity heavy chain variable domain, and one TNF–α binding entity light chain variable domain;

c. the TL1A binding entity light chain variable domain is comprised in a light chain separate from the TL1A binding entity heavy chain variable domain, the TNF–α binding entity heavy chain variable domain, and the TNF–α binding entity light chain variable domain;

d. the TL1A binding entity heavy chain variable domain is comprised in a heavy chain separate from the TL1A binding entity light chain variable domain, the TNF–α binding entity heavy chain variable domain, and the TNF–α binding entity light chain variable domain;

e. the TNF–α binding entity heavy chain variable domain is comprised in a heavy chain separate from the TNF–α binding entity light chain domain, the TL1A binding entity heavy chain variable domain, and the TL1A binding entity light chain variable domain;

f. the heavy chain comprising the TL1A binding entity heavy chain variable domain is covalently bound to the light chain comprising the TL1A binding entity light chain variable domain;

g. the heavy chain comprising the TNF–α binding entity heavy chain variable domain is covalently bound to the light chain comprising the TNF–α binding entity light chain variable domain; and

h. the heavy chain comprising the TL1A binding entity heavy chain variable domain is covalently bound to the heavy chain comprising the TNF–α binding entity heavy chain variable domain.

36. The antigen binding protein of claim 35, wherein:

a. the TL1A binding entity light chain sequence comprises:

i. an LCDR1 sequence selected from Tables A, B, and C (SEQ ID NOS: 98, 104, 110, 116, 122, 128, 467, 469, 635, 642, 646, 651, 658, 661, 668, 226, 229, 232, 235, 241, 238 and) and

ii. an LCDR2 sequence selected from Tables A, B, and C (SEQ ID NOS: 100, 106, 112, 118, 124, 636, 643, 647, 652, 662, 227, 230, 233, 242, and 473);

iii. an LCDR3 sequence selected from Tables A, B, and C (SEQ ID NOS: 102, 108, 263, 120, 126, 637, 638, 631, 653, 663, 228, 231, 234, 237, 243, 240, and 674),

b. the TL1A binding entity heavy chain sequence comprises:

i. an HCDR1 sequence selected from Tables A, B, and C (SEQ ID NOS: 164, 170, 182, 188, 639, 654, 655, 632, 664, 253, 256, 259, 262, 268, 271, and 265),

ii. an HCDR2 sequence selected from Tables A, B, and C (SEQ ID NOS: 166, 172, 178, 184, 190, 196, 202, 483, 485, 640, 204, 644, 648, 633, 656, 659, 660, 665, 666, 114, 669, 254, 172, 260, 184, 269, 272, 266, and 254), and

iii. an HCDR3 sequence selected from Tables A, B, and C (SEQ ID NOS: 168, 174, 180, 186, 192, 489, 641, 645, 650, 657, 676, 657, 667, 671, 255, 258, 261, 264, 267, 270, 273, 672, and 675);

c. the TNF–α binding entity light chain sequence comprises:

i. an LCDR1 sequence selected from Table G (SEQ ID NOS:92, 152, 140, and 146),

ii. an LCDR2 sequence selected from Table G (SEQ ID NOS: 94, 154, 112, and 148), and

iii. an LCDR3 sequence selected from Table G (SEQ ID NOS: 96, 156, 144, and 150); and

d. the TNF–α binding entity heavy chain sequence comprises:

i. an HCDR1 sequence selected from Table G (SEQ ID NOS:158, 218, 206, and 212),

ii. an HCDR2 sequence selected from Table G (SEQ ID NOS:160, 220, 208, and 214), and

iii. an HCDR3 sequence selected from Table G (SEQ ID NOS: 162, 222, 210, and 216).

37. The antigen binding protein of claim 36, wherein:

a. the TL1A binding entity light chain sequence comprises:

i. an LCDR1 sequence selected from Table A (SEQ ID NOS: 98, 104, 110, 116, 122, 128, 467, and 469),

ii. an LCDR2 sequence selected from Table A (SEQ ID NOS: 100, 106, 112, 118, and 124);

iii. an LCDR3 sequence selected from Table A (SEQ ID NOS: 102, 108, 263, 120, and 126),

b. the TL1A binding entity heavy chain sequence comprises:

i. an HCDR1 sequence selected from Table A (SEQ ID NOS: 164, 170, 182, and 188),

ii. an HCDR2 sequence selected from Table A (SEQ ID NOS: 166, 172, 178, 184, 190, 196, 202, 483, and 485), and

iii. an HCDR3 sequence selected from Table A (SEQ ID NOS: 168, 174, 180, 186, 192, and 489).

38. The antigen binding protein of claim 35, wherein the antigen binding protein comprises variable region sequences selected from an antigen binding protein in Table I, SEQ ID NOS:

a. 42, 286, 288, and 290;

b. 42, 44, 292, and 294;

c. 42, 44, 296, and 298;

d. 42, 44, 300 and 302;

e. 42, 44, 304, and 306;

f. 42, 44, 308, and 310;

g. 312, 314, 288, and 290;

h. 312, 314, 292, and 294;

i. 312, 314, 300 and 302;

j. 312, 314, 304, and 306;

k. 312, 314, 308, and 310;

l. 42, 318, 288, and 290;

m. 42, 318, 292, and 294;

n. 42, 318, 296, and 298;

o. 42, 318, 304, and 306;

p. 42, 318, 308, and 310;

q. 320, 322, 288, and 290;

r. 320; 322, 292, and 294;

s. 320, 322, 31, and 298;

t. 320, 322, 300, and 302;

u. 320, 322, 304, and 306; or

v. 320, 322, 308, and 310.

39. The antigen binding protein of claim 38, wherein the antigen binding protein comprises sequences selected from an antigen binding protein in Table J, SEQ ID NOS:

i. 132, 136, 134, and 130;

j. 132, 136, 239, and 138;

k. 132, 136, 253, and 323;

l. 132, 136, 327, and 325;

m. 132, 136, 331, and 329;

n. 132, 136, 335, and 329;

o. 337, 339, 134, and 130;

p. 337, 339, 323, and 253;

q. 337, 339, 327, and 325;

r. 337, 339, 331, and 329;

s. 337, 339, 335, and 329;

t. 132, 316, 239, and 138;

u. 132, 316, 323, and 253;

v. 132, 316, 335, and 329;

w. 132, 316, 331, and 329;

x. 333, 316, 134, and 130;

y. 333, 316, 239, and 138;

z. 333, 463, 323, and 253;

aa. 333, 463, 331, and 329;

bb. 333, 463, 335, and 329;

cc. 341, 343, 134, and 130;

dd. 341, 343, 239, and 138;

ee. 341, 343, 323, and 253;

ff. 341, 343, 327, and 325;

gg. 341, 343, 331, and 329;

hh. 341, 343, 335, and 329;

ii. 510, 514, 512, and 555;

jj. 510, 514, 541, and 558;

kk. 510, 514, 539, and 562;

ll. 510, 514, 543, and 565;

mm. 510, 514, 543, and 568;

nn. 510, 514, 544, and 568;

oo. 542, 571, 512, and 555;

pp. 542, 571, 541, and 558;

qq. 542, 571, 539, and 562;

rr. 542, 571, 543, and 565;

ss. 542, 571, 543, and 568;

tt. 542, 571, 544, and 568;

uu. 510, 573, 512, and 555;

vv. 510, 573, 541, and 558;

ww. 510, 573, 539, and 562;

xx. 510, 573, 543, and 568;

yy. 510, 573, 544, and 568;

zz. 540, 573, 512, and 555;

aaa. 540, 573, 541, and 558;

bbb. 540, 573, 539, and 562;

ccc. 540, 573, 543, and 568;

ddd. 540, 573, 544, and 568;

eee. 540, 487, 512, and 555;

fff. 540, 487, 541, and 558;

ggg. 540, 487, 539, and 562;

hhh. 540, 487, 543, and 568;

iii. 540, 487, 544, and 568;

jjj. 518, 522, 512, and 555;

kkk. 518, 522, 541, and 558;

lll. 518, 522, 539, and 562;

mmm. 518, 522, 543, and 565;

nnn. 518, 522, 543, and 568;

ooo. 518, 522, 544, and 568;

ppp. 545, 578, 546, and 579;

qqq. 545, 578, 548, and 562;

rrr. 545, 578, 549, and 585;

sss. 545, 578, 551, and 591;

