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(54) Title: SINGLE-CHAIN TNF RECEPTOR 2 AGONIST FUSION PROTEINS

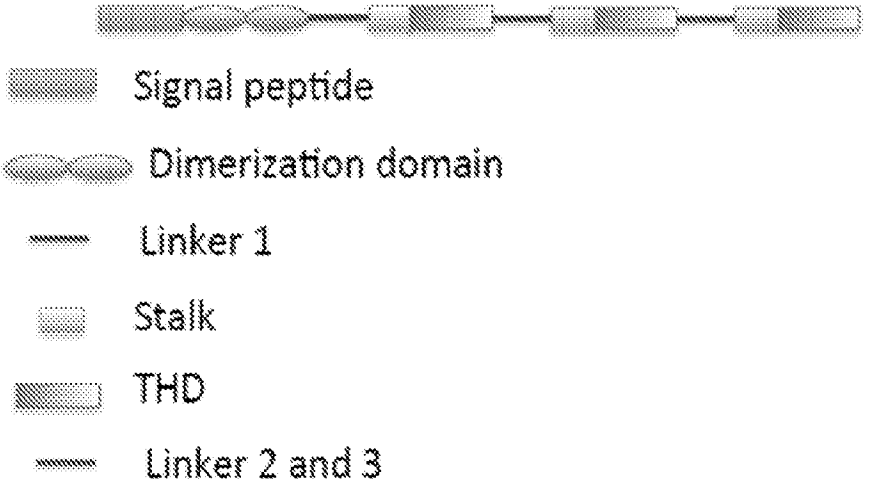

Figure 1A

(57) Abrégé/Abstract:
This invention provides for a fusion protein between a single chain TNFR2 Selective Agonist protein (scTNFR2 Selective Agonist) and a dimerization domain, such as an IgGFc protein. The single chain TNFR2 Selective Agonist moiety provides a therapeutic activity by selectively activating the TNFR2 form of the TNF-α receptor, thus selectively stimulating Tregs and/or increasing myelin deposition.

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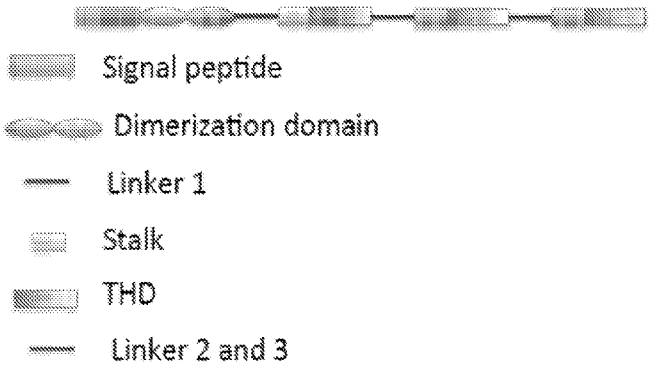

Figure 1A

(57) Abstract: This invention provides for a fusion protein between a single chain TNFR2 Selective Agonist protein (scTNFR2 Selective Agonist) and a dimerization domain, such as an IgGFc protein. The single chain TNFR2 Selective Agonist moiety provides a therapeutic activity by selectively activating the TNFR2 form of the TNF-α receptor, thus selectively stimulating Tregs and/or increasing myelin deposition.

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SINGLE-CHAIN TNF RECEPTOR 2 AGONIST FUSION PROTEINS

[001] This application claims the benefit of U.S. provisional application no. 62/515,643, filed June 6, 2017, and entitled SINGLE-CHAIN TNF RECEPTOR 2 AGONIST FUSION PROTEINS, which application is incorporated herein by reference.

STATEMENT REGARDING FEDERALLY FUNDED RESEARCH

[002] None

REFERENCE TO SEQUENCE LISTING

[003] A listing of the sequences follows the specification and is expressly included in or incorporated herein by reference.

BACKGROUND

I. FIELD OF THE INVENTION

[004] The present invention relates generally to the fields of TNF Receptor 2 agonist molecules and uses thereof.

II. DESCRIPTION OF RELATED ART

[005] Tumor necrosis factor-α (TNF- α) is a cytokine that is responsible for diverse biological effects such as inflammation and immune modulation. It is a target of a variety of therapeutic agents including antibodies such as Humira and Remicade.

SUMMARY

[006] In one embodiment the present disclosure provides a fusion protein comprising a first TNF homology domain (THD) comprising D143N/A145R mutations, wherein the THD has at least 95% identity to SEQ ID NO: 3; a second THD comprising D143N/A145R mutations, wherein the THD has at least 95% identity to SEQ ID NO: 3; a third THD comprising D143N/A145R mutations, wherein the THD has at least 95% identity to SEQ ID NO: 3; an immunoglobulin Fc domain; and a first linker peptide covalently linking the first and second THDs and a second linker covalently linking the second and third THDs.

[007] In some embodiments the linkers in the fusion protein are composed of from 1-31 or 2-15 or 3-10 amino acids and in some embodiments include at least some stalk region from TNF-α.

[008] In some embodiments the Fc in the fusion protein is covalently linked to the N-terminus of the N-terminal THD or the C-terminus of the C-terminal THD.

[009] In some embodiments the Fc is covalently linked to the THD by a linker, although in some embodiments the Fc and THD are directly connected.

[010] In some embodiments the TNFR2 agonist-Fc fusion protein selectively activates TNFR2 over TNFR1, and in some embodiments upon administration to a subject, this fusion protein selectively activates a TNFR2 in the subject over TNFR1 in the subject. In some

embodiments the TNFR2 agonist-Fc fusion protein preferentially activates T regulatory cells in the subject relative to conventional T cells in the subject. In some embodiments the TNFR2 agonist-Fc fusion protein increases myelination in a subject compared to control administration.

[011] In some embodiments the present disclosure provides a nucleic acid encoding a fusion protein as described above.

[012] In some embodiments the present disclosure provides a method of increasing myelin deposition in a patient in need thereof comprising administering a fusion protein as described herein to said patient.

[013] In some embodiments the present disclosure provides method of treating demyelinating disease in a patient in need thereof comprising administering a fusion protein as described herein to said patient. In some embodiments the demyelinating disease is optic neuritis or multiple sclerosis.

[014] In some embodiments the present disclosure provides a method of treating pain in a patient in need thereof comprising administering a fusion protein as described herein to said patient.

[015] It is contemplated that any embodiment of a method or composition described herein can be implemented with respect to any other method or composition described herein.

[016] The use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one."

[017] The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternative are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or."

[018] Throughout this application, the term "about" is used to indicate that a value includes the standard deviation of error for the device or method being employed to determine the value.

[019] As used in this specification and claim(s), the words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing" (and any form of containing, such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, unrecited elements or method steps.

[020] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

DESCRIPTION OF THE DRAWINGS

[021] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of the specification embodiments presented herein.

[022] FIG. 1 Configurations of scTNFR2 agonist fusion proteins. Figure 1A shows domains of scTNFR2 agonist fusion proteins. Figure 1B shows a scTNFR2 agonist fusion protein comprising N-terminal Fc, linker 1, stalk sequence, THD, linker 2, stalk sequence, THD, linker 2, stalk sequence THD. Figure 1C shows a scTNFR2 agonist fusion protein comprising N-terminal Fc, linker 1, stalk sequence variation, THD, linker 2, stalk sequence variation, THD, linker 2, stalk sequence, THD. Figure 1D shows a scTNFR2 agonist fusion protein comprising N-terminal Fc, linker 1, THD, linker 2, THD, linker 2,

THD. Figure 1E shows a scTNFR2 agonist fusion protein comprising N-terminal Fc, linker 1, stalk sequence, THD, linker 2, THD, linker 2, THD. Figure 1F shows a scTNFR2 agonist fusion protein comprising N-terminal Fc, stalk sequence, THD, linker 2, THD, linker 2, THD. Figure 1G shows a scTNFR2 agonist fusion protein comprising N-terminal THD, linker 2, THD, linker 2, THD, linker 1, Fc.

[023] FIG. 2 Depicts sequence of wild type TNF-α. (SEQ ID NO:1) Bold indicates ADAM17 cleavage site between A and V. Italics indicate stalk region (amino acids 57-87). Underline indicates THD (amino acids 88-233). Arrows indicate amino acids to be mutated to form TNFR2 agonist.

[024] FIG 3 Depicts sequence of mature, soluble TNF-α. (SEQ ID NO:2)

[025] FIG. 4 Depicts the TNF homology domain (THD) containing D143N/A145R mutations. (SEQ ID NO:3)

[026] FIG 5 Depicts the sequence from the ADAM17 cleavage site in the stalk region to the C-terminus of the stalk region. (SEQ ID NO:4)

[027] FIG. 6A Version 1- Depicts Human IgG1 Fc sequence (SEQ ID NO:5) with FcγR and C1q knockout (SEQ ID NO:6). The C-terminus of the scTNFR2 agonist can be fused directly to Fc N-terminus. Version 2- Depicts Human IgG1 Fc sequence like Version 1 with the exception that linker GGGGS is placed between the N-terminus of the Fc and C-terminus of the scTNFR2 agonist. (SEQ ID NO:7 and SEQ ID NO:8)

[028] FIG 6B Version 3- Depicts Human IgG1 Fc sequence with FcγR and C1Q knockout. The scTNFR2 agonist is at the Fc C-terminus contains a spacer of $(GGGGS)_n$, wherein n=1=5. (SEQ ID NO:9 and SEQ ID NO:10)

[029] FIG 7A Version 1- Depicts Human IgG4 Fc sequence. (SEQ ID NO:11) and a variant containing Ser to Pro mutation (SEQ ID NO:12) The C-terminus of the scTNFR2 agonist can be fused directly to Fc-N-terminus. Version 2- Depicts Human IgG4 Fc sequence

like Version 1 with the exception that linker GGGGS is placed between the N-terminus of the Fc and C-terminus of the scTNFR2 agonist. (SEQ ID NO:13 and SEQ ID NO:14)

[030] FIG 7B Version 3- Depicts Human IgG4 Fc sequence. The scTNFR2 agonist is at the Fc C-terminus which contains a spacer of $(GGGGS)_n$, wherein n=1=5. (SEQ ID NO:15 and SEQ ID NO: 16)

[031] FIG 8A Version 1- Depicts Human IgG2 Fc sequence (SEQ ID NO:17) with C1q knockout (SEQ ID NO:18). The C-terminus of the scTNFR2 agonist can be fused directly to Fc-N-terminus. Version 2- Depicts Human IgG2 Fc sequence like Version 1 with the exception that linker GGGGS is placed between the N-terminus of the Fc and C-terminus of the scTNFR2 agonist. (SEQ ID NO:19 and SEQ ID NO:20)

[032] FIG 8B Version 3- Depicts Human IgG2 Fc sequence with C1Q knockout. The scTNFR2 agonist is at the Fc C-terminus contains a spacer of $(GGGGS)_n$, wherein n=1=5. (SEQ ID NO:21 and SEQ ID NO:22)

[033] FIG 9 Depicts human IgG sequence including a C-terminal extension (SEQ ID NO:40).

[034] FIG 10- Figure 10a Electrophoregram of SEQ ID NO:101 under non-reducing conditions. Figure 10b Electrophoregram of SEQ ID NO:101 under non-reducing conditions.

[035] FIG 11- Figure 11A Binding of TNF Variants to immobilized TNFR1. Figure 11B Binding of TNF Variants to immobilized TNFR2

[036] FIG 12- Figure 12a Kym-1 Cell Viability assay in the presence of TNF variants. Figure 12b Kym-1 Cell Viability assay in the presende of TNF variants.

DESCRIPTION

[037] TNF-α is found in both soluble forms and transmembrane forms as a homotrimer. The transmembrane precursor is cleaved, resulting in soluble form. The soluble and transmembrane form signal through two distinct receptors, TNFR1 and TNFR2, resulting in distinct biological effects. Soluble TNF-α (sTNF-α) signaling through TNFR1 is

thought to mediate inflammation while transmembrane TNF-α (tmTNF-α) signaling through TNFR2 is thought to modulate immune response, stimulation of regulatory T-cells (Tregs) and myelin regulation.

[038] While current products and methods of inhibiting TNF- α are effective and account for a significant therapeutic market, the current therapies are not without deleterious side effects. These range from immunosuppression to demyelination of neurons. For instance, therapeutics that are effective immunomodulators in the periphery are contraindicated for treatment of neuroinflammatory disorders. Currently marketed TNF-α inhibitors are labeled with a BLACK BOX WARNING specifically warning against treatment of neurological diseases because they cause demyelination resulting in worsening of the condition. These current TNF-α inhibitors block signaling by both soluble and tmTNF-α, resulting in the beneficial anti-inflammatory effects but also leading to deleterious side effects. Accordingly, there is a significant need for the development of molecules that stimulate signaling through the TNFR2 but not TNFR1.

[039] Accordingly, the present disclosure provides novel TNFR2 agonist molecules. These find use as improved compositions and methods for treating disorders such as, but not limited to pain, nerve injury and/or demyelinating diseases such as, but not limited to multiple sclerosis and optic neuritis.

Definitions

[040] "At least a percent (eg. 97%) sequence identify to Sequence ID No. X" as used herein refers to the extent to which the sequence of two or more nucleic acids or polypeptides is the same. The percent identity between a sequence of interest and a second sequence over a window of evaluation, e.g., over the length of the sequence of interest, may be computed by aligning the sequences, determining the number of residues (nucleotides or amino acids) within the window of evaluation that are opposite an identical residue allowing the introduction of gaps to maximize identity, dividing by the total number of residues of the sequence of interest or the second sequence (whichever is greater) that fall

within the window, and multiplying by 100. When computing the number of identical residues needed to achieve a particular percent identity, fractions are to be rounded to the nearest whole number. Percent identity can be calculated with the use of a variety of computer programs. For example, computer programs such as BLAST2, BLASTN, BLASTP, Gapped BLAST, etc., generate alignments and provide percent identity between sequences of interest. The algorithm of Karlin and Altschul (Karlin and Altschul, Proc. Natl. Acad. Sci USA 67:22264-2268, 1990) modified as in Karlin and Altschul, Proc. Natl. Acad. Sci USA 90:5873-5877, 1993 is incorporated into the NBLAST and XBLAST programs of Altschul et al. (Altschul, et al. J. Mol. Biol. 215:403-410, 1990). To obtain gapped alignments for comparison purposes, Gapped BLAST is utilized as described in Altschul et al. (Altschul, et al. Nucleic Acids Res. 25: 3389-3402, 1997). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs may be used. A PAM250 or BLOSUM62 matrix may be used. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (NCBI). See the Web site having URL world-wide web address of: "ncbi.nlm.nih.gov" for these programs. In a specific embodiment, percent identity is calculated using BLAST2 with default parameters as provided by the NCBI.

[041] "N-terminus" refers to the end of a peptide or polypeptide that bears an amino group in contrast to the carboxyl end bearing a carboxyl acid group.

[042] "C-terminus" refers to the end of a peptide or polypeptide that bears a carboxylic acid group in contrast to the amino terminus bearing an amino group.

[043] "C-terminal IgG Fc protein moiety" refers to a portion of a fusion protein that derives from two identical protein fragments, each having a hinge region, a second constant domain, and a third constant domains of the IgG molecule's two heavy chains, and consisting of the carboxy-terminal heavy chains disulphide bonded to each other through the hinge region. It is functionally defined as that part of the IgG molecule that interacts with the complement protein C1q and the IgG-Fc receptors (FcγR), mediating

Complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC) effector functions. The sequence can be modified to decrease effector functions, to increase circulating half-life, and to eliminate glycosylation sites.

Single-chain TNF-α Variants

[044] The single chain TNF-α variant fusion proteins described herein are generally composed of contiguous amino acids having the following domain structure:

[045] DD-L1-THD-L2-THD-L3-THD or THD-L2-THD-L3-THD-L1-DD, where DD is a dimerization domain as described herein. L1, L2 and L3 are linkers that may be the same or different and THD is a TNF-a homology domain as defined herein. In preferred embodiments the fusion protein is encoded by contiguous nucleotides and expressed as a single contiguous polypeptide.

[046] "N-terminal human TNF-α variant protein moiety" or "N-terminal scTNFR2 Agonist (scTNFR2)" refers to an N-terminal domain of a fusion protein that is derived from a wild type TNF-α protein structurally and functionally defined herein and that is composed of three THDs.

[047] "C-terminal human TNF-α variant protein moiety" or "C-terminal scTNFR2 Agonist (scTNFR2)" refers to a C-terminal domain of a fusion protein that is derived from a wild type TNF-α protein structurally and functionally defines above.

Tregs

[048] "Tregs" or "Treg cells" refer to Regulatory T cells. Regulatory T cells are a class of T cells that suppress the activity of other immune cells, and arc defined using flow cytometry by the cell marker phenotype CD4+CD25+FOXP3+. Because FOXP3 is an intracellular protein and requires cell fixation and permeablization for staining, the cell surface phenotype CD4+CD25+CD127- can be used for defining live Tregs. Tregs also

include various Treg subclasses, such as tTregs (thymus-derived) and pTregs (peripherally-derived, differentiated from naive T cells in the periphery).

Peptide Linkers

[049] "Peptide linker" is defined as an amino acid sequence located between the two proteins comprising a fusion protein, such that the linker peptide sequence is not derived from either partner protein. Peptide linkers are incorporated into fusion proteins as spacers in order to promote proper protein folding and stability of the component protein moieties, to improve protein expression, or to enable better bioactivity of the two fusion partners (Chen, et al., 2013, Adv Drug Deliv Rev. 65(10):1357-69). Peptide linkers can be divided into the categories of unstructured flexible peptides or rigid structured peptides.

Fc Fusion Proteins

[050] An "Fc fusion protein" is a protein made by recombinant DNA technology in which the translational reading frame of the Fc domain of a mammalian IgG protein is fused to that of another protein ("Fc fusion partner") to produce a novel single recombinant polypeptide. Fc fusion proteins are typically produced as disulfide-linked dimers, joined together by disulfide bonds located in the hinge region.

Functional Activation

[051] "Bioactivity" refers to the measurement of biological activity in a quantitative cell-based in vitro assay.

[052] "Functional activation of Treg cells" is defined a TNF-α-mediated response in Tregs. Assay readouts for functional activation of Treg cells includes stimulation of pSTAT5, Treg cell proliferation, and stimulation of the levels of Treg effector proteins.

Design and Construction

[053] There are multiple options for the design and construction of an Fc fusion protein, and the choices among these design options are presented below to permit the generation of a molecule with the desired biological activity and pharmaceutical characteristics. Key design options are: (1) the nature of the TNF-α Selective Agonist, (2) the choice of the dimerization domain protein moiety, i.e. Fc, (3) the configuration of fusion partners in the fusion protein, and (4) the amino acid sequence at the junction between the dimerization domain and the fusion partner protein as well as between the three THDs.

General Methods

[054] In general, preparation of the fusion proteins of the invention can be accomplished by procedures disclosed herein and by recognized recombinant DNA techniques involving, e.g., polymerase chain amplification reactions (PCR), preparation of plasmid DNA, cleavage of DNA with restriction enzymes, preparation of oligonucleotides, ligation of DNA, isolation of mRNA, introduction of the DNA into a suitable cell, transformation or transfection of a host, culturing of the host. Additionally, the fusion molecules can be isolated and purified using chaotropic agents and well known electrophoretic, centrifugation and chromatographic methods. See generally, Sambrook et al., Molecular Cloning: A Laboratory Manual (2nd ed. (1989); and Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons, New York (1989) for disclosure relating to these methods.

[055] The genes encoding the fusion proteins of this invention involve restriction enzyme digestion and ligation as the basic steps employed to yield DNA encoding the desired fusions. The ends of the DNA fragment may require modification prior to ligation, and this may be accomplished by filling in overhangs, deleting terminal portions of the fragment(s) with nucleases (e.g., ExoIII), site directed mutagenesis, or by adding new base pairs by PCR. Polylinkers and adaptors may be employed to facilitate joining of selected fragments. The expression construct is typically assembled in stages employing rounds of restriction, ligation, and transformation of *E. coli.* Numerous cloning vectors suitable for construction of the expression construct are known in the art (λ.ZAP and

pBLUESCRIPT SK-1, Stratagene, LaJolla, Calif., pET, Novagen Inc., Madison, Wis.--cited in Ausubel et al., 1999) and the particular choice is not critical to the invention. The selection of cloning vector will be influenced by the gene transfer system selected for introduction of the expression construct into the host cell. At the end of each stage, the resulting construct may be analyzed by restriction, DNA sequence, hybridization and PCR analyses.