uuu. 545, 578, 552, and 591;

vvv. 547, 595, 546, and 579;

www. 547, 595, 548, and 582;

xxx. 547, 595, 549, and 585;

yyy. 547, 595, 550, and 588;

zzz. 547, 595, 551, and 591;

aaaa. 547, 595, 552, and 591;

bbbb. 132, 599, 134, and 598;

cccc. 132, 599, 239, and 601;

dddd. 132, 599, 323, and 603;

eeee. 132, 599, 327, and 605;

ffff. 132, 599, 331, and 607;

gggg. 132, 599, 335, and 607;

hhhh. 337, 609, 134, and 598;

iiii. 337, 609, 239, and 601;

jjjj. 337, 609, 323, and 603;

kkkk. 337, 609, 327, and 605;

llll. 337, 609, 335, and 607;

mmmm. 132, 611, 134, and 598;

nnnn. 132, 611, 239, and 601;

oooo. 132, 611, 323, and 603;

pppp. 132, 611, 331, and 607;

qqqq. 132, 611, 335, and 607;

rrrr. 333, 611, 134, and 598;

ssss. 333, 611, 239, and 601;

tttt. 333, 611, 323, and 603;

uuuu. 333, 611, 331, and 607;

vvvv. 333, 611, 335, and 607;

wwww. 333, 613, 134, and 598;

xxxx. 333, 613, 239, and 601;

yyyy. 333, 613, 323, and 603;

zzzz. 333, 613, 335, and 607;

aaaaa. 341, 615, 134, and 598;

bbbbb. 341, 615, 239, and 601;

ccccc. 341, 615, 323, and 603;

ddddd. 341, 615, 327, and 605;

eeeee. 341, 615, 331, and 607;

fffff. 341, 615, 335, and 607;

ggggg. 333, 613, 331, and 607;

hhhhh. 132, 463, 239, and 138;

iiiii. 132, 463, 331, and 329;

jjjjj. 132, 463, 335, and 320;

kkkkk. 510, 487, 512, and 555;

lllll. 510, 487, 541, and 558;

mmmmm. 510, 487, 543, and 568;

nnnnn. 510, 487, 544, and 568;

ooooo. 132, 613, 134, and 598;

ppppp. 132, 613, 323, and 603;

qqqqq. 132, 613, 331, and 607;

rrrrr. 132, 613, 335, and 607;

sssss. 132, 463, 134, and 130;

ttttt. 540, 616, 512, and 508;

uuuuu. 540, 620, 512, and 508;

vvvvv. 540, 616, 541, and 558;

wwwww. 540, 616, 539, and 562;

xxxxx. 540, 620, 539, and 562;

yyyyy. 538, 623, 512, and 502;

zzzzz. 537, 626, 512, and 508;

aaaaaa. 546, 514, 194, and 535;

bbbbbb. 536, 616, 194, and 535;

cccccc. 536, 620, 194, and 535;

dddddd. 546, 487, 194, and 535;

eeeeee. 471, 136, 473, and 198;

ffffff. 176, 136, 520, and 198;

gggggg. 475, 477, 520, and 198;

hhhhhh. 471, 463, 473, and 198;

iiiiii. 176, 463, 473, and 198;

jjjjjj. 471, 465, 473, and 198;

kkkkkk. 176, 463, 570, and 198;

llllll. 471, 465, 473, and 198;

mmmmmm. 176, 465, 520, and 198;

nnnnnn. 510, 514, 512, and 508;

oooooo. 518, 522, 512, and 508;

pppppp. 540, 616, 512, and 508;

qqqqqq. 540, 620, 512, and 508; and

rrrrrr. 537, 626, 512, and 508.

40. The antigen binding protein of claim 39, wherein the antigen binding protein comprises the sequences of iPS No. 376543 (SEQ ID NOS: 510, 516, 512, and 508).

41. The antigen binding protein of claim 35, wherein:

a. one heavy chain comprises substitutions K392D and K409D and the other heavy chain comprises substitutions E356K and D399K using EU numbering; or

b. one heavy chain comprises substitutions K392D, K409D and K370D and the other heavy chain comprises substitutions E356K, D399K and E357K using EU numbering.

42. The antigen binding protein of claim 35, wherein:

a. one heavy chain comprises substitution S183K, the other heavy chain comprises substitution S183E, one light chain comprises substitution S176E, and the other light chain comprises substitution S176K using EU numbering; or

b. one heavy chain comprises substitutions Q39K and S183K, the other heavy chain comprises substitutions Q39E and S183E, one light chain comprises substitutions Q38E and S176E, and the other light chain comprises substitutions Q38K and S176K using EU numbering; or

c. one heavy chain comprises substitutions G44K and S183K, the other heavy chain comprises substitutions G44E and S183E, one light chain comprises substitutions G100E and S176E, and the other light chain comprises substitutions G100K and S176K using EU numbering; or

d. one heavy chain comprises substitution S183K, the other heavy chain comprises substitution S183E, one light chain comprises substitution S176E, and the other light chain comprises substitution S176K using EU numbering.

43. The antigen binding protein of claim 41, wherein:

a. one heavy chain comprises substitution S183K, the other heavy chain comprises substitution S183E, one light chain comprises substitution S176E, and the other light chain comprises substitution S176K using EU numbering; or

b. one heavy chain comprises substitutions Q39K and S183K, the other heavy chain comprises substitutions Q39E and S183E, one light chain comprises substitutions Q38E and S176E, and the other light chain comprises substitutions Q38K and S176K using EU numbering; or

c. one heavy chain comprises substitutions G44K and S183K, the other heavy chain comprises substitutions G44E and S183E, one light chain comprises substitutions G100E and S176E, and the other light chain comprises substitutions G100K and S176K using EU numbering; or

d. one heavy chain comprises substitution S183K, the other heavy chain comprises substitution S183E, one light chain comprises substitution S176E, and the other light chain comprises substitution S176K using EU numbering.

44. The antigen binding protein of claim 35, wherein the bispecific antigen binding protein comprises two IgG1 heavy chains comprising substitutions R292C, V302C and N297G using EU numbering.

45. The antigen binding protein of claim 41, wherein the bispecific antigen binding protein comprises two IgG1 heavy chains comprising substitutions R292C, V302C and N297G using EU numbering.

46. The antigen binding protein of claim 40, wherein the bispecific antigen binding protein comprises two IgG1 heavy chains comprising substitutions R292C, V302C and N297G using EU numbering.

47. The antigen binding protein of claim 43, wherein the bispecific antigen binding protein comprises two IgG1 heavy chains comprising substitutions R292C, V302C and N297G using EU numbering.

48. The antigen binding protein of claim 23, wherein:

a. the antigen binding protein comprises two light chains and two heavy chains:

b. the heavy and light chains comprise IgG1, IgG2, or IgG4 constant domains;

c. the antigen binding protein comprises two TL1A binding entity heavy chain variable domains, two TL1A binding entity light chain variable domains, two TNF–α binding entity heavy chain variable domains, and two TNF–α binding entity light chain variable domains;

d. each light chain comprises a TL1A binding entity light chain variable domain sequence; and

e. each heavy chain comprises a TL1A binding entity heavy chain variable domain sequence, a TNF–α binding entity heavy chain variable domain sequence and a TNF–α binding entity light chain variable domain sequence.

49. The antigen binding protein of claim 48, wherein:

a. the TL1A binding entity light chain sequence comprises:

i. an LCDR1 sequence selected from Tables A, B, and C (SEQ ID NOS: 98, 104, 110, 116, 122, 128, 467, 469, 635, 642, 646, 651, 658, 661, 668, 226, 229, 232, 235, 241, 238 and) and

ii. an LCDR2 sequence selected from Tables A, B, and C (SEQ ID NOS: 100, 106, 112, 118, 124, 636, 643, 647, 652, 662, 227, 230, 233, 242, and 473);

iii. an LCDR3 sequence selected from Tables A, B, and C (SEQ ID NOS: 102, 108, 263, 120, 126, 637, 638, 631, 653, 663, 228, 231, 234, 237, 243, 240, and 674),

b. the TL1A binding entity heavy chain sequence comprises:

i. an HCDR1 sequence selected from Tables A, B, and C (SEQ ID NOS: 164, 170, 182, 188, 639, 654, 655, 632, 664, 253, 256, 259, 262, 268, 271, and 265),

ii. an HCDR2 sequence selected from Tables A, B, and C (SEQ ID NOS: 166, 172, 178, 184, 190, 196, 202, 483, 485, 640, 204, 644, 648, 633, 656, 659, 660, 665, 666, 114, 669, 254, 172, 260, 184, 269, 272, 266, and 254), and

iii. an HCDR3 sequence selected from Tables A, B, and C (SEQ ID NOS: 168, 174, 180, 186, 192, 489, 641, 645, 650, 657, 676, 657, 667, 671, 255, 258, 261, 264, 267, 270, 273, 672, and 675);

c. the TNF–α binding entity light chain sequence comprises:

i. an LCDR1 sequence selected from Table G (SEQ ID NOS:92, 152, 140, and 146),

ii. an LCDR2 sequence selected from Table G (SEQ ID NOS: 94, 154, 112, and 148), and

iii. an LCDR3 sequence selected from Table G (SEQ ID NOS: 96, 156, 144, and 150); and

d. the TNF–α binding entity heavy chain sequence comprises:

i. an HCDR1 sequence selected from Table G (SEQ ID NOS:158, 218, 206, and 212),

ii. an HCDR2 sequence selected from Table G (SEQ ID NOS:160, 220, 208, and 214), and

iii. an HCDR3 sequence selected from Table G (SEQ ID NOS: 162, 222, 210, and 216).

). The antigen binding protein of claim 49, wherein:

a. the TL1A binding entity light chain sequence comprises:

i. an LCDR1 sequence selected from Table A (SEQ ID NOS: 98, 104, 110, 116, 122, 128, 467, and 469),

ii. an LCDR2 sequence selected from Table A (SEQ ID NOS: 100, 106, 112, 118, and 124);

iii. an LCDR3 sequence selected from Table A (SEQ ID NOS: 102, 108, 263, 120, and 126),

b. the TL1A binding entity heavy chain sequence comprises:

i. an HCDR1 sequence selected from Table A (SEQ ID NOS: 164, 170, 182, and 188),

ii. an HCDR2 sequence selected from Table A (SEQ ID NOS: 166, 172, 178, 184, 190, 196, 202, 483, and 485), and

iii. an HCDR3 sequence selected from Table A (SEQ ID NOS: 168, 174, 180, 186, 192, and 489).

51. The antigen binding protein of claim 48, wherein:

a. the TL1A binding entity heavy chain variable domain comprises a heavy chain variable domain sequence selected from Table D (SEQ ID NOS: 8, 12, 16, 20, 24, 28, and 32);

b. the TL1A binding entity light chain variable domain comprises a light chain variable domain sequence selected from Table D (SEQ ID NOS: 6, 10, 14, 18, 22, 26, and 30);

c. the TNF–α binding entity light chain variable domain sequence is selected from Table H (SEQ ID NOS: 2, 42, 38, and 34) and

d. the TNF–α binding entity heavy chain variable domain sequence is selected from Table H (SEQ ID NOS: 4, 44, 318, 40, and 36).

52. The antigen binding protein of claim 48, wherein the anti-TNF-α binding entity heavy chain variable domain comprises a cysteine residue at position 44 and the anti-TNF-α light chain binding domain comprises a cysteine residue at position 100, numbering as in Kabat.

53. The antigen binding protein of claim 48 wherein the protein comprises the heavy and light chain sequences of an antigen binding protein selected from Table L, SEQ ID NOS:

a. 355 and 447,

b. 357 and 447,

c. 359 and 447,

d. 361 and 447,

e. 363 and 447,

f. 365 and 58,

g. 367 and 70,
h. 369 and 70,
i. 371 and 70,
j. 373 and 70,
k. 375 and 70,
l. 377 and 70,
m. 391 and 467,
n. 391 and 66,
o. 395 and 66,
p. 397 and 66,
q. 399 and 66,
r. 401 and 66,
s. 403 and 66,
t. 405 and 66
u. 407 and 447,
v. 409 and 447,
w. 411 and 66,
x. 413 and 66,
y. 415 and 66,
z. 417 and 66,
aa. 419 and 70,
bb. 421 and 70,
cc. 423 and 70, and
dd. 425 and 70.

54. The antigen binding protein of claim 48 wherein:

a. the light chain sequence comprises SEQ ID NO: 453 and the heavy chain sequence comprises SEQ ID NO: 441;
b. the light chain sequence comprises SEQ ID NO: 451 and the heavy chain sequence comprises SEQ ID NO: 433; or
c. the light chain sequence comprises SEQ ID NO: 453 and the heavy chain sequence comprises SEQ ID NO: 445.

55. The antigen binding protein of claim 23, wherein:

a. the antigen binding protein comprises two light chains and two heavy chains:

b. the heavy and light chains comprise IgG1, IgG2, or IgG4 constant domains;

c. the antigen binding protein comprises two TL1A binding entity heavy chain variable domains, two TL1A binding entity light chain variable domains, two TNF–α binding entity heavy chain variable domains, and two TNF–α binding entity light chain variable domains;

d. each light chain comprises a TNF–α binding entity light chain sequence; and

e. each heavy chain comprises a TNF–α binding entity heavy chain sequence, a TL1A binding entity heavy chain binding domain sequence and a TL1A binding entity light chain binding domain sequence.

56. The antigen binding protein of claim 55, wherein:

a. the TL1A binding entity light chain sequence comprises:

i. an LCDR1 sequence selected from Tables A, B, and C (SEQ ID NOS: 98, 104, 110, 116, 122, 128, 467, 469, 635, 642, 646, 651, 658, 661, 668, 226, 229, 232, 235, 241, 238 and) and

ii. an LCDR2 sequence selected from Tables A, B, and C (SEQ ID NOS: 100, 106, 112, 118, 124, 636, 643, 647, 652, 662, 227, 230, 233, 242, and 473);

iii. an LCDR3 sequence selected from Tables A, B, and C (SEQ ID NOS: 102, 108, 263, 120, 126, 637, 638, 631, 653, 663, 228, 231, 234, 237, 243, 240, and 674),

b. the TL1A binding entity heavy chain sequence comprises:

i. an HCDR1 sequence selected from Tables A, B, and C (SEQ ID NOS: 164, 170, 182, 188, 639, 654, 655, 632, 664, 253, 256, 259, 262, 268, 271, and 265),

ii. an HCDR2 sequence selected from Tables A, B, and C (SEQ ID NOS: 166, 172, 178, 184, 190, 196, 202, 483, 485, 640, 204, 644, 648, 633, 656, 659, 660, 665, 666, 114, 669, 254, 172, 260, 184, 269, 272, 266, and 254), and

iii. an HCDR3 sequence selected from Tables A, B, and C (SEQ ID NOS: 168, 174, 180, 186, 192, 489, 641, 645, 650, 657, 676, 657, 667, 671, 255, 258, 261, 264, 267, 270, 273, 672, and 675);

c. the TNF–α binding entity light chain sequence comprises:

i. an LCDR1 sequence selected from Table G (SEQ ID NOS:92, 152, 140, and 146),

ii. an LCDR2 sequence selected from Table G (SEQ ID NOS: 94, 154, 112, and 148), and

iii. an LCDR3 sequence selected from Table G (SEQ ID NOS: 96, 156, 144, and 150); and

d. the TNF–α binding entity heavy chain sequence comprises:

i. an HCDR1 sequence selected from Table G (SEQ ID NOS:158, 218, 206, and 212),

ii. an HCDR2 sequence selected from Table G (SEQ ID NOS:160, 220, 208, and 214), and

iii. an HCDR3 sequence selected from Table G (SEQ ID NOS: 162, 222, 210, and 216).

57. The antigen binding protein of claim 56, wherein:

a. the TL1A binding entity light chain sequence comprises:

i. an LCDR1 sequence selected from Table A (SEQ ID NOS: 98, 104, 110, 116, 122, 128, 467, and 469),

ii. an LCDR2 sequence selected from Table A (SEQ ID NOS: 100, 106, 112, 118, and 124);

iii. an LCDR3 sequence selected from Table A (SEQ ID NOS: 102, 108, 263, 120, and 126),

b. the TL1A binding entity heavy chain sequence comprises:

i. an HCDR1 sequence selected from Table A (SEQ ID NOS: 164, 170, 182, and 188),

ii. an HCDR2 sequence selected from Table A (SEQ ID NOS: 166, 172, 178, 184, 190, 196, 202, 483, and 485), and

iii. an HCDR3 sequence selected from Table A (SEQ ID NOS: 168, 174, 180, 186, 192, and 489).