[056] Site-directed mutagenesis is typically used to introduce specific mutations into the genes encoding the fusion proteins of this invention by methods known in the art. See, for example, U.S. Patent Application Publication 2004/0171154; Storici et al., 2001, Nature Biotechnology 19: 773-776; Kren et al., 1998, Nat. Med. 4: 285-290; and Calissano and Macino, 1996, Fungal Genet. Newslett. 43: 15-16. Any site-directed mutagenesis procedure can be used in the present invention. There are many commercial kits available that can be used to prepare the variants of this invention.

[057] Various promoters (transcriptional initiation regulatory region) may be used according to the invention. The selection of the appropriate promoter is dependent upon the proposed expression host. Promoters from heterologous sources may be used as long as they are functional in the chosen host.

[058] Various signal sequences may be used to facilitate expression of the proteins described herein. Signal sequence are selected or designed for efficient secretion and processing in the expression host may also be used. A signal sequence, which is homologous to the TCR coding sequence or the mouse IL-2 coding sequence may be used for mammalian cells. Other suitable signal sequence/host cell pairs include the *B. subtilis* sacB signal sequence for secretion in *B. subtilis*, and the *Saccharomyces cerevisiae* α-mating factor or *P. pastoris* acid phosphatase phoI signal sequences for *P. pastoris* secretion. The signal sequence may be joined directly through the sequence encoding the signal peptidase cleavage site to the protein coding sequence, or through a short nucleotide bridge.

[059] Elements for enhancing transcription and translation have been identified for eukaryotic protein expression systems. For example, positioning the cauliflower mosaic virus (CaMV) promoter 1000 bp on either side of a heterologous promoter may elevate transcriptional levels by 10- to 400-fold in plant cells. The expression construct should also include the appropriate translational initiation sequences. Modification of the expression construct to include a Kozak consensus sequence for proper translational initiation may increase the level of translation by 10 fold.

[060] The expression cassette(s) are joined to appropriate vectors compatible with the host that is being employed. The vector must be able to accommodate the DNA sequence coding for the fusion proteins to be expressed. Suitable host cells include eukaryotic and prokaryotic cells, preferably those cells that can be easily transformed and exhibit rapid growth in culture medium. Specifically preferred hosts cells include prokaryotes such as *E. coli, Bacillus subtillus*, etc. and eukaryotes such as animal cells and yeast strains, e.g., *S. cerevisiae*. Mammalian cells are generally preferred, particularly HEK, J558, NSO, SP2-O or CHO. Other suitable hosts include, e.g., insect cells such as Sf9. Conventional culturing conditions are employed. See Sambrook, supra. Stable transformed or transfected cell lines can then be selected. In vitro transcription-translation systems can also be employed as an expression system.

[061] Nucleic acid encoding a desired fusion protein can be introduced into a host cell by standard techniques for transfecting cells. The term "transfecting" or "transfection" is intended to encompass all conventional techniques for introducing nucleic acid into host cells, including calcium phosphate co-precipitation, DEAE-dextran-mediated transfection, lipofection, electroporation, microinjection, viral transduction and/or integration. Suitable methods for transfecting host cells can be found in Sambrook et al. supra, and other laboratory textbooks.

[062] Alternatively, one can use synthetic gene construction for all or part of the construction of the proteins described herein. This entails in vitro synthesis of a designed polynucleotide molecule to encode a polypeptide molecule of interest. Gene synthesis can

be performed utilizing a number of techniques, such as the multiplex microchip-based technology described by Tian, et. al., (Tian, et. al., Nature 432:1050-1054) and similar technologies wherein oligonucleotides are synthesized and assembled upon photo-programmable microfluidic chips.

[063] The fusion proteins of this invention are isolated from harvested host cells or from the culture medium. Standard protein purification techniques are used to isolate the proteins of interest from the medium or from the harvested cells. In particular, the purification techniques can be used to express and purify a desired fusion protein on a large-scale (i.e. in at least milligram quantities) from a variety of approaches including roller bottles, spinner flasks, tissue culture plates, bioreactor, or a fermentor.

The TNFR2 Selective Agonist Moiety and Fusion Proteins

[064] In one embodiment the molecules described herein are single-chain, trimeric TNF-α molecules. By "single-chain" is meant that a single polypeptide comprises 3 THDs as described herein.

[065] The single chain TNF-α variant fusion proteins described herein are generally composed of contiguous amino acids having the following domain structure: DD-L1-THD-L2-THD-L3-THD or THD-L2-THD-L3-THD-L1-DD, where DD is a dimerization domain as described herein. L1, L2 and L3 are linkers that may be the same or different and THD is a TNF-a homology domain as defined herein. In preferred embodiments the fusion protein is encoded by contiguous nucleotides and expressed as a single contiguous polypeptide.

[066] Full length human TNF-α has the sequence as set forth in Fig 2 (SEQ ID NO:1). It is a type 2 transmembrane protein that is cleaved by the protease ADAM17 to produce the cleaved, soluble TNF-α and uncleaved transmembrane TNF-α. Both soluble and transmembrane molecules signal through cognate receptors. Soluble TNF-a signals primarily through TNFR1, while transmembrane TNF-α signals primarily through

TNFR2. The cleaved, soluble TNF-α has the sequence shown in SEQ ID NO:2. C-terminal to the cleavage site is a domain that forms the TNF-homology domain (THD), which is a sequence and structurally similar domain found in members of the TNF superfamily, that makes up the receptor binding domain of the molecule. Of note, a region N-terminal to the THD domain and including the ADAM17 cleavage site is a domain of the molecule referred to as the "stalk region". This stalk region does not appear to be found in the receptor-binding portion of the molecule. Accordingly, domains of TNF-α include from N- to C- terminus: N-terminal intracellular domain, a transmembrane domain, stalk region, ADAM17 cleavage site within the stalk region and THD domain. The transmembrane domain terminates at amino acid 56. The stalk region is defined as amino acids 57-87 of the full-length sequence. The ADAM17 cleavage site is found between amino acids 76/77. The THD domain begins at amino acid 88 and extends to amino acid 233. This is summarized in FIG. 2.

[067] Mutations in the THD have been identified that abrogate binding to TNFR1 and result in a molecule that agonizes TNFR2. The mutations are D143N and A145R, wherein the numbering is based on the sequence of soluble TNF-α. This corresponds to D219N and A221R wherein the numbering is based on the full length TNF-α sequence. That is, at these positions, the native sequences of D and A are mutated to an N and R, respectively. These mutations will be referred to as TNFR2 agonist sequences herein.

[068] Accordingly, the present disclosure provides single-chain TNFR2 agonists (scTNFR2) comprising a first, second and third THD domain comprising the TNFR2 agonist sequences. In some embodiments all three of the THD domains of the scTNFR2 agonist comprise the TNFR2 agonist sequences. Sequence of the THD domain comprising the TNFR2 agonist sequences is found in SEQ ID NO:3.

[069] The variants of this invention optionally include conservatively substituted variants that apply to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refer to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid

does not encode an amino acid sequence, to essentially identical sequences. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed base and/or deoxyinosine residues (Batzer et al., Nucleic Acid Res. 19:5081 (1991); Ohtsuka et al., J. Biol. Chem. 260:2605-2608 (1985); Rossolini et al., Mol. Cell. Probes 8:91-98 (1994)). Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are silent variations, which are one species of conservatively modified variations. Every nucleic acid sequence herein that encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid that encodes a polypeptide is implicit in each described sequence.

[070] With regard to conservative substitution of amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a conservatively modified variant where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention.

[071] The following groups each contain amino acids that are conservative substitutions for one another:

1) Alanine (A), Glycine (G);

2) Serine (5), Threonine (T);

3) Aspartic acid (D), Glutamic acid (E);

4) Asparagine (N), Glutamine (Q);

5) Cysteine (C), Methionine (M);

6) Arginine (R), Lysine (K), Histidine (H);

7) Isoleucine (I), Leucine (L), Valine (V); and

8) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

Dimerization Domains

[072] One design choice is the nature of the dimerization domains of the fusion protein. Without being bound by theory, it is thought that dimerization enhances signaling by the TNFR2 agonist and also may improve half-life of the fusion protein. There are many different dimerization domains, such as Fc fusion proteins derived from other dimerizing molecules, such as IgE heavy chain domain 2 (EHD2) and IgM heavy chain domain 2 (MHD2).

Fc protein moiety

[073] The main therapeutic applications of Fc fusion proteins are (1) endowing the fusion partner protein with immunoglobulin Fc effector functions; or (2) increasing the circulating half-life of the fusion partner protein (Czajkowsky, et al., 2012, EMBO Mol Med. 4:1015-28). The primary effector functions of IgG proteins are Complement-Dependent Cytotoxicity (CDC) and Antibody-Dependent Cellular Cytotoxicity (ADCC), functions mediated by Fc binding to complement protein C1q and to IgG-Fc receptors (FcγR), respectively. These effector functions are important when the therapeutic protein

is used to direct or enhance the immune response to a particular antigen target or cell. The fusion protein of this invention is designed solely to increase the circulating half-life of the TNFR2 Selective Agonist moiety, and effector functions are not needed and can even be toxic, and thus in some embodiments not desired. For instance, a scTNFR2 agonist-Fc fusion protein with an effector function-competent Fc can potentially kill the Treg cells that the fusion protein of this invention is seeking to activate and expand, exactly the opposite of the therapeutic goal for autoimmune diseases. There are four human IgG subclasses that differ in effector functions (CDC, ADCC), circulating half-life, and stability (Salfeld, J. G., 2007, Nature Biotechnology 25:1369 -72). IgG1 possesses Fc effector functions, is the most abundant IgG subclass, and is the most commonly used subclass in US FDA-approved therapeutic proteins. IgG2 is deficient in Fc effector functions, but is subject to dimerization with other IgG2 molecules, and is also subject to instability due to scrambling of disulfide bonds in the hinge region. IgG3 possesses Fc effector functions, and has an extremely long, rigid hinge region. IgG4 is deficient in Fc effector functions, has a shorter circulating half-life than the other subclasses, and the IgG4 dimer is biochemically unstable due to only a single disulfide bond in the hinge region leading to the exchange of H chains between different IgG4 molecules. A skilled artisan would recognize that Fc protein moieties from IgG2 and IgG4 do not possess effector functions and can be used in this invention. The skilled artisan would also recognize that Fc sequence modifications have been described in the art that such that the hinge region of IgG2 Fc can be modified to prevent aggregation, or that the hinge region of IgG4 Fc can be modified to stabilize dimers. It will be appreciated by those of ordinary skill in the art that the IgG described in the sequences of the fusion constructs disclosed herein may be changed. That is, where an IgG1 seqeunce is disclosed, this can be exchanged with an IgG2 or IgG4 and the like.

[074] Alternatively, effector function-deficient variants of IgG1 have been generated. One such variant has an amino acid substitution at position N297, the location of an N-linked glycosylation site. Substitution of this asparagine residue removes the glycosylation site and significantly reduces ADCC and CDC activity (Tao, M. H., et al., 1989, J Immunol.

143:2595-2601). This variant is used as an exemplary case in the invention herein. Another effector function deficient IgG1 variant is IgG1(L234F/L235E/P331S) (Oganesyan, et al., 2008, Acta Crystallogr D Biol Crystallogr. 64:700-4), which mutates amino acids in the C1q and FcγR binding sites, and one skilled in the art would consider using these or similar Fc variants to generate effector-deficient and stable scTNFR2 agonist-Fc fusion proteins. Other mutations at these sites, such as L234A and L235A can also be used in the fusion protein described herein. Exemplary IgG sequences and variants are shown in FIG 6-8 and in SEQ ID NOs:5-22.

[075] A skilled artisan would also recognize that forms of Fc protein moieties engineered to be stable monomers rather than dimers (Dumont, J. A., et., al., 2006, BioDrugs 20:151-60; Liu Z, et al., J Biol Chem. 2015 20;290:7535-62) can also be combined with the TNFR2 selective agonist of this invention. In addition, a skilled artisan would recognize that a functionally monomeric heterodimer composed of a TNFR2 agonist-Fc H chain polypeptide combined with an Fc H chain polypeptide and assembled using bispecific antibody technology (Zhu Z, et al., 1997 Protein Sci. 6:781-8) can also be combined with the TNFR2 Selective Agonist of this invention. In addition, a skilled artisan will recognize that Fc variants that lack some or all of the hinge region can be used with this invention.

[076] Fc fusion proteins can be made in two configurations, indicated here as X-Fc, where X, the scTNFR2 agonist fusion partner protein, is at the N-terminus and Fc is at the C-terminus, and Fc-X, where the Fc is at the N-terminus, and the scTNFR2 agonist fusion partner protein is at the C-terminus (FIG. 1). There are examples in the literature showing that different fusion partners can have distinct preferences for N- or C-terminal Fc fusions. For instance, FGF21 has been shown to have a strong preference for the Fc-X configuration. Fc-FGF21 has receptor-activating bioactivity essentially the same as FGF21 itself, while FGF21-Fc has 1000-fold reduced bioactivity (Hecht, et al., 2012, PLoS One. 7(11):e49345). A number of IL2 agonist Fc fusion proteins have been made for various applications, and these have been reported to have good IL-2 bioactivity when

directly fused to Fc in both the Fc-X (Gillies, et al., 1992, Proc Natl Acad Sci, 89:1428-32; Bell, et al., 2015, J Autoimmun. 56:66-80) and X-Fc (Zheng, X. X., et al., 1999, J Immunol. 163:4041-8) configurations. Gavin, et al. (US 20140286898 A1) describes Fc fusion proteins containing IL-2 and certain IL-2 variants in the in the Fc-X configuration that have bioactivity similar to that of the free IL-2 cytokine, but in contrast to the results of Zheng et al, (Zheng, X. X., et al., 1999, J Immunol. 1999, 163:4041-8) found that IL-2 variant fusion proteins in the X-Fc configuration have reduced or no bioactivity. Thus, whether an N-terminal dimerization domain or a C-terminal dimerization within any given fusion protein is preferred is unpredictable.

EHD2

[077] A recently described dimerization domain may also find use in connection with the scTNFR2 agonist described herein. This polypeptide was used to form dimers of other molecules in WO 2013/156148, which is expressly incorporated herein by reference. The EHD2 sequence is

DFTPPTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQ

VMDVDLSTASTTQEGELASTQSELTLSQKHWLSDRTYTCQVTYQ

GHTFEDSTKKCADSN. (SEQ ID NO:23)

MHD2

[078] Another recently described dimerization domain may also find use in connection with the scTNFR2 agonist described herein. This polypeptide was used to form dimers of other molecules in WO 2013/156148. The MHD2 sequence is

AELPPKVSVFVPPRDGFFGNPRKSKLICQATGFSPRQIQVSWLREG

KQVGSGVTTDQVQAEAKESGPTTYKVTSTLTIKESDWLGQSMFT

CRVDHRGLTFQQNASSMCVPD. (SEQ ID NO:24)

Linker

[079] The amino acid sequence at the junction between the Fc and the fusion partner protein can be either (1) a direct fusion of the two protein sequences or (2) a fusion with an intervening linker peptide. Of the 10 Fc fusion proteins that are presently approved by the US FDA for clinical use (TABLE I), 8 are direct fusions of the fusion partner protein with Fc, while 2 possess linker peptides, so many Fc fusion proteins can be functional without linker peptides. Linker peptides are included as spacers between the two protein moieties. Linker peptides can promote proper protein folding and stability of the component protein moieties, improve protein expression, and enable better bioactivity of the component protein moieties (Chen, et al., 2013, Adv Drug Deliv Rev. 65:1357-69). Peptide linkers used in many fusion proteins are designed to be unstructured flexible peptides. A study of the length, sequence, and conformation of linkers peptides between independent structural domains in natural proteins has provided a theoretical basis for the design of flexible peptide linkers (Argos, 1990, J Mol Biol. 211:943-58). Argos provided the guidance that long flexible linker peptides be composed of small nonpolar residues like Glycine and small polar resides like Serine and Threonine, with multiple Glycine residues enabling a highly flexible conformation and Serine or Threonine providing polar surface area to limit hydrophobic interaction within the peptide or with the component fusion protein moieties. Many peptide linkers described in the literature are rich in glycine and serine, such as repeats of the sequence GGGGS (SEQ ID NO:25), although an artisan skilled in the art will recognize that other sequences following the general recommendations of Argos (Argos, 1990, J Mol Biol. 20;211(4):943-58) can also be used. In some embodiments polypeptide sequences from one of the fusion partners may be used as a linker. For instance, N- or C-terminal extensions from TNF-α or a dimerization domain, such as Fc, could be used all or part of the linker between the fusion partners. In some embodiments the C-terminal extension from human IgG finds use as a linker and is shown as: ELQLEESSAEAQDGELDG (SEQ ID NO:41) or a variant of this also finds use as a linker: ELQLEESSAEAQGG (SEQ ID NO:42).

TABLE I

TABLE I. US FDA-approved Fc fusion proteins and their characteristics

DRUG	Fc Isotype	Fusion Partner	N vs C fusion	Linker Peptide	Half-life (days)
Romiplostim	G1	TPO-R peptide	C	Y	3.5
Etanercept	G1	P75 TNFa-R	N	N	4.3
Alefacept	G1	LFA3	N	N	10.1
Rilonacept	G1	IL1-R	N	N	8.6
Abatacept	G1	CTLA4	N	N	16.7
Belatacept	G1	CTLA4 (mut)	N	N	9.8
Aflibercept	G1	VEGF R1 + R2	N	N	n/a
Dulaglutide	G4 (mut)	GLP1	N	Y	3.7
Eloctate	G1	FVIII	N	N	0.8
Alprolix	G1	FIX	N	N	3.6

[080] In some embodiments, particularly when the fusion protein is in the DD-X configuration, the dimerization domain (DD), i.e. Fc, is directly linked to the N-terminus of the single-chain THD, i.e. TNFR2 agonist.

[081] In some embodiments, particularly when the fusion protein is in the DD-X configuration, the linker between the N-terminus of the first THD domain of the scTNFR2 agonist is sequence from TNF-α itself. That is, sequences from the native TNF-α stalk region are used as a linker between the THD domain of the TNFR2 agonist and the C-terminus of the Fc domain. The linker between the THD domain of the TNFR2 agonist and the C-terminus of the Fc domain contains from 1 to 31 amino acids or contains 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or 31 amino acids. The stalk region contains the sequences shown below and the linker using contiguous amino acids from this region may include from 1 to 31 contiguous amino acids of this sequence. The sequence from the first amino acid of the stalk region to last amino acid prior to the THD domain includes: GPQREEFPRDLSLISPLAQAVRSSSRTPSDK (SEQ ID NO:26). In some embodiments

sequences comprising at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 contiguous sequences from the stalk region can be used as a linker between the N-terminus of the scTNF agonist and dimerization domain.

[082] In some embodiments, other linkers, such as combinations of Gly and Ser find use as linkers. In some embodiments linkers using (GGGGS)n, where n = 1-5 find use as linkers between the dimerization domain, i.e. Fc and first THD of the scTNFR2 agonist. In some embodiments, combinations of the stalk region sequences and Gly/Ser amino acids find use as the linker.

[083] In some embodiments a linker peptide of 5, 10, 15, or 20 amino acids will have a maximum fully extended length of 17.5 Å, 35 Å, 52.5 Å, or 70 Å, respectively. The maximal end-to-end length of the peptide linker can also be a guide for defining the characteristics of a peptide linker in this invention. The goal of a linker peptide within the current invention is to enable attainment of an appropriate conformation and orientation of the individual fusion protein moieties to allow the engagement of the TNFR2 Selective Agonist moiety with its cognate receptor and allow the binding of the Fc moiety to the FcRn to enable fusion protein recycling and a prolonged circulating half-life. Since the factors influencing these interactions are difficult to predict, the requirement for and the proper length of a linker peptide must be empirically tested and determined. Many Fc fusion proteins do not require linker peptides, as evidenced by the 8 out of 10 US FDA-approved Fc fusion proteins lacking such peptides listed in Table I. In contrast, Dulaglutide, a fusion of GLP-1 and Fc, contains a 15 residue peptide linker which has a strong influence on bioactivity (Glaesner, U.S. Pat. No. 7,452,966 B2).