58. The antigen binding protein of claim 55, wherein:

a. the TL1A binding entity heavy chain variable domain comprises a heavy chain variable domain sequence selected from Table D (SEQ ID NOS: 8, 12, 16, 20, 24, 28, and 32);

b. the TL1A binding entity light chain variable domain comprises a light chain variable domain sequence selected from Table D (SEQ ID NOS: 6, 10, 14, 18, 22, 26, and 30);

c. the TNF–α binding entity light chain variable domain sequence is selected from Table H (SEQ ID NOS: 2, 42, 38, and 34) and

d. the TNF–α binding entity heavy chain variable domain sequence is selected from Table H (SEQ ID NOS: 4, 44, 318, 40, and 36).

59. The antigen binding protein of claim 55, wherein the TL1A binding entity heavy chain variable domain comprises a cysteine residue at position 44 and the TL1A binding entity light chain binding domain comprises a cysteine residue at position 100, numbering as in Kabat.

60. The antigen binding protein of claim 55, wherein the protein comprises the heavy and light chain sequences of an antigen binding protein selected from Table M, SEQ ID NOS:

a. 82 and 46,

b. 284 and 46,

c. 286 and 46,

d. 441 and 453

e. 312 and 46,

f. 445 and 453,

g. 314 and 84,

h. 320 and 84,

i. 322 and 84,

j. 345 and 84,

k. 347 and 78,

l. 349 and 78,

m. 351 and 78,

n. 353 and 78,

o. 379 and 46,

p. 381 and 46,

q. 383 and 84,

r. 385 and 84,

s. 387 and 78,

t. 389 and 78,

u. 433 and 451,

v. 427 and 449,

w. 429 and 449,

x. 431 and 451,

y. 433 and 451,

z. 435 and 451,

aa. 437 and 451,

bb. 439 and 453,

cc. 441 and 453,

dd. 443 and 453, and

ee. 445 and 453.

61. The antigen binding protein of claim 55 comprising SEQ ID NOS:

a. 441 and 453;

b. 433 and 451; or

c. 445 and 453.

62. One or more isolated nucleic acids encoding the antigen binding protein of any one of claims 1 to 61 and 105 to 135.

63. One or more expression vectors comprising the nucleic acid or acids of claim 62.

64. A host cell comprising the one or more expression vectors of claim 63.

65. A method for the preparation of an antigen binding protein, comprising:

a. culturing the host cell of claim 64 under conditions that allow expression of the antigen binding protein; and

b. recovering the antigen binding protein from the culture.

66. A pharmaceutical composition comprising the antigen binding protein of any one of claims 1 to 61 and a pharmaceutically acceptable diluent, excipient or carrier.

67. A method for treating a condition associated with TL1A and/or TNF-α in a patient in need thereof, comprising administering to the patient an effective amount of the antigen binding protein of any one of claims 1 to 61 and 105 to 135.

68. The method of claim 67, wherein the condition is inflammatory bowel disease, Crohn's disease, or ulcerative colitis.

69. Use of the antigen binding protein of any one of claims 1 to 61 and 105 to 135 in the preparation of a medicament for treating a condition associated with TL1A in a patient in need thereof.

70. The use of claim 68 wherein the condition is inflammatory bowel disease, Crohn's disease or ulcerative colitis.

71. The antigen binding protein of any one of claims 1 to 61 and 105 to 135 for use in a method for treating a condition associated with TL1A and/or TNF-α in a patient in need thereof.

72. The antigen binding protein of claim 71 wherein the condition is inflammatory bowel disease, Crohn's disease, or ulcerative colitis.

73. An antigen binding protein specific for TL1A wherein:

a. the antigen binding protein has one or two light chain variable domains and one or two heavy chain variable domains;

b. the light chain variable domain comprises a LCDR1, LCDR2, and LCDR3;

c. the heavy chain variable domain comprises a HCDR1, HCDR2, and HCDR3; and

d. the HCDR1 has a sequence selected from Tables A, B, and C (SEQ ID NOS: 164, 170, 182, 188, 777, 783, 792, 798, 804, 809, 815, 819, 265, 836, 847, 857, 639, 654, 655, 632, 664, 955, 979, 994, 1010, 1017, 1022, 1027, 1028, 1029, 1037, 1045, 1087, 253, 256, 259, 262, 268, 271, 265, and 950);

e. the HCDR2 has a sequence selected from Tables A, B, and C (SEQ ID NOS: 166, 172, 178, 184, 190, 196, 202, 483, 485, 779, 785, 794, 800, 811, 817, 821, 827, 832, 838, 843, 849, 853, 859, 864, 640, 204, 678, 679, 644, 648, 649, 633, 656, 659, 660, 665, 666, 677, 114, 227, 230, 691, 715, 669, 711, 971, 972, 973, 974, 975, 980, 981, 982, 983, 984, 995, 996, 997, 998, 0999, 1011, 1023, 1030, 1038, 1039, 1040, 1041, 1042, 1046, 1047, 1048, 1058, 1059, 1060, 1061, 1062, 1066, 1067, 1068, 1069,

1070, 1074, 1088, 1089, 1090, 1095, 1096, 1097, 1102, 1103, 1104, 1108, 1109, 1110, 1111, 1112, 254, 260, 184, 269, 272, 266, 951, 953, 956, and 963); and

f. the HCDR3 sequence is selected from Tables A, B, and C (SEQ ID NOS: 168, 174, 180, 186, 192, 489, 641, 645, 650, 657, 676, 657, 667, 671, 255, 258, 261, 264, 267, 270, 273, 672, and 675).

74. The antigen binding protein of claim 73, wherein the HCDR3 comprises a sequence selected from Table A (SEQ ID NOS: 168, 174, 180, 186, 192, 489, 781, 787, 796, 802, 807, 813, 823, 829, 834, 840, 845, 851, 855, and 861).

75. The antigen binding protein of claim 73, wherein the HCDR3 has a sequence selected from antibodies and 9C8 and 3B3 (SEQ ID NO: 180 and 192).

76. The antigen binding protein of claim 74, wherein:

a. the HCDR1 has a sequence selected from Table A (SEQ ID NOS: 164, 170, 182, 188, 777, 783, 792, 798, 804, 809, 815, 819, 265, 836, 847, and 857) and

b. the HCDR2 has a sequence selected from Table A (SEQ ID NOS: 166, 172, 178, 184, 190, 196, 202, 483, 485, 779, 785, 794, 800, 811, 817, 821, 827, 832, 838, 843, 849, 853, 859, and 864).

77. The antigen binding protein of claim 75 wherein:

a. the HCDR1 has a sequence selected from antibodies 9C8 and 3B3 (SEQ ID NO: 170 and 188) and

b. the HCDR2 has a sequence selected from antibodies 9C8 and 3B3 (SEQ ID NO: 178 and 190).

78. The antigen binding protein of claim 73 wherein the LCDR3 has a sequence selected from Tables A, B, and C (SEQ ID NOS:102, 108, 263, 120, 126, 687, 693, 701, 707, 717, 727, 733, 739, 745, 751, 757, 763, 767, 771, 775, 637, 638, 631, 653, 663, 637, 233, 940, 978, 989, 990, 991, 992, 993, 1005, 1006, 1007, 1008, 1009, 1034, 1035, 1036, 1055, 1056, 1057, 1065, 1086, 1093, 1094, 1099, 1100, 1101, 1107, 228, 231, 234, 237, 243, 240, 674, 936, 938, 701, 707, , 949, , 942, and 944).

79. The antigen binding protein of claim 76 wherein the LCDR3 has a sequence selected from Table A (SEQ ID NOS: 102, 108, 263, 120, 126, 687, 693, 701, 707, 717, 727, 733, 739, 745, 751, 757, 763, 767, 771, and 775).

80. The antigen binding protein of claim 77 wherein the LCDR3 has a sequence selected from antibodies 9C8 and 3B3 (SEQ ID NOS: 263 and 126).