[084] In the context of the single-chain TNFR2 agonist, other linkers may be found between the THD domains. That is, a linker may be found between the first and second and then the second third THD domain of the TNFR2 agonist. The linkers may be the same or may be different. In some embodiments the linkers may be any linker outlined herein including GGGGS linkers. In some embodiments the linker may comprise multiple unites of the

GGGGS sequence as described as $(GGGGS)_n$, wherein n=1, 2, 3, 4, 5, 6, 7, 8, 9 or 10. In some embodiments sequences from the stalk region from find use as linkers between the THDs. In addition, in some embodiments, combinations of Gly/Ser amino acids as well as contiguous amino acids from the stalk region find use as linkers between THDs. Linker between the first and second THDs may be the same or different from the linker between the second and third THD but generally both linkers will be comprised of $(GGGGS)_n$, wherein n=1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 and/or contiguous sequences from the stalk region.

[085] In other embodiments, particularly in the X-DD configuration, a linker may be placed between the C-terminus of the third THD domain and the N-terminus of dimerization domain, i.e. Fc domain. Again this can be Gly/Ser linkers as described herein and may comprise $(GGGGS)_n$, where n=1-5.

Fusion Proteins

[086] Accordingly, the present disclosure provides scTNFR2 fusion proteins comprising a dimerization domain, three THD's each comprising the D143N/A145R mutations to confer selectivity for TNFR2, and a linker between each of the THDs. In some embodiments the dimerization domain is at the N-terminus of the scTNFR2 agonist domain, while in other embodiments the dimerization domain as at the C-terminus of the molecule.

[087] Fusion proteins disclosed herein comprise the following formulas: DD-L1-THD-L2-THD-L3-THD or THD-L2-THD-L3-THD-L1-DD, where DD is a dimerization domain as described herein. Dimerization domains are selected from IgG1, IgG2 an IgG4 Fc domains lacking effector function. In one embodiment the Fc is from IgG2 and lacking effector function. In one embodiment the Fc is from IgG4. In one embodiment the dimerization domain is EHD2 or MHD2. Then the dimerization domain is at the N-terminus of the scTNFR2 agonist protein, the linker (L1) is preferably $(GGGGS)_n$ where n=1-5, although in some embodiments the L1 linker comprises some or all of the stalk

region from TNF-α. All fusion proteins of the invention disclosed herein contain THD with the TNFR2 agonist selective sequences D143N/A145R and are referred to below as THD. Linkers (L2 and L3) between the first and second, and second and third THD may also be constructed from GGGGS, G/S linkers or from some or all of the stalk region. When the dimerization domain is at the C-terminus of the scTNFR2 agonist protein there may not be a linker, or the linker may comprise $(GGGGS)_n$ where n=1-5. Preferred configurations of fusion proteins include:

- DD-L1-THD-L2-THD-L3-THD, wherein DD is the IgG4 Fc with mutation(s) eliminating effector function, L1, L2 and L3 are GGGGS;
- DD-L1-THD-L2-THD-L3-THD, wherein DD is the IgG4 Fc with mutation(s) eliminating effector function, L1 is GGGGSGGGGS (SEQ ID NO:27), L2 and L3 are both GGGGs;
- DD-L1-THD-L2-THD-L3-THD, wherein DD is the IgG1 Fc with mutation(s) eliminating effector function, L1 is VRSSSRTPSDK, L2 and L3 are both GGGGS;
- DD-L1-THD-L2-THD-L3-THD, wherein DD is the IgG1 Fc with mutation(s) eliminating effector function, L1 is VRSSSRTPSDK (SEQ ID NO:4), L2 and L3 are both SSRTPSDK;
- DD-L1-THD-L2-THD-L3-THD, wherein DD is the IgG1 Fc with mutation(s) eliminating effector function, L1 is GPQREEFPRDLSLISPLAQAVRSSSRTPSDK, L2 and L3 are both GGGGS;
- DD-L1-THD-L2-THD-L3-THD, wherein DD is the IgG1 Fc with mutation(s) eliminating effector function, L1 is GPQREEFPRDLSLISPLAQAVRSSSRTPSDK, L2 and L3 are both SSRTPSDK (SEQ ID NO:28);

- DD-L1-THD-L2-THD-L3-THD, wherein DD is the IgG1 Fc with mutation(s) eliminating effector function, L1 is GPQREEFPRDLSLISPLAQAVRSSSRTPSDK, L2 and L3 are both VRSSSRTPSDK;

- DD-L1-THD-L2-THD-L3-THD, wherein DD is the IgG1 Fc with mutation(s) eliminating effector function, L1 is GGGGSVRSSSRTPSDK (SEQ ID NO:29), L2 and L3 are both VRSSSRTPSDK;

- DD-L1-THD-L2-THD-L3-THD, wherein DD is the IgG1 Fc with mutation(s) eliminating effector function, L1 is GGGGSVRSSSRTPSDK, L2 and L3 are both GGGGSSSRTPSDK (SEQ ID NO:30);

- DD-L1-THD-L2-THD-L3-THD, wherein DD is the IgG1 Fc with mutations eliminating effector function, L1, L2 and L3 are GGGGS;

- DD-L1-THD-L2-THD-L3-THD, wherein DD is the IgG1 Fc with mutations eliminating effector function, L1 is GGGGSGGGGS (SEQ ID NO:27), L2 and L3 are both GGGGs;

- DD-L1-THD-L2-THD-L3-THD, wherein DD is the IgG1 Fc with mutations eliminating effector function, L1 is VRSSSRTPSDK, L2 and L3 are both GGGGS;

- DD-L1-THD-L2-THD-L3-THD, wherein DD is the IgG1 Fc with mutations eliminating effector function, L1 is VRSSSRTPSDK (SEQ ID NO:4), L2 and L3 are both SSRTPSDK;

- DD-L1-THD-L2-THD-L3-THD, wherein DD is the IgG1 Fc with mutations eliminating effector function, L1 is GPQREEFPRDLSLISPLAQAVRSSSRTPSDK, L2 and L3 are both GGGGS;

- DD-L1-THD-L2-THD-L3-THD, wherein DD is the IgG1 Fc with mutations eliminating effector function, L1 is GPQREEFPRDLSLISPLAQAVRSSSRTPSDK, L2 and L3 are both SSRTPSDK (SEQ ID NO:28);

- DD-L1-THD-L2-THD-L3-THD, wherein DD is the IgG1 Fc with mutations eliminating effector function, L1 is GPQREEFPRDLSLISPLAQAVRSSSRTPSDK, L2 and L3 are both VRSSSRTPSDK;

- DD-L1-THD-L2-THD-L3-THD, wherein DD is the IgG1 Fc with mutations eliminating effector function, L1 is GGGGSVRSSSRTPSDK (SEQ ID NO:29), L2 and L3 are both VRSSSRTPSDK;

- DD-L1-THD-L2-THD-L3-THD, wherein DD is the IgG1 Fc with mutations eliminating effector function, L1 is GGGGSVRSSSRTPSDK, L2 and L3 are both GGGGSSSRTPSDK (SEQ ID NO:30);

- DD-L1-THD-L2-THD-L3-THD, wherein DD is the IgG4 Fc, L1, L2 and L3 are GGGGS;

- DD-L1-THD-L2-THD-L3-THD, wherein DD is the IgG4 Fc, L1 is GGGGSGGGGS, L2 and L3 are both GGGGs;

- DD-L1-THD-L2-THD-L3-THD, wherein DD is the IgG4 Fc, L1 is VRSSSRTPSDK, L2 and L3 are both GGGGS;

- DD-L1-THD-L2-THD-L3-THD, wherein DD is the IgG4 Fc, L1 is VRSSSRTPSDK, L2 and L3 are both SSRTPSDK;

- DD-L1-THD-L2-THD-L3-THD, wherein DD is the IgG4 Fc, L1 is GPQREEFPRDLSLISPLAQAVRSSSRTPSDK, L2 and L3 are both GGGGS;

- DD-L1-THD-L2-THD-L3-THD, wherein DD is the IgG4 Fc, L1 is GPQREEFPRDLSLISPLAQAVRSSSRTPSDK, L2 and L3 are both SSRTPSDK;
- DD-L1-THD-L2-THD-L3-THD, wherein DD is the IgG4 Fc, L1 is GPQREEFPRDLSLISPLAQAVRSSSRTPSDK, L2 and L3 are both VRSSSRTPSDK;
- DD-L1-THD-L2-THD-L3-THD, wherein DD is the, L1 is GGGGSVRSSSRTPSDK, L2 and L3 are both VRSSSRTPSDK;
- DD-L1-THD-L2-THD-L3-THD, wherein DD is the IgG4 Fc, L1 is GGGGSVRSSSRTPSDK, L2 and L3 are both GGGGSSSRTPSDK;
- THD-L2-THD-L3-THD-L1-DD, wherein DD is the IgG1 Fc with mutations eliminating effector function, L1, L2 and L3 are GGGGS;
- THD-L2-THD-L3-THD-L1-DD, wherein DD is the IgG1 Fc with mutations eliminating effector function, L1 is GGGGS and L2 and L3 are SSRTPSDK;
- THD-L2-THD-L3-THD-DD, wherein DD is the IgG1 Fc with mutations eliminating effector function, L2 and L3 are GGGGS and scTNFR2 agonist domain is fused directly to DD;
- THD-L2-THD-L3-THD-DD, wherein DD is the IgG1 Fc with mutations eliminating effector function, L2 and L3 are SSRTPSDK and scTNFR2 agonist domain is fused directly to DD;
- THD-L2-THD-L3-THD-DD, wherein DD is the IgG1 Fc with mutations eliminating effector function, L2 and L3 are GGGGSSSRTPSDK and scTNFR2 agonist domain is fused directly to DD;

- THD-L2-THD-L3-THD-DD, wherein DD is the IgG1 Fc with mutations eliminating effector function, L2 and L3 are VRSSSRTPSDK and scTNFR2 agonist domain is fused directly to DD;
- THD-L2-THD-L3-THD-L1-DD, wherein DD is the IgG4 Fc, L1, L2 and L3 are GGGGS;
- THD-L2-THD-L3-THD-L1-DD, wherein DD is the IgG4 Fc, L1 is GGGGS and L2 and L3 are SSRTPSDK;
- THD-L2-THD-L3-THD-DD, wherein DD is the IgG4 Fc, L2 and L3 are GGGGS and scTNFR2 agonist domain is fused directly to DD;
- THD-L2-THD-L3-THD-DD, wherein DD is the IgG4 Fc, L2 and L3 are SSRTPSDK and scTNFR2 agonist domain is fused directly to DD;
- THD-L2-THD-L3-THD-DD, wherein DD is the IgG4, L2 and L3 are GGGGSSSRTPSDK and scTNFR2 agonist domain is fused directly to DD;
- THD-L2-THD-L3-THD-DD, wherein DD is the IgG4 Fc, L2 and L3 are VRSSSRTPSDK and scTNFR2 agonist domain is fused directly to DD;
- THD-L2-THD-L3-THD-L1-DD, wherein DD is the IgG4 Fc with mutation(s) eliminating effector function, L1, L2 and L3 are GGGGS;
- THD-L2-THD-L3-THD-L1-DD, wherein DD is the IgG4 Fc with mutation(s) eliminating effector function, L1 is GGGGS and L2 and L3 are SSRTPSDK;
- THD-L2-THD-L3-THD-DD, wherein DD is the IgG4 Fc with mutation(s) eliminating effector function, L2 and L3 are GGGGS and scTNFR2 agonist domain is fused directly to DD;

- THD-L2-THD-L3-THD-DD, wherein DD is the IgG4 Fc with mutations eliminating effector function, L2 and L3 are SSRTPSDK and scTNFR2 agonist domain is fused directly to DD;
- THD-L2-THD-L3-THD-DD, wherein DD is the IgG4 Fc with mutations eliminating effector function, L2 and L3 are GGGGSSSRTPSDK and scTNFR2 agonist domain is fused directly to DD;
- THD-L2-THD-L3-THD-DD, wherein DD is the IgG4 Fc with mutations eliminating effector function, L2 and L3 are VRSSSRTPSDK and scTNFR2 agonist domain is fused directly to DD.

[088] In some embodiments, the Fc-scTNFR2 agonist fusion protein comprises the sequence as shown in SEQ ID NO:31, 32, 34, or 35. In some embodiments the Fc-scTNFR2 agonist fusion protein comprises the sequence as shown in SEQ ID NOs: 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 118, 119 or 120. In some embodiments the scTNFR2 agonist fusion protein comprises a protein having at least 80% or 85% or 90% or 95% or 96% or 97% or 98% or 99% identity with SEQ ID NO:31, 32, 34 or 35 or SEQ IS NOs: 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 118, 119 or 120. In some embodiments, the present disclosure provides a nucleic acid encoding a protein as set forth in SEQ ID NO:31, 32, 34, or 35 or a protein having at least 80% or 85% or 90% or 95% or 96% or 97% or 98% or 99% identity with SEQ ID NO:31, 32, 34 or 35 or SEQ ID NOs 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 118, 119 or 120. In some embodiments the nucleic acid comprises a nucleic acid sequence having at least 80% or 85% or 90% or 95% or 96% or 97% or 98% or 99% identity with SEQ ID NO:36, 37, 38 or 39. In some embodiments the nucleic acid comprises the sequence shown in SEQ ID NO: 36, 27, 28 or 39.

Bioassays

[089] Robust and quantitative bioassays are necessary for the characterization of the biological activity of candidate proteins. These assays should measure the activation of the TNFR2

receptor, measure the downstream functional consequences of activation in Tregs, and measure therapeutically-relevant outcomes and functions of the activated Tregs. These assays can be used the measure the therapeutic activity and potency of scTNFR2 Selective Agonist molecules, and can also be used for measurement of the pharmacodynamics of an scTNFR2 Selective Agonist in animals or in humans. One assay measures the TNF-α mediated caspase activity. In cells lacking TNFR1 or when TNFR1 cannot signal, this is a measure of TNFR2 activation. Another assay for functional activation measures TNFR2 agonist stimulated proliferation of Treg cells. One of ordinary skill in the art will recognize that Treg proliferation can be measured by tritiated thymidine incorporation into purified Treg cells, by an increase in Treg cell numbers in a mixed population of cells measured by flow cytometry and the frequencies of CD4+CD25+FOXP3+ or the CD4+CD25+CD127- marker phenotypes, by increased expression in Treg cells of proliferation-associated cell cycle proteins, such as Ki-67, or by measurement of the cell division-associated dilution of a vital fluorescent dye such as carboxyfluorescein succinimidyl ester (CFSE) by flow cytometry in Treg cells. Accordingly, in some embodiments the present disclosure provides methods of stimulating or expanding Tregs. In some embodiments the fusion proteins of described herein stimulate the expansion of Tregs more potently that EHD2-TNFR2 agonist (disclosed in Dong et al. PNAS 2016).

[090] Other assays include the Kym-1 cell viability assay disclosed in the examples. In some embodiments the disclosure provides Fc-TNFR2 agonist fusion proteins that reduce viability of Kym-1 cells following culture as descrived herein. In some embodiments the Fc-TNFR2 agonists reduce the viability of Kym-1 cells more than EHD2-TNFR2 agonist (disclosed in Dong et al. PNAS 2016).

Formulation

[091] Pharmaceutical compositions of the fusion proteins of the present invention are defined as formulated for parenteral (particularly intravenous or subcutaneous) delivery according to conventional methods. In general, pharmaceutical formulations will include

fusion proteins of the present invention in combination with a pharmaceutically acceptable vehicle, 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. Methods of formulation are well known in the art and are disclosed, for example, in Remington: The Science and Practice of Pharmacy, Gennaro, ed., Mack Publishing Co., Easton, Pa., 19.sup.th ed., 1995.

[092] As an illustration, pharmaceutical formulations may be supplied as a kit comprising a container that comprises fusion proteins of the present invention. Therapeutic proteins can be provided in the form of an injectable solution for single or multiple doses, as a sterile powder that will be reconstituted before injection, or as a prefilled syringe. 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 fusion proteins of the present invention is contraindicated in patients with known hypersensitivity to fusion proteins of the present invention.

[093] The scTNFR2 selective agonist fusion proteins of this invention can be incorporated into compositions, including pharmaceutical compositions. Such compositions typically include the protein and a pharmaceutically acceptable carrier. As used herein, the term "pharmaceutically acceptable carrier" includes, but is not limited to, saline, solvents, dispersion media, coatings, antibacterial and antifungal agents isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Supplementary active compounds (e.g., antibiotics) can also be incorporated into the compositions.

[094] A pharmaceutical composition is formulated to be compatible with its intended route of administration. The scTNFR2 selective agonist fusion proteins of the invention is likely that to be administered through a parenteral route. Examples of parenteral routes of administration include, for example, intravenous, intradermal, and subcutaneous. Solutions or suspensions used for parenteral application can include the following

components: a sterile diluent such as water for injection, saline solution, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfate; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as mono- and/or di-basic sodium phosphate, hydrochloric acid or sodium hydroxide (e.g., to a pH of about 7.2-7.8, e.g., 7.5). The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[095] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, or phosphate buffered saline (PBS). In all cases, the composition should be sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The maintenance of the required particle size in the case of dispersion may be facilitated by the use of surfactants, e.g., Polysorbate or Tween. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition.

[096] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions

are prepared by incorporating the active compound into a sterile vehicle, which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[097] In one embodiment, the scTNFR2 selective agonist fusion protein is prepared with carriers that will protect the scTNFR2 selective agonist fusion protein against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Such formulations can be prepared using standard techniques.

[098] The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

Administration

[099] Fusion proteins of the present invention will preferably be administered by the parenteral route. The subcutaneous route is the preferred route, but intravenous, intramuscular, and subdermal administration can also be used. For subcutaneous or intramuscular routes, depots and depot formulations can be used. For certain diseases specialized routes of administration can be used. For instance, for eye diseases, such as but not limited to optic neuritis, intraocular injection can be used. Fusion proteins can be used in a concentration of about 0.1 to 10 mcg/ml of total volume, although concentrations in the range of 0.01 mcg/ml to 100 mcg/ml may be used. In some embodiments peripheral administration is used to treat neurological disorders. In some embodiments intrathecal administration is used, which can deliver the fusion proteins into the spinal fluid which can bypass the blood brain barrier.

[0100] Determination of dose is within the level of ordinary skill in the art. Dosing is daily or weekly over the period of treatment, or may be at another intermittent frequency. Intravenous administration will be by bolus injection or infusion over a typical period of one to several hours. Sustained release formulations can also be employed. In general, a therapeutically effective amount of fusion proteins of the present invention is an amount sufficient to produce a clinically significant change in the treated condition, such as a clinically significant change in circulating Treg cells, a clinically significant change in Treg cells present within a diseased tissue, or a clinically significant change in a disease symptom.

[0101] The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the half maximal effective concentration (EC_{50}; i.e., the concentration of the test compound which achieves a half-maximal stimulation of Treg cells) with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the EC_{50} as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by enzyme-linked immunosorbent assays.

[0102] As defined herein, a therapeutically effective amount of a scTNFR2 selective agonist fusion protein (i.e., an effective dosage) depends on the polypeptide selected and the dose frequency. For instance, single dose amounts in the range of approximately 0.01 to 50 mg/kg of patient body weight can be administered; in some embodiments, about 0.05 to 10 mg/kg, or 0.1 to 25 mg/kg of patient body weight can be administered; in some embodiments about 0.5 to 10 mg/kg of patient body weight can be administered. In some embodiments about 0.1 mg/kg, 0.5 mg/kg, 1 mg/kg, 2.5 mg/kg, 5mg/kg, 7.5 mg/kg, 10

mg/kg, or 20 mg/kg or 40 mg/kg or 50 mg/kg of patient body can be administered. In some embodiments, for instance when intraocular administration is used, the concentration per patient body weight is in appropriate measure to use. Rather, a total of 0.5mg, or 1mg or 1.5 mg or 2mg or 2.5mg or 3mg or 3.5 mg or 4 mg or 5 mg of fusion protein are administered in each eye. The compositions can be administered from one time per day to one or more times per week, or one or more times per month; including once every other day, or twice a week or twice a month. The skilled artisan will appreciate that certain factors may influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, the level of Treg cells present in the patient, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of the TNFR2 selective agonist fusion protein of the invention is likely to be a series of treatments.

Diseases

[0103] Some of the diseases that can benefit from the therapy of this invention have been noted. However, the role of Treg cells in autoimmune diseases is a very active area of research, and additional diseases will likely be identified as treatable by this invention. Autoimmune diseases are defined as human diseases in which the immune system attacks its own proteins, cells, and tissues. A comprehensive listing and review of autoimmune diseases can be found in The Autoimmune Diseases (Rose and Mackay, 2014, Academic Press).

[0104] As disclosed herein, even when administered peripherally, scTNFR2 agonist proteins may be used to treat neurological disorders, particularly those characterized by elevated TNF-α. In one embodiment the scTNFR2 molecules disclosed herein find use in treating neurological disorders, e.g., by reducing inflammation in the brain, protecting myelination of neurons and/or promoting remyelination of neurons. Accordingly, neurological disorders particularly amenable to the methods disclosed herein include art-recognized inflammatory neurodegenerative diseases, which may result in the destruction

of myelin or may include other neurological disorders not necessarily characterized by myelin destruction but are characterized by elevated levels of TNF-α.

[0105] In one embodiment, neurodegenerative diseases are a group of diseases typified by deterioration of neurons and/or their myelin sheath. This destruction of neurons eventually leads to dysfunction and disabilities. Often inflammation, thought to be mediated by microglial cells, is found to be a component of neurodegenerative diseases and adds to the pathogenesis of the neurodegeneration. Collectively, these diseases comprise the art-recognized neurodegenerative diseases. Neuroinflammation may occur years prior to any considerable loss of neurons in some neurodegenerative disorders. For example, 70% of dopaminergic neurons are lost from the substantia nigra before patients begin to manifest the clinical signs of Parkinson's disease, see, e.g., Factor and Weiner (2008) Parkinson's Disease: Diagnosis and Clinical Management. Many different types of immune cells, including macrophages, neutrophils, T cells, astrocytes, and microglia, can contributed to the pathology of immune-related diseases, like Multiple Sclerosis (M.S.), Parkinson's disease, Huntington's disease, dementia (including but not exclusively diseases like Alzheimer's disease, frontotemporal dementia, trauma related dementia (punch drunk), HIV-associated and Lewy Body dementia), amyotrophic lateral sclerosis (ALS), prion diseases, etc. More specifically, in MS the injury to myelin is mediated by an inflammatory response and M.S. Pathogenesis is exacerbated when leukocytes infiltrate the CNS.

[0106] Accordingly, neurodegenerative diseases include but are not limited to: multiple sclerosis (MS), Optic Neuritis, Parkinson's disease, amyloidosis (e.g., Alzheimer's disease), amyotrophic lateral sclerosis (ALS), HIV-associated dementia, stroke/cerebral ischemia, head trauma, spinal cord injury, Huntington's disease, migraine, cerebral amyloid angiopathy, AIDS, age-related cognitive decline; mild cognitive impairment and prion diseases in a mammal, and preferably in a human.

[0107] Multiple sclerosis (MS) is a chronic inflammatory neurodegenerative disease of the central nervous system (CNS) that affects approximately 1,100,000 people all over the

world, in particular affects young adults. MS is characterized pathologically by demyelination of neural tissue, which results clinically in one of many forms of the disease, ranging from benign to chronic-progressive patterns of the disease state. More specifically, five main forms of multiple sclerosis have been described: 1) benign multiple sclerosis; 2) relapsing-remitting multiple sclerosis (RRMS); 3) secondary progressive multiple sclerosis (SPMS); 4) primary progressive multiple sclerosis (PPMS); and 5) progressive-relapsing multiple sclerosis (PRMS). Chronic progressive multiple sclerosis is a term used to collectively refer to SPMS, PPMS, and PRMS. The relapsing forms of multiple sclerosis are SPMS with superimposed relapses, RRMS and PRMS.

[0108] Throughout the course of the disease there is a progressive destruction of the myelin sheath surrounding axons. Since intact myelin is essential in the preservation of axonal integrity systematic destruction eventually leads, clinically, to various neurological dysfunctions including numbness and pain, problems with coordination and balance, blindness, and general cognitive impairment.

[0109] Parkinson's disease, another inflammatory neurodegenerative disease, is characterized by movement disorders, including muscle rigidity and slow physical movements.

[0110] Amyloidosis develops when certain proteins have altered structure and tend to bind to each building up in particular tissue and blocking the normal tissue functioning. These altered structured proteins are called amyloids. Often amyloidoses is split into two categories: primary or secondary. Primary amyloidoses occur from an illness with improper immune cell function. Secondary amyloidoses usually arise from a complication of some other chronic infectious or inflammatory diseases. Examples of such include Alzheimer's disease and rheumatoid arthritis. The underlying problem in secondary amyloidosis is inflammation.

[0111] Alzheimer's disease is another type of inflammatory neurodegenerative disease. It is exemplified by the increasing impairment of learning and memory, although the disease

may manifest itself in other ways indicating altered cognitive ability. Throughout the disease the progressive loss of neurons and synapses in the cerebral cortex leads to gross atrophy of the neural tissue. Although the cause of Alzheimer's is unknown, many believe that inflammation plays an important role and clinical studies have shown that inflammation considerably contributes to the pathogenesis of the disease.

[0112] Amyotrophic lateral sclerosis is another debilitating neurological disorder. In ALS a link between inflammation and the disease has been suggested.

[0113] In one embodiment, the neurological disorder is any disorder characterized by elevated TNF-α, and can include disorders such as stroke, depression, post-traumatic stress syndrome and traumatic brain injury.

[0114] In some embodiments, the disorders that can be treated by the scTNFR2 fusion proteins described herein include demyelinating disorders, such as but not limited to multiple sclerosis (MS), including primary progressive or relapsing-remitting MS, or optic neuritis. Other disorders such as, but not limited to, pain, which may include neuropathic pain, may be treated with the TNFR2 agonists described herein.

Other Fusion Proteins

[0115] Because the purpose of the Fc protein moiety in this invention is solely to increase circulating half-life, one skilled in the art will recognize that the scTNFR2 selective agonist moiety could be fused to the N-terminus of other proteins to achieve the same goal of increasing molecular size and reducing the rate of renal clearance, using the structure-activity relationships discovered in this invention. The scTNFR2 selective agonist could be fused to the N-terminus of serum albumin (Sleep. D., et al., 2013, Biochim Biophys Acta.1830:5526-34), which both increases the hydrodynamic radius of the fusion protein relative to the TNFR2 moiety and is also recycled by the FcRN. A skilled artisan would also recognize that the scTNFR2 selective agonist moiety of this invention could also be fused to the N-terminus of recombinant non-immunogenic amino acid polymers. Two examples of non-immunogenic amino acid polymers developed for

this purpose are XTEN polymers, chains of A, E, G, P, S, and T amino acids (Schellenberger, V., et. al., 2009, Nat Biotechnol. 27:1186-90)), and PAS polymers, chains of P, A, and S amino acid residues (Schlapschy, M., et. al., 2007, Protein Eng Des Scl. 20:273-84).

Combination treatments

[0116] Treatments that currently are available for MS include glatiramer acetate, interferonβ, natalizumab, and mitoxanthrone. In general, these drugs suppress the immune system in a nonspecific fashion and only marginally limit the overall progression of disease. (Lubetzki et al. (2005), Curr. Opin. Neurol. 18:237-244). Thus, there exists a need for developing therapeutic strategies to better treat MS. As described herein, scTNFR2 find use in treating MS. These molecules find particular use when combined with currently available MS therapies as known in the art and as described herein. For instance, scTNFR2 agonists may be combined in a therapeutic regimen with glatiramer acetate, interferon-β, natalizumab, and mitoxanthron or other molecules, such as bardoxolone methyl or variants thereof.

[0117] As another example, in the treatment of Alzheimer's Disease (AD), a scTNFR2 agonist protein may be administered to an individual in combination therapy with one or more additional therapeutic agents for the treatment of AD. Suitable additional therapeutic agents include, but are not limited to, acetylcholinesterase inhibitors, including, but not limited to, Aricept (donepezil), Exelon (rivastigmine), metrifonate, and tacrine (Cognex); non-steroidal anti-inflammatory agents, including, but not limited to, ibuprofen and indomethacin; cyclooxygenase-2 (Cox2) inhibitors such as Celebrex; and monoamine oxidase inhibitors, such as Selegilene (Eldepryl or Deprenyl). Dosages for each of the above agents are known in the art. For example, Aricept is generally administered at 50 mg orally per day for 6 weeks, and, if well tolerated by the individual, at 10 mg per day thereafter.

[0118] In one embodiment, treatment of the scTNFR2 agonist in a therapeutic regimen in combination with the co-therapies as described herein results in synergistic efficacy as compared to either of the treatments alone. By "synergistic" is meant that efficacy is more than the result of additive efficacy of the two treatments alone.

[0119] In one embodiment treatment of the scTNFR2 agonist in a therapeutic regimen includes the combination of steroidal anti-inflammatory molecules, such as but not limited to dexamethasone and the like or non-steroidal anti-inflammatory molecules.

[0120] In addition, the scTNFR2 agonist may be formulated alone as a topical therapy or used in combination with or treated in a regimen with corticosteroids for treatment of autoimmune skin disorders such as psoriasis, eczema and burns (including sunburn). For instance, bath solutions and moisturizers, mineral oil and petroleum jelly which may help soothe affected skin and reduce the dryness which accompanies the build-up of skin on psoriatic plaques may be used formulated with or in a therapeutic regimen with scTNFR2 agonist as described herein. In addition, medicated creams and ointments applied directly to psoriatic plaques can help reduce inflammation, remove built-up scale, reduce skin turn over, and clear affected skin of plaques. Ointment and creams containing coal, tar, dithranol (anthralin), corticosteroids like desoximetasone (Topicort), fluocinonide, vitamin D3 analogs (for example, calcipotriol), and retinoids find use when combined with scTNFR2 agonist for topical application to the skin for treatment of autoimmune skin disorders.

[0121] All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

[0122] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that

certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

[0123] While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the present invention.

III. EXAMPLES

[0124] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLE 1
GENERATION AND CHARACTERIZATION OF TNFR2 SELECTIVE AGONIST

[0125] Example 1 Expression of TNFR2 agonist molecules

[0126] TNFR2-selective TNF variants, which are composed of a covalently stabilized human TNFR2-selective (D143N/A145R) single-chain TNF ($scTNF_{R2}$) were fused to Fc dimerization domains resulting in a protein that is, with respect to TNF domains, hexameric ($Fc–scTNF_{R2}$). The purity of the recombinant proteins was confirmed by

SDS/PAGE and immunoblot analysis. Under reducing conditions, the TNF variants exhibited an appropriate molecular mass. Under nonreducing conditions the expected dimer was observed. The oligomerization state of Fc–scTNFR2 was further characterized by capillary electrophoresis. Fc–scTNFR2 elutes as a single major peak, indicating high purity. An exemplary electropherogram is shown in Figure 1 for SEQ ID NO:101.

[0127] Each gene sequence was cloned into a high expression mammalian vector. Each completed construct was sequence confirmed before proceeding to DNA scale up. Each DNA expression construct was scaled up to the appropriate amount for transfection. The plasmid DNA was run on agarose gel for quality assessment and sequence confirmed before proceeding to transfection. Suspension HEK293 cells were seeded in a shake flask and were expanded using serum-free chemically defined medium. On the day of transfection, the expanded cells were seeded into a new flask with fresh medium. Each DNA construct was transiently transfected into HEK293 cells using standard methods. The cells were maintained as a batch-fed culture until the end of the production run. The conditioned media from the transient production run was harvested and clarified by centrifugation and filtration. The supernatant was loaded over a Protein A column pre-equilibrated with binding buffer. Washing buffer was passed through the column until the OD280 value (NanoDrop, Thermo Scientific) was measured to be zero. The target protein was eluted with a low pH buffer, fractions were collected, and the OD280 value of each fraction was recorded. Fractions containing the target protein were pooled and filtered through a 0.2 μm membrane filter. The protein concentration was calculated from the OD280 value and the calculated extinction coefficient. CE-SDS analysis of the target protein was performed using LabChip GXII (Perkin Elmer).

[0128] Example 2 TNFR2 Binding

[0129] TNF receptor selectivity of Fc–scTNFR2 is analyzed by binding studies with immobilized huTNFR1–Fc and huTNFR2–Fc fusion proteins. Fc–scTNFR2 does not interact with huTNFR1, but the fusion protein efficiently binds to huTNFR2. In contrast,

soluble human TNF (huTNF) efficiently binds to huTNFR1, whereas it less effectively with huTNFR2.

[0130] Wells were coated with 1 μg/mL hTNFR1-Fc or hTNFR2-Fc in PBS, 4 °C overnight then blocked with 3% milk in PBS, RT 1.5 hours. Primary incubation: TNF variant proteins, RT 1 hour (starting from 60 nM, 1:3 dilution). Primary detection antibody: 1 ug/mL TNF alpha monoclonal antibody (F6C5), RT 1 hour. Secondary detection antibody: HRP conjugated goat anti-mouse antibody (1:5000 dilution), RT 1 hour. Data shown in Figure 2. Calculated binding affinity follows:

Binding to TNFR1

TNF Variant	Kd (nM)
TNF-α	1.12
SEQ ID NO:101	n/a
SEQ ID NO:102	n/a
EHD-scTNFr2	n/a
SEQ ID NO:113	n/a
SEQ ID NO:114	Did not express
SEQ ID NO:115	n/a
SEQ ID NO:116	n/a
SEQ ID NO:117	n/a
IgG4 Control	n/a

Binding to TNFR2

TNF Variant	Kd (nM)
TNF-α	0.90
SEQ ID NO:101	0.44
SEQ ID NO:102	0.27
EHD-scTNFr2	0.33
SEQ ID NO:113	0.21
SEQ ID NO:114	Did not express
SEQ ID NO:115	0.28
SEQ ID NO:116	0.30
SEQ ID NO:117	n/a
IgG4 Control	n/a

[0131] Example 3 Cell based TNFR2 assay

[0132] Fc–scTNFR2 does not activate TNFR1-dependent cell death in L929, verifying that Fc–scTNFR2 had lost affinity for TNFR1 due to the mutations D143N/A145R. In contrast, Fc-scTNFR2 efficiently induced cell death in Kym-1 cells, which endogenously express both TNF receptors and are highly sensitive to endogenous TNF-induced TNFR1 mediated cytotoxicity. Thus, TNFR2 signaling can be measured as an increase in cell death in Kym-1 cells.

[0133] Kym-1 cells (1.5 × 104 cells/well) were grown in 96-well white opaque cell culture plates (Perkin Elmer) overnight. The cells were incubated with 8 concentrations of TNF muteins (100, 10, 1, 0.1, 0.01, 0.001, 0.0001 and 0.00001 ng/mL) in triplicates for 24h at 37 °C and 5% CO2. Cell viability was analyzed at 24 h by Cell Titer Glo assay (Promega). SEQ ID NO:114 did not express and therefore could not be tested. SEQ ID NO:117 did not induce cell death under any concentrations consistent with its inability to bind TNFR2 as shown in Example 2.

Sequence Listing

SEQ ID NO:1 (Full length TNF-a)
MSTESMIRDVELAEEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTLFCLLHFGVIGPQR
EEFPRDLSLISPLAQAVRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVEL
RDNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLDFAESGQVYFGIIAL

SEQ ID NO:2 (Soluble TNF-a)
VRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLDFAESGQVYFGIIAL

SEQ ID NO:3 (THD Domain with TNFR2 Agonist Scquences)
PVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL

SEQ ID NO:4 (Sequence from the ADAM17 cleavage site in the stalk region to the C-terminus of the stalk region)
VRSS SRTPSDK

SEQ ID NO:5 (Human IgG1 Fc)
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVYTLPPSRDELT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEA
LHNHYTQKSLSLSPGK

SEQ ID NO: 6 (Human IgG1 Fc with FcgR and C1q knockout)
EPKSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAKGQPREP
QVYTLPPSRDELT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEA
LHNHYTQKSLSLSPGK

SEQ ID NO:7 (Human IgG1 Fc with N-terminal linker)
GGGGSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP

QVYTLPPSRDELT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEA
LHNHYTQKSLSLSPGK

SEQ ID NO:8 (Human IgG1 Fc with FcgR and C1q knockout and linker)
GGGGSEPKSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAKGQPREP
QVYTLPPSRDELT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEA
LHNHYTQKSLSLSPGK

SEQ ID NO:9 (Human IgG1 Fc with C-terminal linker)
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVYTLPPSRDELT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEA
LHNHYTQKSLSLSP(GGGGS) n=1-5

SEQ ID NO:10 (Human IgG1 Fc with FcgR and C1q knockout and C-terminal linker)
EPKSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAKGQPREP
QVYTLPPSRDELT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEA
LHNHYTQKSLSLSP(GGGGS) n=1-5

SEQ ID NO:11 (Human IgG4 Fc)
ESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY
VDGVEVHNA
KTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREP
QVYTLPPSQEEMT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQ
EGNVFSCSVMHEA
LHNHYTQKSLSLSLGK

SEQ ID NO: 12 (Human IgG4 Fc with Ser to Pro mutation)
ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY
VDGVEVHNA
KTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREP
QVYTLPPSQEEMT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQ
EGNVFSCSVMHEA
LHNHYTQKSLSLSLGK

SEQ ID NO:13 (Human IgG4 Fc with N-terminal linker)
GGGGSESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEV
QFNWYVDGVEVHNA
KTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREP
QVYTLPPSQEEMT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQ
EGNVFSCSVMHEA
LHNHYTQKSLSLSLGK

SEQ ID NO:14 (Human IgG4 Fc with Ser to Pro Mutation and N-terminal linker)
GGGGSESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEV
QFNWYVDGVEVHNA
KTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREP
QVYTLPPSQEEMT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQ
EGNVFSCSVMHEA
LHNHYTQKSLSLSLGK

SEQ ID NO:15 (Human IgG4 Fc with C-terminal linker)
ESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY
VDGVEVHNA
KTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREP
QVYTLPPSQEEMT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQ
EGNVFSCSVMHEA
LHNHYTQKSLSLSL(GGGGS) n=1-5

SEQ ID NO:16 (Human IgG4 Fc with Ser to Pro Mutation and C-terminal linker)
ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY
VDGVEVHNA
KTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREP
QVYTLPPSQEEMT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQ
EGNVFSCSVMHEA
LHNHYTQKSLSLSL(GGGGS) n=1-5

SEQ ID NO: 17 (Human IgG2 Fc)
ERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWY
VDGVEVHNA
KTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREP
QVYTLPPSREEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMH
EALHNHYTQKSLSLSPGK

SEQ ID NO:18 (Human IgG2 Fc with C1q knockout)
ERKSSVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYV
DGVEVHNA
KTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPASIEKTISKTKGQPREP
QVYTLPPSREEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMH
EALHNHYTQKSLSLSPGK

SEQ ID NO: 19 (Human IgG2 Fc with N-terminal linker)
GGGGSERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQ
FNWYVDGVEVHNA
KTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREP
QVYTLPPSREEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMH
EALHNHYTQKSLSLSPGK

SEQ ID NO:20 (Human IgG2 Fc with C1q knockout and N-terminal linker)
GGGGSERKSSVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQ
FNWYVDGVEVHNA
KTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPASIEKTISKTKGQPREP
QVYTLPPSREEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMH
EALHNHYTQKSLSLSPGK

SEQ ID NO:21 (Human IgG2 Fc with C-terminal linker)
ERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWY
VDGVEVHNA
KTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREP
QVYTLPPSREEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMH
EALHNHYTQKSLSLSP(GGGGS) n=1-5

SEQ ID NO:22 (Human IgG2 Fc with C1q knockout and C-terminal linker)
ERKSSVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYV
DGVEVHNA
KTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPASIEKTISKTKGQPREP
QVYTLPPSREEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMH
EALHNHYTQKSLSLSP(GGGGS) n=1-5

SEQ ID NO:23 (EHD2 dimerization domain)
DFTPPTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQ
VMDVDLSTASTTQEGELASTQSELTLSQKHWLSDRTYTCQVTYQ
GHTFEDSTKKCADSN

SEQ ID NO:24 (MHD2 dimerization domain)
AELPPKVSVFVPPRDGFFGNPRKSKLICQATGFSPRQIQVSWLREG
KQVGSGVTTDQVQAEAKESGPTTYKVTSTLTIKESDWLGQSMFT
CRVDHRGLTFQQNASSMCVPD

SEQ ID NO:25 (linker)
GGGGS

SEQ ID NO:26 (TNF-α stalk region)
GPQREEFPRDLSLISPLAQAVRSSSRTPSDK

SEQ ID NO:27 (linker)
GGGGSGGGGS

SEQ ID NO:28 (stalk based linker)
SSRTPSDK

SEQ ID NO:29 (G/S Stalk based linker)
GGGGSVRSSSRTPSDK

SEQ ID NO:30 (G/S Stalk based linker)
GGGGSSSRTPSDK

SEQ ID NO:31 (IgG1 Fc with mutations; $(GGGGS)_2$; THDR2; stalk linker; THDR2; stalk linker; THDR2)
**EPKSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KALPASIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE
SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT**