81. The antigen binding protein of claim 78 wherein:

a. the LCDR1 has a sequence selected from Tables A, B, and C (SEQ ID NOS: 98, 104, 110, 116, 122, 128, 467, 469, 683, 689, 235, 697, 146, 709, 713, 719, 723, 229, 226, 741, 747, 753, 773, 635, 642, 646, 651, 658, 661, 668, 759, 743, 765, 977, 986, 987, 1001, 1016, 1021, 1025, 1031, 1032, 1033, 1044, 1053, 1064, 1072, 1078, 1079, 1080, 1106, 226, 229, 232, 235, 241, 238, , 1113, , 146, and 947) and

b. the LCDR2 has a sequence selected from Tables A, B, and C (SEQ ID NOS: 100, 106, 112, 118, 124, 235, 699, 705, 721, 725, 731, 737, 749, 755, 761, 769, 636, 643, 647, 652, 662, 789, 988, 1001, 1002, 1004, 1026, 1054, 1073, 1081, 1082, 1083, 1084, 1085, 1092, 1098, 242, 935, 148, 948, and 943).

82. The antigen binding protein of claim 79 wherein:

a. the LCDR1 has a sequence selected from Table A (SEQ ID NOS: 98, 104, 110, 116, 122, 128, 467, 469, 683, 689, 235, 697, 146, 709, 713, 719, 723, 229, 226, 741, 747, 753, and 773) and

b. the LCDR2 has a sequence selected from Table A (SEQ ID NOS: 100, 106, 112, 118, 124, 685, 699, 705, 721, 725, 731, 737, 749, 755, 761, and 769).

83. The antigen binding protein of claim 80 wherein:

a. the LCDR1 has a sequence selected from antibodies 9C8 and 3B3 (SEQ ID NOS: 110 and 122) and

b. the LCDR2 has a sequence selected from antibodies 9C8 and 3B3 (SEQ ID NOS: 112 and 124).

84. The antigen binding protein of claim 73 wherein the heavy chain variable domain comprises a sequence at least about 90% identical to a heavy chain variable domain sequence selected from Table D (SEQ ID NOS: 8, 12, 16, 20, 24, 28, 32, 495, 497, 868, 872, 876, 880, 884, 888, 892, 896, 900, 904, 908, 912, 916, 920, 926, 930, and 934).

85. The antigen binding protein of claim 73 wherein the heavy chain variable domain comprises a heavy chain variable domain sequence selected from Table D (SEQ ID NOS: 8, 12, 16, 20, 24, 28, 32, 495, 497, 868, 872, 876, 880, 884, 888, 892, 896, 900, 904, 908, 912, 916, 920, 926, 930, and 934).

86. The antigen binding protein of claim 73, wherein the light chain variable domain comprises a sequence at least about 90% identical to a light chain variable domain sequence selected from Table D (SEQ ID NOS: 6, 10, 14, 18, 22, 26, 30, 491, 493, 866, 870, 874, 878, 882, 886, 890, 894, 898, 902, 906, 910, 914, 918, 922, 924, 928, and 932,).

87. The antigen binding protein of claim 73, wherein the light chain variable domain comprises a light chain variable domain sequence selected from Table D (SEQ ID NOS: 6, 10, 14, 18, 22, 26, 30, 491, 493, 866, 870, 874, 878, 882, 886, 890, 89 4, 898, 902, 906, 910, 914, 918, 922, 924, 928, and 932).

88. The antigen binding protein of claim 84, wherein the light chain variable domain comprises a sequence at least about 90% identical to a light chain variable domain sequence selected from Table D (SEQ ID NOS: 6, 10, 14, 18, 22, 26, 30, 491, 493, 866, 870, 874, 878, 882, 886, 890, 894, 898, 902, 906, 910, 914, 918, 922, 924, 928, and 932,).

89. The antigen binding protein of claim 85, wherein the light chain variable domain comprises a light chain variable domain sequence selected from Table D (SEQ ID NOS: 6, 10, 14, 18, 22, 26, 30, 491, 493, 866, 870, 874, 878, 882, 886, 890, 89 4, 898, 902, 906, 910, 914, 918, 922, 924, 928, and 932).

90. The antigen binding protein of claim 73 wherein:
    a. the light chain variable domain sequence comprises a light chain variable domain sequence selected from antibodies 9C8 and 3B3 (SEQ ID NOS: 14 and 22) and
    b. the heavy chain variable domain comprises a heavy chain variable domain sequence selected from antibodies 9C8 and 3B3 (SEQ ID NOS: 16 and 24).

91. The antigen binding protein of claim 73 wherein the protein comprises a heavy chain having a heavy chain sequence selected from Table E (SEQ ID NOS: 52, 56, 60, 64, 68, 72, 76, 457, 461, 1118, 1122, 1126, 1130, 1134, 1138, 1142, 1146, 1150, 1154, 1158, 1162, 1166, 1170, 1174, 1178, 1182, 1186 and 1190).

92. The antigen binding protein of claim 73 wherein the protein comprises a light chain having a light chain sequence selected from Table E (SEQ ID NOS: 66, 54, 58, 62, 66, 70, 74, 455, 459, 1116, 1120, 1124, 1128, 1132, 1136, 1140, 1144, 1148, 1152, 1156, 1160, 1164, 1168, 1172, 1176, 1180, 1184 and 1188).

93. The antigen binding protein of claim 73 comprising the light and heavy chain sequences of an antibody selected from Table E, SEQ ID NOS:

a. 50 and 52,

b. 54 and 56,

c. 58 and 60,

d. 62 and 64,

e. 66 and 68,

f. 70 and 72,

g. 74 and 76,

h. 455 and 457,

i. 459 and 461,

j. 1116 and 1118,

k. 1120 and 1122,

l. 1124 and 1126,

m. 1128 and 1130,

n. 1132 and 1134,

o. 1136 and 1138,

p. 1140 and 1142,

q. 1144 and 1146,

r. 1148 and 1150,

s. 1152 and 1154,

t. 1156 and 1158,

u. 1160 and 1162,

v. 1164 and 1166,

w. 1168 and 1170,

x. 1172 and 1174,

y. 1176 and 1178,

z. 1180 and 1182,

aa. 1184 and 1186, and

bb. 1188 and 1190.

94. One or more isolated nucleic acids encoding the antigen binding protein of any one of claims 73 to 93.

95. One or more expression vectors comprising the nucleic acid or acids of claim 94.

96. A host cell comprising the one or more expression vectors of claim 95.

97. A method for the preparation of an antigen binding protein, comprising:

a. culturing the host cell of claim 96 under conditions that allow expression of the antigen binding protein; and

b. recovering the antigen binding protein from the culture.

98. A pharmaceutical composition comprising the antigen binding protein of any one of claims 73 to 93 and a pharmaceutically acceptable diluent, excipient or carrier.

99. A method for treating a condition associated with TL1A in a patient in need thereof, comprising administering to the patient an effective amount of the antigen binding protein of any one of claims 73 to 93.

100. The method of claim 99, wherein the condition is inflammatory bowel disease, Crohn's disease, or ulcerative colitis.

101. Use of the antigen binding protein of any one of claims 73 to 93 in the preparation of a medicament for treating a condition associated with TL1A in a patient in need thereof.

102. The use of claim 101 wherein the condition is inflammatory bowel disease, Crohn's disease, or ulcerative colitis.

103. The antigen binding protein of any one of claims 73 to 93 for use in a method for treating a condition associated with TL1A in a patient in need thereof.

104. The antigen binding protein of claim 103 wherein the condition is inflammatory bowel disease, Crohn's disease, or ulcerative colitis.

105. The antigen binding protein of claim 23, comprising:

a. a first polypeptide comprising a first heavy chain of a first antibody comprising a first heavy chain variable region (VH1) and a first CH1 domain, wherein the first heavy chain is fused through its C-terminus to the N-terminus of a polypeptide comprising a second heavy chain variable region of a second antibody (VH2), wherein the VH2 is fused through its C-terminus to the N-terminus of a second CH1 domain, and wherein the first antibody comprises one of the TL1A binding entity or the TNF-α binding entity and the second antibody comprises the other; wherein:

i. the VH1 or first CH1 domain comprises at least one amino acid substitution to introduce a positively charged amino acid at a residue selected from the group consisting of positions 39, 44, and 183 using EU numbering; and

ii. the VH2 or second CH1 domain comprises at least one amino acid substitution to introduce a negatively charged amino acid at a residue selected from the group consisting of a residue that corresponds to positions 39, 44, and 183 using EU numbering; and

b. a second polypeptide comprising a first light chain of the first antibody of a., wherein the first light chain comprises a first light chain variable region (VL1) and a first CL region; and wherein the VL1 or first CL domain comprises at least one amino acid substitution to introduce a negatively charged amino acid at a residue selected from the group consisting of positions 38, 100, and 176 using EU numbering; and

c. a third polypeptide comprising a second light chain of the second antibody of a., wherein the second light chain comprises a second light chain variable region (VL2) and a second CL region; and wherein the VL1 or first CL domain comprises at least one amino acid substitution to introduce a positively charged amino acid at a residue selected from the group consisting of positions 38, 100, and 176 using EU numbering.

106. The antigen binding protein of claim 105, wherein the first heavy chain is fused to the VH2 via a peptide linker.

107. The antigen binding protein according to claim 106, wherein the peptide linker comprises a sequence selected from the group consisting of $(Gly_3Ser)_2$, $(Gly_4Ser)_2$, $(Gly_3Ser)_3$, $(Gly_4Ser)_3$, $(Gly_3Ser)_4$, $(Gly_4Ser)_4$, $(Gly_3Ser)_5$, $(Gly_4Ser)_5$, $(Gly_3Ser)_6$, and $(Gly_4Ser)_6$.

108. The antigen binding protein according to claim 105, wherein:

a. the VH1 or first CH1 domain comprises a mutation selected from the group consisting of Q39K, G44K, and S183K using EU numbering;

b. the VH2 or second CH1 domain comprises a mutation selected from the group consisting of Q39E, G44E, and S183E using EU numbering;

c. the VL1 or first CL domain comprises a mutation selected from the group consisting of Q38E, G100E, and S176E using EU numbering; and

d. the VL2 or second CL domain comprises a mutation selected from the group consisting of Q38K, G100K, and S176K using EU numbering.

109. The antigen binding protein according to claim 108, wherein:
    a. the first CH1 domain comprises a S183K mutation using EU numbering;
    b. the second CH1 domain comprises a S183E mutation using EU numbering;
    c. the first CL domain comprises a S176E mutation using EU numbering; and
    d. the second CL domain comprises a S176K mutation using EU numbering.

110. The antigen binding protein according to claim 108, wherein:
    a. the VH1 comprises a Q39K mutation and the first CH1 domain comprises a S183K mutation using EU numbering;
    b. the VH2 comprises a Q39E mutation and the second CH1 domain comprises a S183E mutation using EU numbering;
    c. the VL1 comprises a Q38E mutation and the first CL domain comprises a S176E mutation using EU numbering; and
    d. the VL2 comprises a Q38K mutation and the second CL domain comprises a S176K mutation using EU numbering.

111. The antigen binding protein according to claim 108, wherein:
    a. the first CH1 domain comprises G44K and S183K mutations using EU numbering;
    b. the second CH1 domain comprises G44E and S183E mutations using EU numbering;
    c. the first CL domain comprises G100E and S176E mutations using EU numbering; and
    d. the second CL domain comprises G100K and S176K mutations using EU numbering.

112. The antigen binding protein of claim 23, comprising:
    a. a first polypeptide comprising a first heavy chain of a first antibody comprising a first heavy chain variable region (VH1) and a first CH1 domain, wherein the first heavy chain is fused through its C-terminus to the N-terminus of a polypeptide comprising a second heavy chain variable region of a second antibody (VH2), wherein the VH2 is fused through its C-terminus to the N-terminus of a second CH1 domain, and wherein the first antibody comprises one of the TL1A binding entity and the TNF-α binding entity and the second antibody comprises the other; wherein:
        i. the VH1 or first CH1 domain comprises at least one amino acid substitution to introduce a negatively charged amino acid at a residue selected from the group consisting of positions 39, 44, and 183 using EU numbering; and

ii. the VH2 or second CH1 domain comprises at least one amino acid substitution to introduce a positively charged amino acid at a residue selected from the group consisting of a residue that corresponds to positions 39, 44, and 183 using EU numbering; and

a. a second polypeptide comprising a first light chain of the first antibody of a., wherein the first light chain comprises a first light chain variable region (VL1) and a first CL region; and wherein the VL1 or first CL domain comprises at least one amino acid substitution to introduce a positively charged amino acid at a residue selected from the group consisting of positions 38, 100, and 176 using EU numbering; and

b. a third polypeptide comprising a second light chain of the second antibody of a., wherein the second light chain comprises a second light chain variable region (VL2) and a second CL region;. and wherein the VL1 or first CL domain comprises at least one amino acid substitution to introduce a negatively charged amino acid at a residue selected from the group consisting of positions 38, 100, and 176 using EU numbering.

113. The antigen binding protein according to claim 112, wherein the first heavy chain is fused to the VH2 via a peptide linker.

114. The antigen binding protein according to claim 113, wherein the peptide linker comprises a sequence selected from the group consisting of $(Gly_3Ser)_2$, $(Gly_4Ser)_2$, $(Gly_3Ser)_3$, $(Gly_4Ser)_3$, $(Gly_3Ser)_4$, $(Gly_4Ser)_4$, $(Gly_3Ser)_5$, $(Gly_4Ser)_5$, $(Gly_3Ser)_6$, and $(Gly_4Ser)_6$.

115. The antigen binding protein according to claim 112, wherein:

a. the VH1 or first CH1 domain comprises a mutation selected from the group consisting of Q39E, G44E, and S183E using EU numbering;

b. the VH2 or second CH1 domain comprises a mutation selected from the group consisting of Q39K, G44K, and S183K using EU numbering;

c. the VL1 or first CL domain comprises a mutation selected from the group consisting of Q38K, G100K, and S176K using EU numbering; and

d. the VL2 or second CL domain comprises a mutation selected from the group consisting of Q38E, G100E, and S176E using EU numbering.

116. The antigen binding protein according to claim 115, wherein:

a. the first CH1 domain comprises a S183E mutation using EU numbering;

b. the second CH1 domain comprises a S183K mutation using EU numbering;

c. the first CL domain comprises a S176K mutation using EU numbering; and

d. the second CL domain comprises a S176E mutation using EU numbering.

117. The antigen binding protein according to claim 115, wherein:

a. the VH1 comprises a Q39E mutation and the first CH1 domain comprises a S183E mutation using EU numbering;

b. the VH2 comprises a Q39K mutation and the second CH1 domain comprises a S183K mutation using EU numbering;

c. the VL1 comprises a Q38K mutation and the first CL domain comprises a S176K mutation using EU numbering; and

d. the VL2 comprises a Q38E mutation and the second CL domain comprises a S176E mutation using EU numbering.

118. The antigen binding protein according to claim 115, wherein:

a. the first CH1 domain comprises G44E and S183E mutations using EU numbering;

b. the second CH1 domain comprises G44K and S183K mutations using EU numbering;

c. the first CL domain comprises G100K and S176K mutations using EU numbering; and

d. the second CL domain comprises G100E and S176E mutations using EU numbering.

119. The antigen binding protein of claim 23, comprising:

a. a first polypeptide comprising a first heavy chain of a first antibody comprising a first heavy chain variable region (VH1) and a first CH1 domain, wherein the first heavy chain is fused through its C-terminus to the N-terminus of a polypeptide comprising a second heavy chain variable region of a second antibody (VH2), wherein the VH2 is fused through its C-terminus to the N-terminus of a second CH1 domain, and wherein the first antibody comprises one of the TL1A binding entity and the TNF-α binding entity and the second antibody comprises the other; wherein:

i. the VH1 or first CH1 domain comprises at least one amino acid substitution to introduce a charged amino acid at a residue selected from the group consisting of positions 39, 44, and 183 using EU numbering; and

ii. the VH2 or second CH1 domain comprises at least one amino acid substitution to introduce a charged amino acid at a residue selected from the group consisting of a residue that corresponds to positions 39, 44, and 183 using EU numbering,

wherein the charge is the opposite of the substituted residue of the VH1 or first CH1 of the first heavy chain; and

b. a second polypeptide comprising a first light chain of the first antibody of a., wherein the first light chain comprises a first light chain variable region (VL1) and a first CL region; and wherein the VL1 or first CL domain comprises at least one amino acid substitution to introduce a charged amino acid at a residue selected from the group consisting of positions 38, 100, and 176 using EU numbering, wherein;
   i. the charge at position 38 is the opposite of the substituted residue of the VH1 or first CH1 of the first heavy chain at position 39 using EU numbering; the charge at position 100 using EU numbering is the opposite of the substituted residue of the VH1 or first CH1 of the first heavy chain at position 44 using EU numbering; the charge at position 176 is the opposite of the substituted residue of the VH1 or first CH1 of the first heavy chain at position 183 using EU numbering; and

c. a third polypeptide comprising a second light chain of the second antibody of a., wherein the second light chain comprises a second light chain variable region (VL2) and a second CL region; and wherein the VL2 or second CL domain comprises at least one amino acid substitution to introduce a charged amino acid at a residue selected from the group consisting of positions 38, 100, and 176 using EU numbering, wherein:
   i. the charge at position 38 is the opposite of the substituted residue of the VH2 or second CH1 of the second heavy chain at position 39; the charge at position 100 is the opposite of the substituted residue of the VH2 or second CH1 of the second heavy chain at position 44; the charge at position 176 is the opposite of the substituted residue of the VH2 or second CH1 of the second heavy chain at position 183.