QKSLSLSP*GGGGSGGGGS* PVAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL*VRSSSRTPSDK*PVAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL*VRSSSRTPSDK*PVAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL

SEQ ID NO:32 (IgG1 Fc with mutations; G/S stalk linker; THDR2; G/S short stalk linker; THDR2; G/S short stalk linker; THDR2)
EPKSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP*GGGGSVRSSSRTPSDK*PVAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL*GGGGSSSRTPSDK*PVAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL*GGGGSSSRTPSDK*PVAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL

SEQ ID NO:33 (Soluble TNF-a sequence with TNFR2 agonist mutations)
VRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL

SEQ ID NO: 34 (scTNFR2 agonist with C-terminal IgG1 with mutations. This is soluble TNF-a sequence, which includes VRSSSRTPSDK at N-terminus prior to THD domain.)
VRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL
VRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL
VRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL
EPKSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN

KALPASIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE
SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSP

SEQ ID NO:35 (scTNFR2 agonist with C-terminal IgG1 with mutations. This is soluble TNF-a sequence, which includes VRSSSRTPSDK at N-terminus prior to THD domain and GGGGS prior to IgG1.)
VRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL
VRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL
VRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL*GGGGS*
EPKSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KALPASIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE
SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSP

SEQ ID NO:36 nucleic acid encoding SEQ ID NO:31 optimized for *Mus musculus* expression by www.jcat.de
GAGCCCAAGAGCAGCGACAAGACCCACACCTGCCCCCCCTGCCCCGCCCC 50
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GGAGGTGCACAACGCCAAGACCAAGCCCAGGGAGGAGCAGTACAACAGCA 250
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CGAGAAGACCATCAGCAAGGCCAAGGGCCAGCCCAGGGAGCCCCAGGTGT 400
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GCACAACCACTACACCCAGAAGAGCCTGAGCCTGAGCCCCGGCGGCGGCG 700
GCAGCGGCGGCGGCGGCAGCCCCGTGGCCCACGTGGTGGCCAACCCCCAG 750
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CAACGGCGTGGAGCTGAGGGACAACCAGCTGGTGGTGCCCAGCGAGGGCC 850
TGTACCTGATCTACAGCCAGGTGCTGTTCAAGGGCCAGGGCTGCCCCAGC 900
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GACCAAGGTGAACCTGCTGAGCGCCATCAAGAGCCCCTGCCAGAGGGAGA 1000

CCCCCGAGGGCGCCGAGGCCAAGCCCTGGTACGAGCCCATCTACCTGGGC 1050
GGCGTGTTCCAGCTGGAGAAGGGCGACAGGCTGAGCGCCGAGATCAACAG 1100
GCCCGACTACCTGAACTTCAGGGAGAGCGGCCAGGTGTACTTCGGCATCA 1150
TCGCCCTGGTGAGGAGCAGCAGCAGGACCCCCAGCGACAAGCCCGTGGCC 1200
CACGTGGTGGCCAACCCCCAGGCCGAGGGCCAGCTGCAGTGGCTGAACAG 1250
GAGGGCCAACGCCCTGCTGGCCAACGGCGTGGAGCTGAGGGACAACCAGC 1300
TGGTGGTGCCCAGCGAGGGCCTGTACCTGATCTACAGCCAGGTGCTGTTC 1350
AAGGGCCAGGGCTGCCCCAGCACCCACGTGCTGCTGACCCACACCATCAG 1400
CAGGATCGCCGTGAGCTACCAGACCAAGGTGAACCTGCTGAGCGCCATCA 1450
AGAGCCCCTGCCAGAGGGAGACCCCCGAGGGCGCCGAGGCCAAGCCCTGG 1500
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GCCAGGTGTACTTCGGCATCATCGCCCTGGTGAGGAGCAGCAGCAGGACC 1650
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CCAGCTGCAGTGGCTGAACAGGAGGGCCAACGCCCTGCTGGCCAACGGCG 1750
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ATCTACAGCCAGGTGCTGTTCAAGGGCCAGGGCTGCCCCAGCACCCACGT 1850
GCTGCTGACCCACACCATCAGCAGGATCGCCGTGAGCTACCAGACCAAGG 1900
TGAACCTGCTGAGCGCCATCAAGAGCCCCTGCCAGAGGGAGACCCCCGAG 1950
GGCGCCGAGGCCAAGCCCTGGTACGAGCCCATCTACCTGGGCGGCGTGTT 2000
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SEQ ID NO:37 nucleic acid encoding SEQ ID NO:32 optimized for *Mus musculus* expression by www.jcat.de

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GATCGCCGTGAGCTACCAGACCAAGGTGAACCTGCTGAGCGCCATCAAGA 1000
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CCACGTGGTGGCCAACCCCCAGGCCGAGGGCCAGCTGCAGTGGCTGAACA 1750
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CTGGTGGTGCCCAGCGAGGGCCTGTACCTGATCTACAGCCAGGTGCTGTT 1850
CAAGGGCCAGGGCTGCCCCAGCACCCACGTGCTGCTGACCCACACCATCA 1900
GCAGGATCGCCGTGAGCTACCAGACCAAGGTGAACCTGCTGAGCGCCATC 1950
AAGAGCCCCTGCCAGAGGGAGACCCCCGAGGGCGCCGAGGCCAAGCCCTG 2000
GTACGAGCCCATCTACCTGGGCGGCGTGTTCCAGCTGGAGAAGGGCGACA 2050
GGCTGAGCGCCGAGATCAACAGGCCCGACTACCTGAACTTCAGGGAGAGC 2100
GGCCAGGTGTACTTCGGCATCATCGCCCTG

SEQ ID NO:38 nucleic acid encoding SEQ ID NO:34 optimized for *Mus musculus* expression by www.jcat.de

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ACGCCCTGCTGGCCAACGGCGTGGAGCTGAGGGACAACCAGCTGGTGGTG 150
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CCAGGTGCTGTTCAAGGGCCAGGGCTGCCCCAGCACCCACGTGCTGCTGA 700
CCCACACCATCAGCAGGATCGCCGTGAGCTACCAGACCAAGGTGAACCTG 750
CTGAGCGCCATCAAGAGCCCCTGCCAGAGGGAGACCCCCGAGGGCGCCGA 800
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CAGCAGCAGGACCCCCAGCGACAAGCCCGTGGCCCACGTGGTGGCCAACC 1000
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CCAGCACCCACGTGCTGCTGACCCACACCATCAGCAGGATCGCCGTGAGC 1200
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GGAGACCCCCGAGGGCGCCGAGGCCAAGCCCTGGTACGAGCCCATCTACC 1300
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CCTGCCCCGCCCCCGAGGCCGCCGGCGGCCCCAGCGTGTTCCTGTTCCCC 1500
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CAGTACAACAGCACCTACAGGGTGGTGAGCGTGCTGACCGTGCTGCACCA 1700
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CCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACC 1950
CCCCCCGTGCTGGACAGCGACGGCAGCTTCTTCCTGTACAGCAAGCTGAC 2000
CGTGGACAAGAGCAGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGA 2050
TGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGCCTGAGCCTGAGC 2100
CCC

SEQ ID NO:39 nucleic acid encoding SEQ ID NO:35 optimized for *Mus musculus* expression by www.jcat.de

GTGAGGAGCAGCAGCAGGACCCCCAGCGACAAGCCCGTGGCCCACGTGGT 50
GGCCAACCCCCAGGCCGAGGGCCAGCTGCAGTGGCTGAACAGGAGGGCCA 100
ACGCCCTGCTGGCCAACGGCGTGGAGCTGAGGGACAACCAGCTGGTGGTG 150
CCCAGCGAGGGCCTGTACCTGATCTACAGCCAGGTGCTGTTCAAGGGCCA 200
GGGCTGCCCCAGCACCCACGTGCTGCTGACCCACACCATCAGCAGGATCG 250
CCGTGAGCTACCAGACCAAGGTGAACCTGCTGAGCGCCATCAAGAGCCCC 300
TGCCAGAGGGAGACCCCCGAGGGCGCCGAGGCCAAGCCCTGGTACGAGCC 350
CATCTACCTGGGCGGCGTGTTCCAGCTGGAGAAGGGCGACAGGCTGAGCG 400
CCGAGATCAACAGGCCCGACTACCTGAACTTCAGGGAGAGCGGCCAGGTG 450
TACTTCGGCATCATCGCCCTGGTGAGGAGCAGCAGCAGGACCCCCAGCGA 500
CAAGCCCGTGGCCCACGTGGTGGCCAACCCCCAGGCCGAGGGCCAGCTGC 550
AGTGGCTGAACAGGAGGGCCAACGCCCTGCTGGCCAACGGCGTGGAGCTG 600
AGGGACAACCAGCTGGTGGTGCCCAGCGAGGGCCTGTACCTGATCTACAG 650
CCAGGTGCTGTTCAAGGGCCAGGGCTGCCCCAGCACCCACGTGCTGCTGA 700
CCCACACCATCAGCAGGATCGCCGTGAGCTACCAGACCAAGGTGAACCTG 750

CTGAGCGCCATCAAGAGCCCCTGCCAGAGGGAGACCCCCGAGGGCGCCGA 800
GGCCAAGCCCTGGTACGAGCCCATCTACCTGGGCGGCGTGTTCCAGCTGG 850
AGAAGGGCGACAGGCTGAGCGCCGAGATCAACAGGCCCGACTACCTGAAC 900
TTCAGGGAGAGCGGCCAGGTGTACTTCGGCATCATCGCCCTGGTGAGGAG 950
CAGCAGCAGGACCCCCAGCGACAAGCCCGTGGCCCACGTGGTGGCCAACC 1000
CCCAGGCCGAGGGCCAGCTGCAGTGGCTGAACAGGAGGGCCAACGCCCTG 1050
CTGGCCAACGGCGTGGAGCTGAGGGACAACCAGCTGGTGGTGCCCAGCGA 1100
GGGCCTGTACCTGATCTACAGCCAGGTGCTGTTCAAGGGCCAGGGCTGCC 1150
CCAGCACCCACGTGCTGCTGACCCACACCATCAGCAGGATCGCCGTGAGC 1200
TACCAGACCAAGGTGAACCTGCTGAGCGCCATCAAGAGCCCCTGCCAGAG 1250
GGAGACCCCCGAGGGCGCCGAGGCCAAGCCCTGGTACGAGCCCATCTACC 1300
TGGGCGGCGTGTTCCAGCTGGAGAAGGGCGACAGGCTGAGCGCCGAGATC 1350
AACAGGCCCGACTACCTGAACTTCAGGGAGAGCGGCCAGGTGTACTTCGG 1400
CATCATCGCCCTGGGCGGCGGCGGCAGCGAGCCCAAGAGCAGCGACAAGA 1450
CCCACACCTGCCCCCCCTGCCCCGCCCCCGAGGCCGCCGGCGGCCCCAGC 1500
GTGTTCCTGTTCCCCCCCAAGCCCAAGGACACCCTGATGATCAGCAGGAC 1550
CCCCGAGGTGACCTGCGTGGTGGTGGACGTGAGCCACGAGGACCCCGAGG 1600
TGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACC 1650
AAGCCCAGGGAGGAGCAGTACAACAGCACCTACAGGGTGGTGAGCGTGCT 1700
GACCGTGCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGG 1750
TGAGCAACAAGGCCCTGCCCGCCAGCATCGAGAAGACCATCAGCAAGGCC 1800
AAGGGCCAGCCCAGGGAGCCCCAGGTGTACACCCTGCCCCCCAGCAGGGA 1850
CGAGCTGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTGAAGGGCTTCT 1900
ACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAAC 1950
AACTACAAGACCACCCCCCCCGTGCTGGACAGCGACGGCAGCTTCTTCCT 2000
GTACAGCAAGCTGACCGTGGACAAGAGCAGGTGGCAGCAGGGCAACGTGT 2050
TCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAG 2100
AGCCTGAGCCTGAGCCCC

SEQ ID NO:40 Human IgG1 sequence including C-terminal extension
EPKSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY
KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
LHNHYTQKSLSLSPELQLEESSAEAQDGELDG

SEQ ID NO:41 Linker from C-terminus of Human IgG
ELQLEESSAEAQDGELDG

SEQ ID NO:42 Linker variant derived from C-terminus of Human IgG
ELQLEESSAEAQGG

SEQ ID NO: 100: EHD2:GGGSGGGTGSEFLA-SSRTPSDK:THDR2:GGGGS-
SSRTPSDK:THDR2:GGGGS-SSRTPSDK:THDR2

DFTPPTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQ
VMDVDLSTASTTQEGELASTQSELTLSQKHWLSDRTYTCQVTYQ
GHTFEDSTKKCADSN GGGSGGGTGSEFLASSRTPSDK
PVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIALGGGGSSS
RTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIALGGGGSSS
RTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL

SEQ ID NO:101 I4SP: GGGGS: sTNFR2: GGGGS- SSRTPSDK:THDR2: GGGGS-
SSRTPSDK:THDR2

ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY
VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIS
KAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK
GGGGSVRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIALGGGGSSS
RTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIALGGGGSSS
RTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL

SEQ ID NO: 102: I4SP: GGGGS: sTNFR2: SSRTPSDK-THDR2: SSRTPSDK:THDR2

ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY
VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIS
KAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK
GGGGSVRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIALSSRTPSDK
PVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE

TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL
SSRTPSDK PVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL

SEQ ID NO: REL103: I4SP: GGGGS: sTNFR2: VRSSSRTPSDK :THDR2: VRSSSRTPSDK:THDR2

ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY
VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIS
KAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK
GGGGSVRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIALVRSSSRTP
SDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIALVRSSSRTP
SDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL

SEQ ID NO: 104: I4SP: GGGGSGGGGS:sTNFR2: GGGGS- SSRTPSDK:THDR2: GGGGS-SSRTPSDK:THDR2

ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY
VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIS
KAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK
GGGGSGGGGSVRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIALGGGGSSS
RTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIALGGGGSSS
RTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL

SEQ ID NO:105: I4SP: GGGGSGGGGS: sTNFR2: SSRTPSDK-THDR2: SSRTPSDK:THDR2

ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY
VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIS
KAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP

VLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK
GGGGSGGGGSVRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIALSSRTPSDK
PVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL
SSRTPSDK PVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL

SEQ ID NO:106 I4SP: GGGGSGGGGS: sTNFR2: VRSSSRTPSDK :THDR2:
VRSSSRTPSDK:THDR2

ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY
VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIS
KAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK
GGGGSGGGGSVRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIALVRSSSRTP
SDK PVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL
VRSSSRTPSDK PVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL

SEQ ID NO:107 sTNFR2: GGGGS- SSRTPSDK:THDR2: GGGGS-
SSRTPSDK:THDR2:GGGGS:I4SP

VRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIALGGGGS
SSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIALGGGGS
SSRTPSDK PVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIALGGGGSES
KYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVD
GVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKA
KGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
DSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 108: sTNFR2: SSRTPSDK:THDR2: SSRTPSDK:THDR2:GGGGS:I4SP

VRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL
SSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL
SSRTPSDK PVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIALGGGGSES
KYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVD
GVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKA
KGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
DSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

SEQ ID NO:109: sTNFR2: VRSSSRTPSDK:THDR2:VRSSSRTPSDK:THDR2:GGGGS:I4SP

VRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIALVRS
SSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIALVRS
SSRTPSDK PVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIALGGGGSES
KYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVD
GVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKA
KGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
DSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 110: sTNFR2: GGGGS- SSRTPSDK:THDR2:GGGGS-
SSRTPSDK:THDR2:14SP

VRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIALGGGGS
SSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIALGGGGS
SSRTPSDK PVAHVVANPQAEGQLQWLNRRANALLANGVELR

DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIALESKYGPP
CPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEV
HNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQP
REPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
SFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 111: sTNFR2: SSRTPSDK:THDR2: SSRTPSDK:THDR2:I4SP

VRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL
SSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL
SSRTPSDK PVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIALESKYGPP
CPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEV
HNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQP
REPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
SFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

SEQ ID NO:112 sTNFR2: VRSSSRTPSDK :THDR2: VRSSSRTPSDK :THDR2:4SP

VRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL
VRSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL
VRSSRTPSDK PVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIALESKYGPP
CPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEV
HNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQP
REPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
SFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

SEQ ID NO: **113** IgG1: GGGGS: sTNFR2: GGGGS- SSRTPSDK:THDR2: GGGGS-
SSRTPSDK:THDR2

EPKSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI

EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY
KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGS
VRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL
GGGGS
SSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL
GGGGS
SSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL

SEQ ID NO: 114
IgG2: GGGGS: sTNFR2: GGGGS- SSRTPSDK:THDR2: GGGGS- SSRTPSDK:THDR2

ERKSSVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYV
DGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPASIEKTIS
KTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
MLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGS
VRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL
GGGGS
SSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL
GGGGS
SSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL

SEQ ID NO: 115
sTNFR2: GGGGS- SSRTPSDK:THDR2: GGGGS- SSRTPSDK:THDR:GGGGS-IgG1*

VRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL
GGGGS
SSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE

TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL
GGGGS
SSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL
GGGGS
EPKSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY
KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 116
sTNFR2: GGGGS- SSRTPSDK:THDR2: GGGGS- SSRTPSDK:THDR:GGGGS-IgG2

VRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL
GGGGS
SSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL
GGGGS
SSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL
GGGGS
ERKSSVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYV
DGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPASIEKTIS
KTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
MLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:117:
IgG4: sTNFR2:THDR2:THDR2

ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY
VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIS
KAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK
VRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL
SSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL

SSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL

SEQS ID NO: 118: I4SP: GGGGS: sTNFR2: GGGGS- THDR2: GGGGS- THDR2

ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY
VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIS
KAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK
GGGGSVRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIALGGGGSPV
AHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIALGGGGSPV
AHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL

SEQS ID NO: 119: I4SP: GGGGSGGGGS: sTNFR2: GGGGS- THDR2: GGGGS- THDR2

ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY
VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIS
KAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK
GGGGSGGGGSVRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIALGGGGSPV
AHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIALGGGGSPV
AHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL

SEQ ID NO: 120: I4SP: GGGGSGGGGS: sTNFR2: SSRTPSDK-THDR2:
SSRTPSDK:THDR2

ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY
VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIS
KAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK
GGGGSGGGGSVRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR

DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIALSSRTPSDK
PVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL
SSRTPSDK PVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL

SEQ ID NO:121- Codon optimized nucleic acid sequence encoding SEQ ID NO 101

GAG TCC AAG TAT GGG CCA CCT TGT CCA CCA TGC CCA GCC CCC GAA TTT
CTT GGT GGC CCT TCA GTC TTT CTC TTC CCA CCC AAACCC AAA GAT ACT C
TT ATG ATT TCT CGA ACC CCC GAG GTG ACA TGC GTG GTC GTA GAC GTG A
GT CAG GAA GAC CCA GAG GTT CAGTTC AAC TGG TAT GTC GAC GGC GTA GA
G GTG CAT AAC GCC AAG ACT AAA CCC CGA GAA GAG CAG TTT AAC TCC AC
T TAC AGA GTGGTG AGT GTC TTG ACC GTC CTG CAT CAG GAC TGG CTT AAC
GGC AAA GAG TAT AAA TGT AAA GTT AGC AAT AAA GGA CTC CCA AGTAGC
ATT GAA AAA ACC ATC AGT AAA GCA AAG GGC CAA CCA AGA GAG CCC CAG
GTG TAT ACC CTT CCA CCC AGT CAG GAG GAA ATGACC AAA AAC CAA GTT T
CC CTT ACT TGC CTT GTT AAG GGA TTC TAC CCC TCA GAC ATT GCT GTA G
AG TGG GAG TCC AAT GGT CAGCCT GAG AAT AAT TAC AAA ACA ACA CCT CC
T GTG TTG GAC AGC GAC GGA TCT TTC TTT CTC TAT AGT CGA CTC ACT GT
G GAC AAATCA AGA TGG CAG GAG GGG AAT GTG TTC TCA TGC TCA GTA ATG
CAT GAA GCC CTG CAC AAT CAC TAC ACA CAA AAG AGT CTC TCTCTG TCC
CTT GGA AAG GGT GCA CCT GGG AGC GTG CGC TCT TCA AGC CGC ACA CCA
TCT GAT AAG CCT GTG GCA CAT GTC GTT GCAAAT CCA CAA GCA GAG GGA C
AA CTT CAG TGG TTG AAC AGG CGC GCC AAC GCA TTG CTC GCC AAC GGT G
TC GAG CTG CGG GAC AACCAG CTG GTC GTA CCT AGT GAG GGT CTG TAC TT
G ATC TAC AGC CAA GTA CTG TTC AAA GGG CAG GGC TGT CCC AGC ACC CA
T GTTCTC TTG ACT CAT ACC ATA TCA CGA ATC GCA GTA AGT TAC CAG ACT
AAA GTG AAC CTG CTT TCC GCT ATC AAA AGT CCC TGT CAAAGA GAG ACT
CCA GAA GGG GCT GAG GCT AAA CCT TGG TAC GAA CCA ATT TAT CTG GGA
GGT GTG TTC CAG CTT GAG AAA GGA GATCGC CTT TCA GCT GAG ATC AAT C
GA CCA GAT TAT TTG AAT TTT CGA GAG AGC GGC CAA GTT TAT TTT GGC A
TA ATC GCA TTG GGTGGT GGT GGT AGC TCC TCA CGC ACT CCA TCT GAC AA
G CCA GTT GCT CAT GTC GTA GCT AAT CCC CAG GCA GAG GGA CAA CTT CA
ATGG CTG AAC AGA AGG GCA AAC GCC CTG TTG GCC AAT GGT GTG GAG TTG
AGA GAC AAT CAG CTG GTT GTC CCT TCT GAG GGA CTTTAT CTT ATA TAT
AGC CAA GTG TTG TTC AAA GGT CAA GGG TGC CCC TCA ACT CAT GTT CTG
TTG ACC CAT ACC ATA AGT CGA ATCGCA GTG AGT TAC CAA ACA AAG GTC A
AT CTC TTG TCC GCC ATA AAG AGC CCC TGC CAA CGG GAA ACA CCC GAA G
GA GCC GAG GCAAAA CCA TGG TAC GAA CCA ATA TAC CTC GGG GGA GTG TT
C CAG CTG GAG AAG GGA GAC CGA CTT TCA GCT GAA ATC AAC AGG CCCGAC
TAT CTT AAC TTC AGG GAG TCA GGG CAG GTC TAC TTT GGA ATA ATA GCA

TTG GGC GGA GGC GGA TCC AGC AGC AGA ACT CCTAGC GAC AAG CCC GTT
GCT CAT GTC GTA GCC AAT CCA CAA GCC GAA GGC CAG CTG CAG TGG CTT
AAT CGA CGG GCC AAT GCC CTGTTG GCA AAC GGA GTC GAG CTT AGG GAT A
AT CAG CTC GTT GTT CCA AGT GAA GGA TTG TAT TTG ATC TAC AGC CAA G
TT CTG TTCAAG GGT CAG GGT TGC CCC TCT ACC CAT GTT TTG TTG ACA CA
C ACA ATC AGT CGC ATT GCT GTA TCC TAT CAA ACC AAG GTC AATTTG CTG
TCC GCA ATC AAG AGC CCA TGC CAG AGA GAG ACT CCA GAA GGC GCA GAA
GCT AAG CCC TGG TAC GAG CCA ATT TAC CTTGGC GGG GTT TTC CAG CTT
GAG AAA GGA GAT AGG CTG AGC GCA GAA ATC AAT CGG CCC GAC TAC TTG
AAT TTC CGC GAA AGC GGTCAA GTG TAT TTT GGT ATC ATA GCA CTT

SEQ ID NO:122- Codon optimized nucleic acid sequence encoding SEQ ID NO 102

GAA TCT AAG TAC GGT CCC CCT TGT CCA CCA TGT CCA GCC CCC GAG TTT
CTC GGA GGG CCC AGT GTC TTT CTT TTC CCT CCT AAACCC AAG GAT ACT C
TC ATG ATT AGC CGA ACA CCT GAA GTA ACA TGT GTT GTT GTG GAC GTT A
GT CAA GAA GAC CCC GAA GTT CAATTT AAC TGG TAT GTG GAT GGC GTA GA
G GTA CAC AAC GCA AAG ACT AAA CCA CGA GAA GAG CAG TTC AAC TCC AC
T TAT CGA GTAGTT AGT GTG TTG ACA GTA CTC CAT CAA GAC TGG CTC AAC
GGC AAA GAA TAT AAG TGT AAA GTT AGT AAC AAA GGA CTC CCC AGTAGC
ATT GAA AAG ACT ATC TCC AAG GCA AAA GGG CAA CCA AGG GAG CCC CAG
GTG TAT ACC TTG CCA CCC TCA CAA GAG GAG ATGACA AAG AAC CAG GTC A
GT CTC ACC TGT CTG GTT AAG GGT TTC TAT CCT TCT GAC ATT GCC GTT G
AA TGG GAG TCT AAC GGC CAGCCT GAA AAT AAC TAC AAG ACT ACA CCT CC
C GTC CTG GAT AGC GAT GGT AGT TTT TTC CTC TAT TCC AGG CTC ACT GT
A GAC AAGTCA AGG TGG CAG GAA GGC AAT GTT TTC AGC TGC TCT GTC ATG
CAT GAG GCA CTC CAC AAT CAT TAT ACA CAA AAA AGT CTC AGTTTG TCC
TTG GGC AAG GGT GGA GGC GGG AGC GTT CGC AGC TCC TCT CGG ACT CCA
AGC GAC AAA CCT GTT GCT CAT GTC GTC GCCAAT CCT CAG GCA GAA GGC C
AA CTG CAA TGG CTG AAC AGA CGC GCT AAT GCA TTG TTG GCC AAC GGC G
TT GAG TTG AGA GAC AACCAA CTC GTT GTA CCC TCC GAG GGA CTT TAT CT
G ATA TAC TCT CAA GTA TTG TTT AAG GGT CAA GGT TGT CCA TCA ACC CA
C GTATTG CTG ACC CAT ACC ATT TCT AGA ATT GCC GTA AGT TAT CAG ACT
AAA GTT AAT TTG TTG AGC GCA ATT AAA AGT CCT TGT CAACGC GAA ACT
CCT GAG GGA GCA GAA GCA AAA CCC TGG TAC GAA CCC ATT TAT TTG GGA
GGG GTA TTT CAG CTG GAA AAG GGG GATCGG CTG TCA GCC GAA ATT AAT C
GC CCT GAT TAT CTG AAC TTC AGA GAA AGC GGT CAA GTC TAC TTC GGC A
TC ATA GCC CTT TCATCT CGC ACA CCA AGT GAT AAG CCC GTT GCT CAC GT
C GTG GCA AAC CCA CAA GCC GAG GGG CAA CTC CAG TGG TTG AAC CGC AG
GGCA AAT GCT CTC TTG GCT AAC GGG GTC GAA TTG AGG GAT AAT CAG CTC
GTT GTC CCT TCC GAA GGA CTG TAT CTG ATC TAC AGCCAA GTA CTG TTC
AAG GGT CAA GGT TGC CCA AGT ACA CAT GTT TTG CTG ACA CAT ACT ATA
AGC CGC ATC GCC GTG TCT TAC CAAACA AAA GTG AAT CTG CTG TCA GCT A
TA AAG AGC CCA TGT CAG AGG GAA ACA CCC GAG GGA GCT GAG GCA AAG C
CC TGG TAC GAACCC ATA TAC TTG GGG GGC GTC TTC CAA CTG GAG AAA GG

T GAC AGG CTC AGT GCA GAG ATA AAC CGC CCC GAC TAC CTG AAT TTTCGA
GAG AGC GGT CAA GTA TAT TTT GGT ATT ATT GCA CTT AGT AGT CGG ACC
CCA TCT GAT AAA CCC GTC GCT CAC GTC GTC GCAAAC CCA CAA GCT GAG
GGG CAG TTG CAG TGG CTT AAT AGG CGC GCT AAC GCT CTG CTT GCT AAT
GGC GTG GAG TTG AGG GAT AATCAA TTG GTC GTT CCC AGC GAG GGT CTG T
AT TTG ATC TAC AGC CAG GTA CTT TTT AAG GGC CAA GGC TGC CCT AGT A
CT CAT GTGCTT CTG ACT CAT ACT ATA TCA AGG ATC GCC GTC AGC TAC CA
A ACC AAG GTT AAT CTC CTT AGT GCT ATC AAA AGC CCA TGT CAACGC GAG
ACT CCC GAG GGC GCC GAA GCC AAA CCC TGG TAC GAG CCC ATA TAC CTG
GGT GGT GTG TTT CAG CTG GAG AAG GGG GACCGA CTT AGT GCA GAG ATT
AAT AGA CCT GAT TAC CTG AAT TTC AGG GAG AGC GGT CAG GTT TAT TTT
GGG ATC ATC GCA CTC

SEQ ID NO:123- - Codon optimized nucleic acid sequence encoding SEQ ID NO 103

GAG TCA AAG TAC GGC CCA CCA TGT CCT CCT TGT CCT GCC CCC GAG TTT
CTG GGT GGC CCA TCC GTC TTC CTC TTT CCA CCT AAACCA AAA GAT ACC C
TC ATG ATC TCT CGG ACA CCC GAA GTT ACC TGC GTC GTC GTC GAC GTC A
GC CAA GAA GAT CCT GAA GTT CAGTTC AAT TGG TAC GTT GAC GGC GTT GA
G GTA CAT AAC GCC AAA ACA AAA CCC CGG GAG GAG CAA TTC AAT TCT AC
T TAT CGG GTGGTT TCA GTT TTG ACC GTG CTG CAT CAG GAC TGG CTC AAC
GGG AAA GAA TAC AAA TGT AAG GTG TCC AAC AAA GGA CTC CCT TCCAGT
ATA GAG AAG ACT ATA TCA AAG GCC AAG GGC CAG CCA CGA GAG CCT CAG
GTA TAC ACC CTG CCC CCT AGC CAA GAG GAG ATGACT AAA AAC CAA GTA A
GT CTG ACA TGC CTT GTC AAG GGG TTC TAT CCT AGT GAT ATT GCC GTA G
AG TGG GAG TCT AAC GGC CAGCCC GAG AAC AAT TAT AAG ACA ACC CCA CC
C GTG CTG GAT TCA GAT GGA TCT TTT TTC TTG TAT AGC CGG CTT ACA GT
A GAT AAATCT CGA TGG CAA GAA GGT AAC GTG TTT AGT TGC TCC GTA ATG
CAC GAG GCA CTC CAT AAT CAC TAT ACT CAA AAA TCC CTC TCCTTG TCT
CTG GGC AAA GGG GGG GGC GGC TCC GTC CGA TCA TCT AGT CGC ACT CCT
TCA GAC AAG CCT GTG GCC CAC GTA GTT GCTAAT CCA CAG GCC GAG GGG C
AA CTC CAA TGG CTC AAC CGC AGA GCC AAC GCA TTG CTG GCT AAC GGC G
TA GAA TTG CGA GAC AATCAG CTT GTG GTA CCT TCC GAG GGA CTG TAC CT
C ATC TAC TCT CAA GTT TTG TTT AAA GGC CAA GGT TGC CCC AGT ACT CA
C GTACTT CTC ACT CAC ACA ATC AGC CGC ATC GCT GTG TCT TAT CAA ACC
AAA GTC AAT TTG CTT TCC GCC ATA AAA AGC CCT TGT CAGCGA GAA ACC
CCT GAA GGA GCT GAA GCT AAA CCA TGG TAC GAG CCC ATC TAT CTC GGC
GGT GTT TTC CAG CTT GAG AAG GGG GATCGG CTT TCC GCC GAG ATT AAT C
GG CCC GAT TAC TTG AAT TTC AGG GAG AGC GGG CAG GTG TAT TTT GGA A
TA ATC GCT CTT GTCCGG TCC TCA TCT CGA ACA CCT AGT GAT AAA CCC GT
A GCC CAC GTA GTT GCA AAT CCC CAG GCC GAA GGT CAA CTG CAG TGG CT
TAAC CGC CGA GCA AAT GCT CTT CTG GCA AAT GGG GTA GAG TTG CGC GAC
AAT CAA TTG GTC GTA CCA AGT GAA GGC CTC TAC CTTATC TAC TCT CAG
GTC CTC TTC AAA GGT CAA GGT TGT CCT TCT ACT CAC GTA CTC CTG ACA
CAT ACA ATA TCT CGC ATT GCA GTATCA TAC CAA ACA AAG GTG AAT CTT C

TC TCC GCT ATA AAA TCA CCC TGC CAA CGA GAG ACA CCT GAA GGT GCA G
AG GCC AAA CCCTGG TAC GAA CCA ATT TAC CTT GGA GGA GTT TTT CAA TT
G GAA AAA GGA GAT AGA CTT AGC GCC GAA ATA AAT AGG CCC GAT TACTTG
AAT TTT AGA GAG TCC GGG CAG GTA TAT TTC GGC ATA ATA GCA CTG GTC
AGG AGT TCC AGC AGG ACT CCC AGC GAT AAG CCCGTC GCA CAC GTG GTT
GCT AAT CCA CAA GCT GAA GGA CAG CTG CAA TGG CTT AAT AGA AGG GCC
AAT GCT CTG TTG GCT AAC GGCGTT GAA CTT CGG GAT AAC CAG CTT GTG G
TG CCC TCC GAA GGT TTG TAT TTG ATC TAT TCA CAA GTT TTG TTC AAA G
GC CAG GGTTGC CCC TCT ACC CAC GTA CTT CTG ACA CAC ACA ATC AGC CG
C ATC GCT GTC TCA TAC CAG ACC AAA GTC AAC TTG TTG TCT GCAATA AAA
TCA CCA TGT CAG CGG GAA ACT CCT GAG GGC GCC GAG GCC AAA CCC TGG
TAT GAG CCA ATC TAC CTT GGT GGC GTA TTTCAG CTT GAA AAA GGA GAC
AGG CTT TCC GCA GAG ATA AAC AGG CCA GAT TAT CTG AAC TTT AGG GAA
TCA GGT CAA GTC TAC TTTGGA ATC ATA GCT CTC

SEQ ID NO:124 - Codon optimized nucleic acid sequence encoding SEQ ID NO 104

GAA TCC AAG TAT GGC CCA CCA TGT CCC CCC TGC CCC GCC CCT GAA TTT
CTT GGC GGA CCC AGC GTA TTT CTG TTC CCA CCA AAGCCC AAG GAC ACA C
TT ATG ATA AGT CGG ACA CCT GAA GTA ACT TGT GTC GTC GTC GAC GTG A
GT CAG GAA GAC CCT GAA GTC CAATTT AAC TGG TAC GTG GAT GGC GTG GA
G GTA CAC AAT GCC AAG ACC AAG CCA CGC GAA GAG CAG TTC AAT TCA AC
A TAT CGG GTCGTT TCC GTC CTG ACC GTA CTG CAC CAA GAT TGG CTC AAT
GGG AAG GAG TAC AAA TGT AAA GTA TCT AAC AAA GGC CTC CCA TCCTCC
ATA GAA AAA ACC ATA AGT AAA GCT AAG GGA CAG CCT CGA GAA CCT CAG
GTC TAC ACA CTG CCC CCA TCT CAA GAA GAA ATGACC AAA AAC CAA GTG A
GT CTT ACT TGT CTG GTG AAA GGT TTC TAT CCA TCC GAC ATT GCC GTA G
AG TGG GAA TCA AAC GGC CAACCT GAG AAT AAC TAC AAA ACT ACT CCT CC
C GTC CTC GAT AGT GAC GGT AGC TTC TTC CTG TAC AGC AGG CTC ACA GT
C GAC AAATCC AGG TGG CAA GAA GGC AAT GTT TTC AGC TGT TCC GTC ATG
CAT GAA GCC CTG CAC AAC CAT TAT ACA CAG AAA AGC TTG AGCCTG TCC
TTG GGT AAA GGT GGA GGG GGG AGT GGG GGT GGT GGG TCT GTG CGA AGC
AGT AGC AGA ACA CCT TCC GAC AAA CCA GTTGCA CAT GTT GTT GCT AAT C
CT CAG GCC GAA GGG CAG CTT CAG TGG CTC AAC AGG AGG GCT AAC GCT T
TG TTG GCT AAC GGT GTAGAG CTC CGC GAT AAC CAA CTT GTA GTG CCT TC
C GAG GGA CTC TAT CTT ATT TAC TCC CAA GTG CTG TTT AAA GGA CAA GG
G TGCCCT AGC ACC CAC GTA TTG CTG ACT CAC ACT ATC AGC AGG ATT GCC
GTC AGC TAC CAG ACT AAA GTT AAC CTT CTG TCA GCT ATAAAA TCA CCC
TGT CAG CGG GAA ACC CCA GAG GGA GCA GAG GCA AAA CCC TGG TAC GAA
CCA ATA TAC TTG GGC GGA GTA TTT CAATTG GAG AAA GGT GAT AGA CTG A
GC GCT GAA ATA AAT CGG CCT GAC TAT CTT AAC TTC CGC GAA TCA GGG C
AG GTG TAT TTC GGCATC ATT GCC CTC GGT GGC GGA GGG AGC TCC TCA AG
G ACT CCA AGC GAT AAG CCA GTG GCT CAC GTA GTG GCC AAT CCA CAA GC
AGAA GGT CAA CTG CAA TGG CTT AAC CGC CGC GCA AAC GCA TTG TTG GCT

AAC GGT GTG GAA TTG AGA GAT AAC CAA TTG GTG GTTCCT TCA GAA GGC CTG TAC CTG ATC TAT AGT CAA GTA CTG TTC AAA GGA CAG GGT TGT CCC AGC ACT CAT GTT CTT CTG ACC CACACT ATT AGT AGA ATA GCC GTA TCA T AT CAA ACC AAA GTC AAC CTT TTG TCT GCC ATA AAA TCC CCC TGC CAA A GA GAA ACA CCCGAA GGA GCC GAG GCC AAA CCT TGG TAC GAG CCA ATA TA C CTG GGG GGC GTT TTC CAA TTG GAA AAG GGC GAT AGG TTG AGC GCTGAG ATA AAT AGG CCA GAT TAT TTG AAT TTC AGG GAA AGC GGG CAA GTG TAC TTC GGG ATC ATA GCC CTG GGC GGG GGT GGG TCAAGC TCT CGC ACT CCC TCA GAC AAG CCC GTT GCA CAT GTG GTG GCT AAT CCA CAG GCT GAG GGA CAG CTG CAG TGG CTG AAT AGACGA GCA AAT GCA CTG CTT GCT AAC GGA G TT GAG CTC CGC GAT AAC CAA CTG GTG GTA CCC TCT GAG GGA CTC TAT T TG ATT TACTCC CAA GTT CTC TTC AAG GGC CAA GGC TGC CCC TCC ACT CA T GTC CTG CTT ACC CAC ACT ATT TCT AGA ATA GCC GTA TCT TACCAG ACC AAG GTC AAC CTC TTG AGT GCA ATA AAG AGT CCC TGT CAA CGA GAA ACT CCA GAA GGC GCC GAA GCT AAG CCA TGG TATGAG CCA ATT TAC CTC GGG GGA GTG TTT CAG CTT GAG AAA GGG GAC AGA CTG AGT GCC GAA ATA AAC CGG CCC GAC TAT CTC AACTTC CGC GAG AGT GGT CAA GTC TAC TTC GGT A TC ATA GCT TTG