120. The antigen binding protein according to claim 119, wherein the first heavy chain is fused to the VH2 via a peptide linker.

121. The antigen binding protein according to claim 120, wherein the peptide linker comprises a sequence selected from the group consisting of $(Gly_3Ser)_2$, $(Gly_4Ser)_2$, $(Gly_3Ser)_3$, $(Gly_4Ser)_3$, $(Gly_3Ser)_4$, $(Gly_4Ser)_4$, $(Gly_3Ser)_5$, $(Gly_4Ser)_5$, $(Gly_3Ser)_6$, and $(Gly_4Ser)_6$.

122. The antigen binding protein according to claim 119, wherein:

a. the VH1 comprises a Q39E mutation and the first CH1 domain comprises a S183K mutation using EU numbering;
b. the VH2 comprises a Q39K mutation and the second CH1 domain comprises a S183E mutation using EU numbering;
c. the VL1 comprises a Q38K mutation and the first CL domain comprises a S176E mutation using EU numbering; and
d. the VL2 comprises a Q38E mutation and the second CL domain comprises a S176K mutation using EU numbering.

123. The antigen binding protein according to claim 122, wherein:
a. the first CH1 domain comprises G44E and S183K mutations using EU numbering;
b. the second CH1 domain comprises G44K and S183E mutations using EU numbering;
c. the first CL domain comprises G100K and S176E mutations using EU numbering; and
d. the second CL domain comprises G100E and S176K mutations using EU numbering.

124. The antigen binding protein according to claim 122, wherein:
a. the VH1 comprises a Q39K mutation and the first CH1 domain comprises a S183E mutation using EU numbering;
b. the VH2 comprises a Q39E mutation and the second CH1 domain comprises a S183K mutation using EU numbering;
c. the VL1 comprises a Q38E mutation and the first CL domain comprises a S176K mutation using EU numbering; and
d. the VL2 comprises a Q38K mutation and the second CL domain comprises a S176E mutation using EU numbering.

125. The antigen binding protein according to claim 122, wherein:
a. the first CH1 domain comprises G44K and S183E mutations using EU numbering;
b. the second CH1 domain comprises G44E and S183K mutations using EU numbering;
c. the first CL domain comprises G100E and S176K mutations using EU numbering; and
d. the second CL domain comprises G100K and S176E mutations using EU numbering.

126. The antigen binding protein of claim 23, comprising:

a. a first polypeptide comprising a first heavy chain (VH2-CH1-CH2-CH3) from a first antibody, wherein the first heavy chain is fused at its carboxyl terminus (optionally through a peptide linker) to a polypeptide comprising VH2-CH1 domains of a second antibody,

b. a second polypeptide comprising a light chain from the first antibody (VL1-CL) and

c. a third polypeptide comprising VL2-CL domains of the second antibody;

wherein the first antibody comprises one of a TL1A binding entity and a TNF-α binding entity and the second antibody comprises the other.

127. The antigen binding protein of claim 126, wherein:

a. CL from the first antibody comprises E at position 230, CH1 from the first antibody comprises K at position 230, CL from the second antibody comprises K at position 230, and CH1 from the second antibody comprises E at position 230;

b. CL from the first antibody comprises E at position 230, CH1 from the first antibody comprises K at position 230, VL from the first antibody comprises E at position 46, VH from the first antibody comprises K at position 46, CL from the second antibody comprises K at position 230, CH1 from the second antibody comprises E at position 230, VL from the second antibody comprises E at position 46, and VH from the second antibody comprises K at position 46; or

c. CL from the first antibody comprises E at position 230, CH1 from the first antibody comprises K at position 230, VL from the first antibody comprises E at position 141, VH from the first antibody comprises K at position 51, CL from the second antibody comprises K at position 230, CH1 from the second antibody comprises E at position 230, VL from the second antibody comprises E at position 51, and VH from the second antibody comprises K at position 141;

wherein all positions are according to EU numbering.

128. The antigen binding protein of claim 23, comprising:

a. a first polypeptide comprising VH1-CL-CH2-CH3 domains of a first antibody fused at their carboxyl terminus (optionally through a peptide linker) to a polypeptide comprising VH2-CH1 domains of a second antibody,

b. a second polypeptide comprising a light chain from the first antibody (VL1-CH1) and

c. a third polypeptide comprising VL2-CL domains of the second antibody;

wherein the first antibody comprises one of a TL1A binding entity or a TNF-α binding entity and the second antibody comprises the other.

129. The antigen binding protein of claim 128, wherein:

a. CL from the first antibody comprises K at position 230, CH1 from the first antibody comprises E at position 230, CL from the second antibody comprises K at position 230, and CH1 from the second antibody comprises E at position 230;

b. CL from the first antibody comprises K at position 230, CH1 from the first antibody comprises E at position 230, VL from the first antibody comprises E at position 46, VH from the first antibody comprises K at position 46, CL from the second antibody comprises K at position 230, CH1 from the second antibody comprises E at position 230, VL from the second antibody comprises E at position 46, and VH from the second antibody comprises K at position 46; or

c. CL from the first antibody comprises K at position 230, CH1 from the first antibody comprises E at position 230, VL from the first antibody comprises E at position 141, VH from the first antibody comprises K at position 51, CL from the second antibody comprises K at position 230, CH1 from the second antibody comprises E at position 230, VL from the second antibody comprises E at position 51, and VH from the second antibody comprises K at position 141;

wherein all positions are according to EU numbering.

130. The antigen binding protein of claim 23, comprising:

a. a first polypeptide comprising a first heavy chain (VH1-CH1-CH2-CH3) of a first antibody, wherein the first heavy chain is fused at its carboxyl terminus (optionally through a peptide linker) to a polypeptide comprising VH2-CL domains of a second antibody,

b. a second polypeptide comprising a light chain from a first antibody (VL1-CL) and

c. a third polypeptide comprising VL2-CH1 domains of the second antibody;

wherein the first antibody comprises one of a TL1A binding entity or a TNF-α binding entity and the second antibody comprises the other.

131. The antigen binding protein of claim 130, wherein:

a. CL from the first antibody comprises E at position 230, CH1 from the first antibody comprises K at position 230, CL from the second antibody comprises E at position 230, and CH1 from the second antibody comprises K at position 230;

b. CL from the first antibody comprises E at position 230, CH1 from the first antibody comprises K at position 230, VL from the first antibody comprises E at position 46, VH from the first antibody comprises K at position 46, CL from the second antibody comprises E at position 230, CH1 from the second antibody comprises K at position 230, VL from the second antibody comprises E at position 46, and VH from the second antibody comprises K at position 46; or

c. CL from the first antibody comprises E at position 230, CH1 from the first antibody comprises K at position 230, VL from the first antibody comprises E at position 141, VH from the first antibody comprises K at position 51, CL from the second antibody comprises E at position 230, CH1 from the second antibody comprises K at position 230, VL from the second antibody comprises E at position 51, and VH from the second antibody comprises K at position 141;

wherein all positions are according to EU numbering.

132. The antigen binding protein of claim 23, comprising:

a. a first polypeptide comprising VH1-CL-CH2-CH3 domains of a first antibody fused at their carboxyl terminus (optionally through a peptide linker) to a polypeptide comprising VH2-CL domains of a second antibody,

b. a second polypeptide comprising VL1-CH1 domains from the first antibody and

c. a third polypeptide comprising VL2-CH1 domains of the second antibody;

wherein the first antibody comprises one of a TL1A binding entity or a TNF-α binding entity and the second antibody comprises the other.

133. The antigen binding protein of claim 132, wherein:

a. CL from the first antibody comprises K at position 230, CH1 from the first antibody comprises E at position 230, CL from the second antibody comprises E at position 230, and CH1 from the second antibody comprises K at position 230;

b. CL from the first antibody comprises K at position 230, CH1 from the first antibody comprises E at position 230, VL from the first antibody comprises E at position 46, VH from the first antibody comprises K at position 46, CL from the second antibody

comprises E at position 230, CH1 from the second antibody comprises K at position 230, VL from the second antibody comprises E at position 46, and VH from the second antibody comprises K at position 46; or

c. CL from the first antibody comprises K at position 230, CH1 from the first antibody comprises E at position 230, VL from the first antibody comprises E at position 141, VH from the first antibody comprises K at position 51, CL from the second antibody comprises E at position 230, CH1 from the second antibody comprises K at position 230, VL from the second antibody comprises E at position 51, and VH from the second antibody comprises K at position 141;

wherein all positions are according to EU numbering.

134. The antigen binding protein according to claims 105, 112, 119, 126, 128, 130, and 132 wherein the antigen binding protein comprises:

a. CDRL1, CDRL2, and CDRL3 amino acid sequences selected from Table 21.2A, SEQ ID NOS:

i. 92, 110, 122, 128, and 146;

ii. 112, 118, 124, 148, and 242;

iii. 96, 108, 120, 126 and 150; and

b. CDRH1, CDRH2, and CDRH3 amino acid sequences selected from Table 21.2B, SEQ ID NOS:

i. 158, 170, 182, 188, and 212;

ii. 160, 190, 196, 214, and 633;

iii. 162, 180, 186, 192 and 216.

135. The antigen binding protein according to claims 105, 112, 119, 126, 128, 130, and 132 wherein the antigen binding protein comprises sequences of an antigen binding protein selected from Table 21.1, SEQ ID NOS:

a. 1254, 1256, and 1258;

b. 1260, 1262, and 1264;

c. 1266, 1268, and 1270;

d. 1272, 1274, and 1276;

e. 1278, 1280, and 1282;

f. 1284, 1286, and 1288;

g. 1290, 1292, and 1294;

h. 1296, 1298, and 1300;

i. 1302, 1304, and 1306;

j. 1308, 1310, and 1312;

k. 1314, 1316, and 1318;

l. 1320, 1322, and 1324;

m. 1326, 1328, and 1330;

n. 1332, 1334, and 1336;

o. 1338, 1340, and 1342;

p. 1344, 1346, and 1348;

q. 1350, 1352, and 1354;

r. 1356, 1358, and 1360;

s. 1362, 1364, and 1366;

t. 1368, 1370, and 1372;

u. 1374, 1376, and 1378;

v. 1380, 1382, and 1384;

w. 1386, 1388, and 1390;

x. 1392, 1394, and 1396;

y. 1398, 1400, and 1402;

z. 1404, 1406, and 1408;

aa. 1410, 1412, and 1414;

bb. 1416, 1418, and 1420;

cc. 1422, 1424, and 1426;

dd. 1428, 1430, and 1432;

ee. 1434, 1436, and 1438;

ff. 1440, 1442, and 1444;

gg. 1446, 1448, and 1450;

hh. 1452, 1454, and 1456;

ii. 1458, 1460, and 1462;

jj. 1464, 1466, and 1468;

kk. 1470, 1472, and 1474;

ll. 1476, 1478, and 1480;

mm. 1482, 1484, and 1486;

nn. 1488, 1490, and 1492;

oo. 1494, 1496, and 1498;

pp. 1500, 1502, and 1504;

qq. 1506, 1508, and 1510;

rr. 1512, 1514, and 1516;

ss. 1518, 1520, and 1522;

tt. 1524, 1526, and 1528;

uu. 1530, 1532, and 1534;

vv. 1536, 1538, and 1540;

ww. 1542, 1544, and 1546;

xx. 1548, 1550, and 1552;

yy. 1554, 1556, and 1558;

zz. 1560, 1562, and 1564;

aaa. 1566, 1568, and 1570;

bbb. 1572, 1574, and 1576;

ccc. 1578, 1580, and 1582;

ddd. 1584, 1586, and 1588;

eee. 1590, 1592, and 1594;

fff. 1596, 1598, and 1600;

ggg. 1602, 1604, and 1606;

hhh. 1608, 1610, and 1612;

iii. 1614, 1616, and 1618;

jjj. 1620, 1622, and 1624;

kkk. 1626, 1628, and 1630;

lll. 1632, 1634, and 1636;

mmm. 1638, 1640, and 1642;

nnn. 1644, 1646, and 1648;

ooo. 1650, 1652, and 1654;

ppp. 1656, 1658, and 1660;

qqq. 1662, 1664, and 1666;

rrr. 1668, 1670, and 1672;

sss. 1674, 1676, and 1678;

ttt. 1680, 1682, and 1684;

uuu. 1686, 1688, and 1690;

vvv. 1692, 1694, and 1696;

www. 1698, 1700, 1702;

xxx. 1704, 1706, and 1708;

yyy. 1710, 1712, and 1714;

zzz. 1716, 1718, and 1720;

aaaa. 1722, 1724, and 1726;

bbbb. 1728, 1730, and 1732;

cccc. 1734, 1736, and 1738;

dddd. 1740, 1742, and 1744;

eeee. 1746, 1748, and 1750;

ffff. 1752, 1754, and 1756;

gggg. 1758, 1760, and 1762;

hhhh. 1764, 1766, and 1768;

iiii. 1770, 1772, and 1774;

jjjj. 1776, 1778, and 1780;

kkkk. 1782, 1784, and 1786;

llll. 1788, 1790, and 1792;

mmmm. 1794, 1796, and 1798;

nnnn. 1800, 1802, and 1804;

oooo. 1806, 1808, and 1810;

pppp. 1812, 1814, and 1816;

qqqq. 1818, 1820, and 1822;

rrrr. 1824, 1826, and 1828;

ssss. 1830, 1832, and 1834;

tttt. 1836, 1838, and 1840;

uuuu. 1842, 1844, and 1846;

vvvv.	1848, 1950, and 1852;
wwww.	1854, 1856, and 1858;
xxxx.	1860, 1862, and 1864;
yyyy.	1866, 1868, and 1870;
zzzz.	1872, 1874, and 1876;
aaaaa.	1878, 1880, and 1882;
bbbbb.	1884, 1886, and 1888;
ccccc.	1890, 1892, and 1894;
ddddd.	1896, 1898, and 1900;
eeeee.	1902, 1904, and 1906;
fffff.	1908, 1910, and 1912;
ggggg.	1914, 1916, and 1918;
hhhhh.	1920, 1922, and 1924;
iiiii.	1926, 1928, and 1930;
jjjjj.	1932, 1934, and 1936;
kkkkk.	1938, 1940, and 1942;
lllll.	1944, 1946, and 1948;
mmmmm.	1950, 1952, and 1954;
nnnnn.	1956, 1958, and 1960;
ooooo.	1962, 1964, and 1966;
ppppp.	1968, 1970, and 1972;
qqqqq.	1974, 1976, and 1978;
rrrrr.	1980, 1982, and 1984;
sssss.	1986, 1988, and 1990;
ttttt.	1992, 1994, and 1996;
uuuuu.	1998, 2000, and 2002;
vvvvv.	2004, 2006, and 2008;
wwwww.	2010, 2012, and 2014;
xxxxx.	2016, 2018, and 2020;
yyyyy.	2022, 2024, and 2026;
zzzzz.	2028, 2030, and 2032;

aaaaaa.	2034, 2036, and 2038;
bbbbbb.	2040, 2042, and 2044;
cccccc.	2046, 2048, and 2050;
dddddd.	2052, 2054, and 2056;
eeeeee.	2058, 2060, and 2062;
ffffff.	2064, 2066, and 2068;
gggggg.	2070, 2072, and 2074;
hhhhhh.	2076, 2078, and 2080;
iiiiii.	2082, 2084, and 2086;
jjjjjj.	2088, 2090, and 2092;
kkkkkk.	2094, 2096, and 2098;
llllll.	2100, 2102, and 2104;
mmmmmm.	2106, 2108, and 2110; and
nnnnnn.	2112, 2114, and 2116.