SEQ ID NO:125 - Codon optimized nucleic acid sequence encoding SEQ ID NO 105

GAG AGT AAA TAC GGC CCA CCT TGT CCT CCC TGC CCT GCT CCA GAG TTC CTT GGC GGG CCT TCC GTC TTC CTG TTT CCC CCC AAGCCA AAG GAC ACA C TG ATG ATT TCA AGA ACC CCA GAG GTC ACC TGT GTC GTT GTA GAT GTT A GT CAA GAG GAT CCA GAG GTG CAATTC AAT TGC TAT GTC GAT GGG GTG GA G GTT CAC AAC GCT AAG ACC AAA CCT CGG GAA GAG CAA TTC AAT TCT AC T TAT CGG GTGGTA AGT GTT CTT ACT GTT TTG CAC CAG GAC TGG TTG AAC GGG AAG GAA TAT AAG TGC AAG GTT AGT AAC AAG GGG CTT CCT TCCAGC ATC GAA AAG ACA ATT AGC AAA GCC AAG GGA CAA CCC CGA GAG CCA CAA GTG TAT ACC CTT CCC CCC TCC CAA GAG GAA ATGACC AAG AAC CAA GTC T CT CTG ACC TGC CTG GTG AAA GGG TTC TAT CCA AGC GAC ATA GCT GTC G AA TGG GAA TCC AAC GGC CAACCC GAA AAT AAC TAT AAA ACA ACA CCT CC C GTC CTG GAT TCC GAT GGG TCA TTT TTC TTG TAT TCA AGA TTG ACC GT G GAT AAAAGC CGC TGG CAG GAG GGG AAC GTT TTT TCA TGT AGT GTA ATG CAT GAA GCT CTT CAT AAC CAT TAT ACA CAG AAA AGT TTG AGTTTG TCA CTC GGT AAA GGT GGA GGA GGG TCC GGT GGC GGT GGC TCA GTG AGA AGT TCT TCT AGG ACC CCT TCC GAC AAA CCC GTTGCC CAC GTT GTC GCA AAT C CA CAA GCT GAA GGG CAG CTT CAG TGG CTC AAT CGG AGA GCA AAT GCT C TC CTT GCC AAC GGA GTCGAA CTG CGC GAC AAC CAA CTC GTC GTT CCC TC C GAG GGC CTG TAT CTG ATC TAT TCA CAA GTG TTG TTC AAA GGT CAA CC T TGTCCA AGT ACC CAT GTC TTG CTG ACA CAC ACA ATA TCA AGA ATA GCA GTC AGC TAT CAA ACA AAA GTG AAT TTG CTC TCT GCC ATCAAA AGT CCC TGC CAA CGC GAG ACT CCT GAA GGT GCT GAA GCA AAA CCC TGG TAT GAA CCT ATA TAT TTG GGT GGC GTC TTT CAACTT GAA AAG GGT GAC AGA CTT T

CT GCC GAG ATA AAC CGG CCA GAC TAT CTG AAC TTT CGA GAG TCC GGT C
AG GTT TAT TTC GGTATC ATT GCC TTG AGC TCT AGA ACA CCT AGC GAC AA
A CCT GTC GCC CAT GTA GTT GCA AAT CCC CAG GCT GAG GGT CAA CTC CA
ATGG CTT AAC AGG CGC GCC AAC GCT CTT CTC GCC AAC GGT GTA GAG CTG
CGC GAT AAT CAA CTG GTG GTT CCT TCC GAG GGA CTTTAT CTG ATA TAT
TCA CAG GTT CTG TTT AAA GGC CAG GGT TGT CCC TCT ACA CAT GTA TTG
TTG ACA CAC ACT ATA TCT CGG ATAGCT GTG AGC TAC CAA ACA AAA GTA A
AT TTG CTG TCT GCT ATC AAG AGT CCA TGT CAG AGG GAA ACC CCC GAA G
GA GCA GAG GCCAAA CCA TGG TAC GAA CCA ATA TAT CTT GGG GGA GTC TT
T CAA TTG GAG AAA GGG GAC CGG TTG AGT GCC GAG ATT AAC CGA CCTGAT
TAC CTT AAT TTC AGG GAG AGC GGT CAA GTT TAC TTC GGC ATA ATA GCC
CTT TCT TCA CGG ACA CCT TCA GAC AAA CCA GTGGCT CAT GTG GTT GCA
AAC CCT CAA GCA GAA GGT CAA TTG CAA TGG CTT AAT CGC AGA GCT AAT
GCC CTT TTG GCA AAC GGT GTGGAG CTT CGG GAT AAT CAG TTG GTG GTT C
CA AGT GAA GGT CTG TAC TTG ATA TAT TCC CAA GTG CTG TTC AAA GGG C
AG GGC TGCCCC TCT ACT CAT GTT CTG CTC ACC CAT ACA ATA TCT AGA AT
C GCT GTG AGC TAC CAG ACT AAG GTC AAT CTT TTG TCA GCA ATAAAA TCA
CCA TGC CAA CGG GAG ACT CCA GAA GGA GCA GAA GCC AAA CCC TGG TAT
GAA CCT ATA TAC CTC GGG GGC GTC TTT CAGCTT GAG AAG GGT GAC AGG
CTG AGC GCT GAA ATT AAT CGG CCC GAC TAC CTT AAC TTT AGA GAA TCC
GGT CAA GTA TAT TTC GGTATT ATT GCC CTC

SEQ ID NO:126- - Codon optimized nucleic acid sequence encoding SEQ ID NO 106

GAG AGC AAA TAT GGC CCA CCC TGC CCC CCA TGT CCT GCC CCA GAA TTC
CTG GGA GGA CCC TCA GTG TTT CTC TTT CCA CCC AAGCCA AAA GAC ACA T
TG ATG ATT TCA AGG ACT CCT GAG GTG ACA TGT GTT GTA GTA GAC GTA T
CA CAG GAG GAT CCT GAA GTC CAGTTC AAC TGG TAC GTC GAC GGC GTT GA
A GTG CAC AAT GCT AAA ACC AAG CCC CGA GAG GAG CAG TTT AAC AGC AC
A TAT CGG GTCGTT TCT GTG CTT ACC GTC TTG CAT CAG GAT TGG CTG AAC
GGA AAA GAA TAT AAA TGC AAG GTC TCA AAC AAG GGG CTT CCA TCTTCA
ATA GAA AAA ACA ATT TCA AAG GCA AAA GGA CAG CCT AGA GAG CCC CAA
GTC TAC ACT CTG CCA CCC AGC CAG GAG GAG ATGACA AAG AAC CAG GTC A
GC CTG ACC TGT CTC GTC AAA GGA TTC TAT CCA TCC GAC ATC GCC GTA G
AA TGG GAG AGT AAT GGA CAGCCT GAA AAC AAC TAT AAG ACC ACT CCC CC
A GTA CTG GAC AGT GAT GGG TCA TTC TTT TTG TAT AGT CGA CTG ACT GT
A GAT AAAAGT CGA TGG CAG GAA GGT AAT GTG TTC TCA TGC AGC GTC ATG
CAC GAG GCC CTG CAC AAC CAT TAT ACA CAG AAG AGT CTG AGTCTT AGC
TTG GGT AAG GGA GGC GGG GGA TCC GGA GGC GGT GGA TCT GTA CGG TCT
TCT AGC AGA ACA CCA AGT GAT AAA CCA GTGGCT CAC GTG GTA GCA AAC C
CC CAA GCT GAG GGG CAG CTT CAA TGG CTT AAT AGA AGG GCT AAC GCT C
TT CTT GCC AAC GGG GTCGAG CTT AGG GAT AAC CAG CTG GTG GTC CCC TC
T GAA GGC TTG TAT CTG ATA TAC TCC CAG GTA CTG TTT AAA GGA CAA GG
C TGTCCC AGC ACT CAT GTA CTG TTG ACA CAT ACT ATA TCA CGC ATA GCT
GTC TCT TAT CAG ACA AAA GTT AAC TTG CTT AGC GCT ATCAAG AGT CCC

TGT CAG AGA GAA ACC CCC GAA GGT GCA GAG GCC AAG CCA TGG TAC GAA
CCT ATT TAC CTT GGA GGC GTT TTC CAACTG GAG AAA GGG GAT CGC CTC T
CC GCC GAA ATA AAC AGG CCC GAT TAT CTG AAC TTC CGA GAG AGC GGC C
AA GTC TAC TTT GGGATA ATC GCT CTC GTG CGG AGC AGT AGC AGA ACC CC
C TCT GAT AAA CCA GTT GCC CAT GTG GTT GCC AAC CCA CAG GCC GAA GG
TCAG CTG CAG TGG CTG AAT CGG AGA GCC AAC GCT CTT CTC GCC AAT GGT
GTG GAA CTC AGG GAT AAC CAA CTG GTT GTC CCA TCTGAA GGT CTT TAT
CTT ATC TAT TCA CAA GTG CTC TTT AAG GGA CAG GGC TGT CCA AGT ACA
CAC GTC TTG CTC ACT CAC ACA ATATCC AGA ATT GCT GTA AGC TAC CAG A
CA AAA GTA AAC CTC CTT AGC GCC ATT AAA AGC CCT TGT CAA AGG GAA A
CA CCT GAG GGAGCC GAA GCC AAA CCA TGG TAC GAA CCC ATA TAT CTC GG
T GGC GTT TTC CAG TTG GAG AAG GGC GAT CGA CTG TCC GCC GAG ATTAAT
CGC CCT GAT TAT CTG AAC TTT CGG GAG TCC GGG CAG GTT TAC TTT GGT
ATA ATC GCA CTG GTA CGC TCA AGC AGT AGA ACTCCC TCA GAC AAA CCA
GTA GCA CAT GTT GTA GCT AAT CCA CAA GCA GAA GGA CAG CTG CAA TGG
CTG AAC CGG AGA GCT AAC GCCCTG CTG GCT AAC GGT GTC GAG TTG CGA G
AT AAT CAG CTT GTC GTG CCT AGC GAG GGG CTC TAC CTT ATT TAT AGT C
AA GTT CTCTTT AAA GGG CAG GGG TGT CCA AGT ACA CAC GTG TTG CTC AC
A CAT ACT ATT TCT CGA ATA GCC GTG TCC TAT CAA ACC AAG GTGAAC CTT
CTC TCC GCT ATC AAA AGC CCT TGC CAA AGA GAA ACA CCC GAA GGC GCC
GAG GCT AAG CCA TGG TAC GAA CCT ATC TATCTC GGG GGT GTT TTT CAA
CTC GAA AAA GGG GAC AGG TTG AGT GCT GAG ATT AAT AGA CCC GAT TAT
TTG AAT TTT AGG GAA TCTGGG CAG GTT TAT TTT GGA ATA ATT GCT CTC

SEQ ID NO:127- Codon optimized nucleic acid sequence encoding SEQ ID NO 107

GTA CGG AGC AGC TCT AGA ACT CCA TCT GAC AAG CCA GTC GCT CAT GTG
GTA GCA AAT CCC CAA GCT GAG GGC CAA CTT CAG TGGTTG AAT CGC AGG G
CT AAC GCT CTG CTC GCC AAT GGA GTA GAA TTG AGG GAT AAT CAG CTC G
TA GTA CCT AGC GAA GGG CTT TACCTC ATA TAT TCT CAG GTT CTG TTT AA
G GGT CAA GGC TGT CCA AGT ACT CAC GTT CTC CTT ACT CAT ACA ATC TC
T CGC ATC GCAGTT TCT TAT CAA ACC AAG GTT AAT TTG CTG AGC GCC ATT
AAG TCA CCA TGC CAG CGC GAA ACC CCC GAA GGT GCC GAA GCA AAACCT
TGG TAT GAG CCC ATT TAC CTT GGC GGT GTG TTT CAG CTG GAG AAG GGG
GAC AGG CTT TCA GCA GAA ATT AAT AGG CCC GACTAT CTT AAT TTC CGG G
AG TCC GGC CAG GTT TAT TTC GGT ATC ATT GCC CTG GGC GGT GGC GGC T
CA TCC TCA CGC ACT CCA TCTGAT AAG CCC GTC GCA CAT GTG GTC GCC AA
T CCT CAG GCA GAG GGG CAA TTG CAA TGG CTT AAC CGC AGG GCA AAC GC
T CTG CTTGCT AAT GGG GTT GAG CTT CGG GAT AAC CAG CTC GTG GTA CCT
TCA GAG GGT TTG TAC TTG ATC TAT TCT CAA GTG CTT TTC AAAGGA CAA
GGT TGC CCA AGC ACC CAT GTG TTG TTG ACC CAT ACT ATT TCC CGG ATA
GCA GTG TCA TAT CAA ACT AAG GTC AAT CTTCTG TCA GCT ATT AAA AGT C
CC TGT CAG AGA GAG ACT CCA GAG GGA GCT GAA GCC AAA CCC TGG TAC G
AG CCC ATA TAT CTT GGAGGG GTG TTC CAG CTC GAG AAA GGC GAC AGA TT
G AGC GCC GAG ATA AAC CGG CCT GAC TAT CTC AAT TTT CGA GAG TCC GG

T CAGGTT TAC TTT GGG ATA ATC GCA CTG GGT GGT GGA GGG TCT AGC TCT
CGC ACA CCA TCC GAT AAG CCA GTA GCT CAT GTG GTG GCCAAC CCT CAA
GCC GAG GGG CAA CTT CAG TGG CTG AAT AGA CGA GCT AAT GCA TTG CTG
GCT AAC GGT GTC GAA CTG AGA GAT AATCAG CTC GTA GTA CCT TCA GAA G
GG CTT TAC CTC ATA TAC TCT CAG GTT TTG TTC AAA GGA CAG GGA TGT C
CT TCA ACT CAC GTCCTT CTC ACT CAC ACT ATA AGT AGA ATC GCT GTA TC
C TAC CAA ACT AAA GTG AAC CTT TTG TCT GCT ATC AAA TCC CCT TGC CA
ACGC GAA ACT CCC GAA GGC GCA GAA GCC AAG CCT TGG TAT GAG CCA ATC
TAC CTC GGA GGA GTT TTT CAG TTG GAA AAG GGT GACAGG CTG AGT GCT
GAA ATC AAC AGG CCC GAT TAT CTG AAC TTC AGG GAA AGC GGA CAA GTG
TAT TTT GGA ATA ATC GCA CTT GGTGGG GGA GGG TCC GAG TCT AAG TAC G
GG CCA CCT TGT CCT CCC TGT CCA GCA CCT GAG TTT TTG GGC GGG CCC A
GT GTA TTC CTGTTT CCA CCC AAA CCT AAG GAT ACC CTG ATG ATA TCA CG
A ACC CCT GAG GTC ACC TGT GTT GTC GTT GAC GTA AGT CAG GAG GACCCA
GAG GTT CAG TTC AAC TGG TAT GTC GAC GGG GTA GAA GTT CAT AAT GCT
AAG ACT AAG CCA AGG GAG GAA CAA TTT AAT TCCACT TAT CGA GTT GTG
AGC GTC CTG ACA GTT TTG CAT CAG GAT TGG CTT AAC GGC AAA GAA TAT
AAG TGC AAG GTT TCA AAT AAAGGT CTG CCT TCT TCC ATA GAA AAA ACA A
TC TCT AAA GCC AAA GGC CAA CCA AGA GAG CCT CAG GTG TAC ACT CTT C
CT CCC TCTCAG GAA GAG ATG ACA AAA AAC CAG GTG TCC TTG ACC TGT CT
C GTT AAG GGG TTC TAT CCA AGC GAT ATT GCT GTT GAG TGG GAATCA AAC
GGG CAG CCT GAG AAT AAT TAC AAG ACC ACA CCC CCA GTT TTG GAT AGC
GAT GGT AGT TTC TTC CTT TAC AGT AGG TTGACC GTT GAT AAG TCC CGG
TGG CAA GAA GGA AAT GTG TTT AGT TGC TCC GTG ATG CAC GAG GCA CTG
CAT AAT CAT TAC ACT CAAAAG AGT CTT AGT CTG AGC TTG GGG AAA

SEQ ID NO:128 - Codon optimized nucleic acid sequence encoding SEQ ID NO 108

GTG CGG AGT AGC AGC AGA ACT CCA TCC GAT AAA CCA GTG GCA CAC GTG
GTC GCT AAT CCC CAA GCA GAA GGG CAG CTC CAA TGGCTG AAC AGG CGG G
CC AAT GCC CTT TTG GCT AAT GGC GTC GAG CTC AGA GAC AAT CAG CTC G
TC GTC CCA TCT GAG GGT CTC TACTTG ATC TAT AGT CAG GTC TTG TTC AA
A GGC CAA GGC TGT CCT AGT ACT CAT GTT CTC CTT ACA CAT ACC ATT TC
A AGG ATA GCAGTC TCA TAT CAG ACT AAA GTC AAT CTC CTG AGT GCA ATT
AAG TCC CCC TGC CAG CGA GAG ACT CCA GAA GGT GCT GAG GCA AAGCCA
TGG TAT GAG CCC ATA TAT CTT GGC GGA GTC TTT CAA CTG GAG AAG GGT
GAC CGG CTC TCC GCA GAG ATT AAC CGG CCT GACTAT CTG AAT TTC AGA G
AG TCT GGC CAG GTT TAC TTT GGC ATT ATC GCA CTT TCC AGT CGG ACC C
CC AGC GAC AAA CCT GTT GCCCAT GTC GTA GCA AAT CCC CAA GCC GAA GG
C CAG TTG CAG TGG CTG AAC AGA CGA GCT AAT GCT TTG TTG GCA AAT GG
G GTG GAGCTT CGG GAC AAT CAA CTC GTG GTA CCA TCT GAA CCC TTC TAC
CTG ATA TAT AGC CAG GTA CTC TTT AAG GGT CAA GGT TGT CCTAGT ACT
CAT GTG CTC TTG ACC CAC ACA ATT TCA AGA ATC GCC GTC AGT TAC CAA
ACC AAG GTT AAT CTG CTT TCT GCC ATA AAGTCT CCC TGC CAA CGC GAA A
CC CCA GAA GGT GCT GAA GCC AAG CCT TGG TAC GAG CCA ATC TAC CTC G

GT GGC GTT TTT CAA CTTGAA AAG GGG GAT CGC CTG TCT GCC GAG ATC AA
C AGG CCA GAC TAC CTG AAC TTC CGA GAA AGT GGG CAA GTC TAT TTT GG
G ATCATA GCC CTG AGC TCT CGG ACC CCC AGC GAC AAG CCT GTT GCC CAC
GTA GTT GCT AAC CCT CAG GCT GAA GGA CAA CTT CAG TGGCTG AAC AGG
AGA GCT AAC GCC CTC CTG GCT AAT GGA GTC GAA CTG AGA GAT AAT CAA
TTG GTC GTA CCA AGC GAG GGA CTG TACCTC ATA TAC TCT CAG GTA CTG T
TT AAG GGC CAA GGA TGT CCA AGT ACC CAT GTA CTT CTC ACA CAT ACA A
TA AGC CGG ATA GCCGTC AGC TAT CAG ACT AAG GTA AAC CTG CTC AGC GC
T ATT AAG AGC CCA TGC CAG CGA GAG ACC CCA GAA GGA GCA GAA GCT AA
ACCC TGG TAC GAG CCA ATA TAT CTT GGA GGA GTC TTT CAA CTG GAG AAG
GGT GAC CGA TTG AGT GCT GAA ATT AAT CGG CCA GATTAT TTG AAC TTC
CGC GAG AGC GGG CAA GTG TAT TTC GGA ATC ATT GCA CTT GGC GGG GGC
GGG AGC GAG TCC AAA TAT GGC CCACCA TGT CCC CCC TGC CCT GCC CCA G
AG TTC CTT GGG GGC CCT TCT GTA TTT CTC TTC CCC CCA AAA CCC AAG G
AT ACT CTT ATGATC AGC AGG ACT CCT GAG GTA ACC TGT GTG GTC GTC GA
C GTA TCA CAA GAG GAT CCA GAG GTA CAG TTT AAT TGG TAT GTA GACGGC
GTG GAA GTC CAC AAT GCT AAA ACT AAG CCC AGA GAG GAG CAG TTT AAT
AGT ACA TAC CGA GTA GTG AGC GTA TTG ACT GTATTG CAT CAG GAC TGG
TTG AAT GGG AAA GAG TAC AAG TGC AAA GTT TCC AAC AAA GGT CTC CCT
TCA TCT ATC GAG AAA ACC ATCTCA AAG GCC AAA GGC CAA CCC AGA GAG C
CT CAA GTA TAC ACT CTG CCA CCC AGC CAA GAA GAG ATG ACT AAG AAT C
AG GTT AGTCTC ACT TGT CTC GTC AAA GGG TTC TAT CCC TCC GAT ATT GC
T GTG GAA TGG GAG AGC AAC GGG CAA CCC GAG AAC AAC TAT AAGACA ACC
CCA CCA GTA CTT GAT AGC GAC GGG TCT TTT TTC CTT TAT TCA CGC CTT
ACA GTT GAT AAA TCT CGG TGG CAG GAA GGGAAC GTT TTC AGC TGT TCT
GTT ATG CAT GAA GCC TTG CAT AAC CAT TAC ACA CAA AAG AGT CTT AGT
TTG TCT CTT GGA AAG

SEQ ID NO:129 - Codon optimized nucleic acid sequence encoding SEQ ID NO 109

GTG CGC AGC AGT TCC AGA ACA CCT AGT GAC AAG CCT GTG GCA CAC GTT
GTG GCC AAT CCT CAA GCT GAA GGT CAG CTC CAA TGGCTT AAT AGA AGG G
CT AAC GCA TTG CTT GCT AAT GGG GTG GAA CTT CGA GAT AAC CAA TTG G
TG GTG CCC TCC GAG GGT CTC TACCTT ATC TAT AGC CAG GTC CTC TTT AA
A GGC CAA GGT TGC CCC AGT ACA CAC GTC CTG CTT ACA CAC ACA ATA TC
C AGA ATA GCAGTC TCA TAC CAG ACC AAG GTA AAT CTG CTT AGC GCT ATT
AAG TCA CCC TGT CAG CGG GAA ACC CCA GAG GGT GCA GAA GCA AAACCA
TGG TAT GAG CCA ATT TAC CTT GGT GGC GTT TTT CAA CTG GAA AAG GGC
GAT AGG TTG AGC GCC GAG ATC AAT AGA CCC GACTAT CTC AAT TTT CGG G
AG TCA GGC CAG GTT TAT TTC GGG ATC ATT GCT TTG GTT CGC TCC TCT A
GC CGC ACC CCT TCC GAT AAACCA GTT CCA CAT CTT CTG GCC AAT CCC CA
G GCT GAA GGC CAG CTT CAG TGG CTC AAC AGA CGG GCT AAT GCC CTC CT
C GCC AATGGG GTC GAG CTG AGG GAC AAC CAA CTT GTG GTC CCC TCA GAA
GGT CTC TAC CTT ATC TAC AGC CAG GTT CTT TTC AAA GGC CAGGGC TGT
CCT TCC ACT CAC GTG CTG TTG ACC CAT ACC ATA TCC CGC ATT GCC GTT

AGC TAT CAA ACC AAA GTC AAC CTT TTG TCTGCA ATT AAG AGT CCA TGC C
AG AGA GAA ACT CCC GAA GGT GCA GAA GCA AAG CCA TGG TAT GAA CCT A
TA TAT CTC GGA GGT GTGTTT CAA CTT GAG AAA GGG GAC AGA CTG AGT GC
C GAA ATA AAT CGC CCT GAT TAT CTT AAT TTC CGA GAG TCT GGG CAA GT
A TATTTT GGA ATT ATT GCC CTC GTG CGA AGC TCT TCA AGG ACC CCA AGT
GAT AAA CCC GTA GCA CAC GTA GTT GCA AAT CCA CAA GCCGAA GGA CAG
TTG CAA TGG CTG AAT AGG CGG GCT AAT GCT TTG CTT GCT AAT GGG GTC
GAG CTG CGG GAT AAC CAG CTT GTC GTGCCA TCT GAA GGA TTG TAC CTG A
TA TAC AGC CAA GTT TTG TTT AAG GGA CAG GGA TGC CCA TCA ACC CAC G
TG CTC CTC ACT CACACT ATT TCT CGA ATT GCC GTA TCA TAT CAG ACT AA
A GTC AAC TTG TTG AGC GCA ATA AAG AGC CCT TGT CAA CGG GAA ACC CC
CGAG GGT GCA GAG GCC AAA CCA TGG TAT GAA CCT ATT TAC CTC GGG GGC
GTC TTT CAG TTG GAA AAA GGT GAT CGG TTG TCC GCTGAG ATT AAC CGA
CCA GAC TAT CTT AAC TTT CGG GAA TCT GGT CAA GTC TAT TTT GGC ATA
ATT GCA TTG GGG GGC GGG GGC TCTGAA TCC AAA TAC GGG CCT CCT TGC C
CC CCT TGC CCA GCA CCA GAA TTT CTC GGG GGC CCA TCA GTT TTT CTT T
TC CCC CCT AAGCCA AAA GAT ACC CTC ATG ATA TCA AGA ACT CCA GAG GT
T ACA TGT GTC GTG GTC GAC GTT AGC CAG GAG GAT CCC GAG GTT CAGTTC
AAT TGG TAC GTG GAT GGA GTT GAA GTG CAC AAT GCC AAA ACA AAA CCA
CGA GAA GAG CAA TTT AAT AGC ACC TAC AGG GTAGTC AGC GTT CTT ACA
GTT TTG CAC CAA GAT TGG CTT AAC GGC AAA GAA TAC AAA TGT AAG GTT
AGT AAT AAA GGA CTC CCC TCATCA ATA GAA AAA ACA ATT TCC AAA GCT A
AA GGC CAG CCT AGG GAA CCT CAA GTG TAC ACA CTT CCT CCA AGT CAA G
AA GAG ATGACA AAG AAC CAG GTC TCA CTC ACT TGT CTC GTC AAA GGT TT
C TAC CCC TCT GAC ATC GCC GTG GAA TGG GAG TCC AAT GGC CAACCT GAG
AAT AAT TAC AAG ACC ACA CCT CCA GTA CTC CAT AGT GAC GGG TCT TTC
TTT TTG TAT TCT AGG TTG ACA GTG GAT AAATCC AGA TGG CAA GAA GGA
AAT GTT TTC TCA TGT TCT GTG ATG CAC GAG GCT CTT CAC AAC CAC TAC
ACT CAA AAG TCT CTG TCTCTT TCC CTT GGC AAA

SEQ ID NO:130 - Codon optimized nucleic acid sequence encoding SEQ ID NO 110

GTC CGA TCA TCT AGT AGG ACC CCT AGC GAC AAG CCA GTT GCA CAC GTG
GTA GCA AAC CCA CAA GCA GAA GGA CAA CTG CAG TGGCTT AAT AGG CGC G
CA AAT GCA TTG CTC GCC AAT GGA GTG GAA CTC CGA GAC AAC CAA TTG G
TA GTG CCT TCC GAA GGA CTC TACCTT ATT TAT AGT CAG GTC CTG TTC AA
A GGG CAA GGT TGC CCC TCA ACA CAC GTA TTG CTG ACA CAC ACC ATA TC
C CGC ATA GCAGTT AGC TAT CAA ACA AAG GTT AAT TTG CTG TCC GCA ATA
AAG AGC CCC TGC CAA CGG GAG ACC CCC GAG GGC GCA GAG GCA AAACCC
TGG TAC GAG CCC ATC TAC TTG GGT GGC GTC TTT CAA CTT GAA AAG GGG
GAT AGG CTG AGC GCT GAA ATT AAC CGC CCC GACTAT TTG AAT TTC CGG G
AA TCT GGC CAA GTA TAC TTT GGT ATT ATT GCC TTG GGT GGT GGA GGT A
GC AGT AGC CGA ACA CCA TCAGAC AAA CCT GTG GCA CAC GTT GTC GCC AA
C CCA CAA GCT GAA GGA CAA CTC CAA TGG TTG AAC AGG CGA GCC AAT GC
C CTC CTTGCA AAT GGC GTA GAA TTG CGA GAT AAT CAG CTT GTT GTT CCT

AGC GAG GGT CTT TAT CTT ATA TAC AGT CAG GTC CTC TTT AAAGGC CAA
GGA TGT CCT AGT ACA CAC GTG CTG CTG ACT CAT ACA ATA AGC CGA ATT
GCC GTA TCC TAT CAG ACT AAG GTC AAC CTTCTG AGC GCT ATT AAA TCC C
CA TGT CAA AGG GAA ACT CCA GAA GGC GCA GAA GCC AAG CCC TGG TAT G
AG CCA ATC TAT CTC GGAGGG GTT TTC CAA TTG GAG AAG GGC GAC CGG CT
T TCT GCT GAA ATC AAT CGA CCT GAT TAT CTC AAC TTT CGA GAG TCA GG
G CAGGTT TAT TTC GGT ATC ATT GCT CTC GGT GGC GGA GGG TCC AGC TCT
AGG ACC CCC TCA GAC AAA CCA GTA GCC CAC GTT GTG GCCAAT CCC CAG
GCA GAA GGT CAG TTG CAG TGG TTG AAT CGG CGC GCT AAT GCA CTC CTC
GCC AAT GGA GTT GAA CTT AGG GAT AATCAA CTC GTA GTC CCC AGC GAA G
GG TTG TAT CTT ATT TAT AGT CAG GTC CTT TTT AAG GGT CAG GGT TGC C
CA TCC ACT CAC GTGTTG CTC ACT CAC ACC ATC AGT CGC ATC GCC GTT TC
C TAT CAG ACC AAG GTT AAT CTC CTG TCC GCT ATA AAG TCC CCA TGT CA
AAGA GAG ACC CCC GAA GGA GCA GAG GCA AAG CCT TGG TAC GAG CCT ATA
TAC TTG GGT GGC GTA TTT CAG TTG GAA AAG GGT GACCGG TTG TCC GCT
GAG ATA AAT CGA CCT GAC TAT CTC AAC TTT CGG GAG TCT GGT CAG GTT
TAC TTT GGG ATT ATA GCA CTG GAGAGC AAA TAC GGA CCC CCC TGT CCT C
CT TGT CCT GCC CCA GAG TTT CTC GGT GGA CCA TCA GTC TTT CTT TTT C
CT CCT AAG CCCAAG GAT ACA TTG ATG ATC TCA CGG ACC CCC GAA GTT AC
C TGC GTG GTT GTT GAT GTA AGT CAG GAG GAT CCC GAA GTC CAA TTCAAT
TGG TAT GTC GAC GGC GTG GAG GTC CAC AAT GCA AAG ACA AAG CCC CGG
GAG GAA CAG TTT AAC AGC ACA TAC CGG GTC GTTAGC GTG TTG ACC GTC
CTT CAT CAA GAT TGG TTG AAC GGC AAA GAG TAC AAG TGC AAG GTT AGC
AAC AAA GGT TTG CCA TCT TCCATC GAG AAA ACA ATA TCT AAG GCC AAA G
GA CAG CCC CGC GAA CCA CAA GTT TAT ACT CTT CCT CCA AGC CAG GAG G
AA ATG ACTAAG AAT CAG GTT TCC CTC ACA TCC CTT GTA AAG GGT TTT TA
T CCC TCA GAT ATT GCA GTT GAG TGG GAG AGC AAT GGT CAG CCCGAG AAT
AAC TAT AAA ACA ACC CCA CCA GTA CTC GAC TCA GAT GGT AGT TTC TTC
CTC TAC TCC AGG TTG ACA GTA GAC AAA AGCCGC TGG CAA GAG GGC AAC
GTA TTC TCT TGC TCA GTG ATG CAT GAA GCA CTG CAT AAT CAC TAC ACA
CAA AAA TCT CTG AGC CTTTCA CTT GGC AAA

SEQ ID NO:131 - Codon optimized nucleic acid sequence encoding SEQ ID NO 111

GTT AGG TCT TCA TCT AGA ACA CCC AGC GAC AAG CCC GTG GCC CAC GTC
GTT GCC AAC CCC CAG GCA GAG GGT CAG CTG CAG TGGCTC AAT AGG CGA G
CT AAC GCC CTT CTC GCT AAC GGT GTG GAG TTG CGC GAT AAC CAA CTG G
TC GTA CCA TCC GAA GGA CTC TATCTG ATT TAT TCT CAA GTC CTG TTT AA
G GGC CAG GGC TGT CCT TCA ACC CAC GTC CTC CTT ACA CAT ACC ATT TC
T AGA ATA GCCGTA TCA TAT CAG ACT AAA GTA AAT CTT TTG TCA GCA ATC
AAA TCT CCA TGC CAA CGG GAG ACC CCA CAC CGA GCA GAA GCT AAACCC
TGG TAC GAA CCC ATA TAT CTG GGC GGT GTC TTC CAG CTT GAG AAG GGG
GAC CGA CTC TCA GCC GAG ATA AAT CGA CCT GACTAT TTG AAC TTC AGA G
AG TCC GGG CAA GTC TAT TTC GGA ATT ATA GCT CTC TCC TCT AGG ACC C
CA TCA GAT AAA CCA GTT GCCCAT GTC GTG GCT AAT CCC CAG GCT GAA GG

C CAA CTG CAA TGG CTT AAC CGC CGG GCC AAT GCT TTG CTC GCC AAC GG
T GTA GAGTTG CGC GAC AAC CAA CTG GTA GTC CCT AGC GAA GGG CTG TAC
CTG ATC TAC TCC CAA GTT CTT TTT AAA GGC CAA GGT TGT CCTAGT ACC
CAC GTA CTT CTG ACC CAT ACT ATA TCT CGG ATA GCT GTG AGT TAC CAG
ACA AAG GTT AAC CTT CTT TCC GCC ATC AAAAGT CCT TGC CAA AGG GAA A
CA CCT GAA GGT GCA GAA GCC AAG CCC TGG TAT GAG CCA ATT TAT CTG G
GC GGA GTC TTC CAA CTCGAG AAG GGG GAT AGA CTG AGC GCT GAG ATA AA
C AGA CCA GAC TAT CTG AAT TTT AGG GAG TCA GGC CAG GTA TAC TTT GG
A ATAATC GCC CTC TCA TCA AGG ACT CCC TCC GAC AAA CCA GTA GCA CAC
GTA GTG GCA AAT CCC CAG GCA GAA GGA CAG CTC CAG TGGCTG AAT CGG
CGG GCA AAC GCC CTG CTC GCT AAC GGG GTC GAA CTT AGG GAC AAC CAG
CTT GTT GTG CCA TCC GAA GGT TTG TACCTG ATA TAT TCT CAA GTT CTC T
TT AAA GGC CAG GGG TGT CCT TCT ACT CAT GTG CTG TTG ACT CAT ACA A
TA TCA CGG ATT GCAGTT TCC TAT CAA ACT AAA GTA AAC TTG CTT TCA GC
T ATC AAG AGT CCA TGC CAA AGG GAG ACA CCT GAA GGG GCA GAG GCT AA
ACCC TGG TAC GAG CCT ATT TAC CTC GGG GGC GTT TTT CAG CTG GAA AAA
GGA GAT CGG TTG TCA GCT GAA ATC AAC AGA CCC GACTAT CTG AAC TTT
CGC GAG TCA GGT CAG GTT TAT TTT GGC ATT ATT GCC CTG GAA AGC AAG
TAC GGT CCT CCT TGT CCA CCA TGCCCT GCT CCA GAA TTC TTG GGG GGA C
CA TCA GTG TTT CTG TTC CCC CCC AAA CCA AAG GAC ACC TTG ATG ATA A
GC CGA ACC CCAGAA GTG ACC TGT GTC GTA GTT GAT GTA AGT CAA GAA GA
T CCA GAG GTC CAA TTC AAC TGG TAC GTT GAC GGT GTC GAG GTA CATAAC
GCC AAA ACC AAG CCT CGC GAA GAG CAG TTT AAC TCC ACA TAT AGG GTG
GTA AGT GTG CTC ACA GTG CTG CAT CAA GAC TGGCTT AAC GGG AAG GAA
TAC AAG TGT AAA GTC TCC AAT AAG GGA CTT CCC TCT AGC ATA GAA AAA
ACT ATA TCT AAA GCA AAG GGTCAA CCA CGC GAA CCA CAG GTA TAT ACA C
TC CCC CCT AGC CAG GAG GAA ATG ACC AAA AAC CAA GTA TCT TTG ACC T
GT CTG GTGAAA GGC TTT TAC CCA TCT GAT ATC GCA GTT GAA TGG GAG TC
A AAT GGC CAA CCC GAA AAT AAC TAC AAG ACA ACT CCT CCC GTGCTC GAC
TCT GAC GGA TCA TTC TTC CTT TAC TCT CGC CTC ACC GTA GAT AAG AGC
CGC TGG CAA GAG GGT AAC GTA TTC AGT TGTAGC GTG ATG CAT GAG GCT
CTT CAT AAC CAT TAT ACA CAA AAG TCC CTC AGC CTT TCT CTG GGA AAG

SEQ ID NO:132 - Codon optimized nucleic acid sequence encoding SEQ ID NO 112

GTC CGC TCA TCA TCA AGA ACC CCA AGC GAC AAA CCT GTG GCC CAC GTT
GTT GCC AAT CCA CAA GCC GAG GGG CAG CTG CAG TGGCTT AAC AGG AGA G
CA AAC GCT CTT CTT GCC AAC GGC GTA GAG CTT CGA GAC AAC CAA CTT G
TC GTA CCT TCT GAA GGT CTG TACCTC ATC TAT AGT CAA GTA CTT TTT AA
A GGA CAG GGT TGT CCA AGT ACA CAT GTA CTT CTG ACC CAC ACA ATA TC
C AGG ATA GCCGTG TCA TAC CAC ACA AAG GTC AAT CTG TTG TCT GCA ATT
AAG TCA CCA TGC CAA AGA GAA ACC CCA GAA GGT GCA GAA GCA AAGCCA
TGG TAT GAG CCA ATA TAT CTG GGC GGC GTC TTT CAG CTC GAG AAG GGA
GAC CGG CTG TCT GCA GAA ATC AAC AGG CCT GACTAC CTC AAC TTC AGG G
AG AGT GGC CAG GTG TAT TTT GGA ATA ATT GCA TTG GTT AGA AGT TCT C

GC ACA CCA TCC GAT AAA CCAGTC GCC CAC GTT GTA GCT AAT CCA CAA GC
C GAG GGA CAG CTG CAA TGG CTG AAT CGA CGG GCC AAT GCA TTG CTG GC
T AAT GGGGTA GAG CTT CGC GAT AAT CAA CTT GTG GTC CCA TCA GAG GGT
CTT TAC CTC ATA TAC TCC CAA GTC CTT TTC AAA GGC CAA GGTTGT CCT
TCT ACA CAT GTG CTT TTG ACC CAC ACT ATT TCT AGA ATC GCA GTG TCA
TAC CAG ACT AAG GTC AAC CTG CTC TCA GCTATT AAG TCA CCC TGC CAA A
GG GAA ACT CCC GAG GGT GCC GAG GCC AAA CCT TGG TAT GAA CCT ATC T
AC CTT GGG GGA GTG TTCCAA CTG GAG AAG GGC GAT AGA TTG AGT GCC GA
G ATA AAT CGG CCA GAT TAT TTG AAC TTC AGA GAG AGC GGA CAA GTC TA
C TTCGGT ATA ATA GCA TTG GTG CGC AGT AGC CGA ACT CCC TCC GAT AAG
CCA GTC GCC CAT GTT GTC GCA AAC CCT CAG GCA GAG GGACAG CTT CAA
TGG CTC AAT CGC CGC GCC AAT GCC TTG CTT GCC AAC GGT GTT GAA CTG
AGG GAC AAC CAG TTG GTC GTT CCT AGCGAA GGT TTG TAT CTT ATC TAT A
GC CAG GTA CTG TTC AAA GGG CAA GGG TGT CCT AGT ACC CAT GTG CTC C
TC ACA CAT ACC ATATCA AGA ATT GCA GTT AGT TAT CAG ACC AAG GTA AA
T CTC CTG AGT GCA ATA AAA TCC CCC TGC CAG CGG GAG ACT CCA GAG GG
GGCT GAG GCC AAA CCA TGG TAC GAG CCC ATC TAT CTC GGT GGA GTC TTT
CAG CTG GAA AAG GGA GAT CGC CTT TCT GCA GAG ATTAAT AGG CCA GAT
TAC CTG AAT TTC CGC GAG AGT GGG CAA GTT TAC TTC GGT ATC ATA GCC
CTT GAA AGC AAA TAC GGC CCT CCATGC CCC CCC TGC CCT GCA CCC GAG T
TC CTG GGC GGT CCC TCT GTG TTC TTG TTC CCC CCA AAG CCC AAG GAC A
CC CTC ATG ATATCC AGG ACA CCA GAA GTA ACT TGC GTT GTC GTC GAT GT
G TCC CAG GAA GAT CCA GAA GTT CAA TTT AAC TGG TAT GTC GAT GGTGTG
GAA GTG CAT AAT GCA AAA ACT AAG CCT CGA GAA GAA CAA TTC AAC TCT
ACA TAT CGC GTC GTC AGT GTG TTG ACT GTC CTCCAC CAA GAC TGG CTG
AAT GGC AAA GAG TAC AAG TGC AAA GTG TCC AAT AAG GGC CTT CCA TCT
TCA ATT GAG AAA ACC ATT AGTAAG GCA AAG GGT CAG CCC CGG GAA CCA C
AG GTC TAT ACA TTG CCC CCT AGC CAA GAG GAG ATG ACC AAG AAC CAA G
TC TCA CTCACC TGT CTG GTA AAG GGA TTT TAC CCT AGT GAT ATC GCT GT
C GAA TGG GAA AGC AAC GGT CAG CCC GAG AAC AAT TAC AAA ACCACT CCA
CCA GTG CTC GAC TCA GAC GGC TCT TTT TTC CTT TAC TCA CGG TTG ACT
GTA GAT AAA TCC CGC TGG CAG GAG GGC AATGTT TTC AGC TGT AGT GTT
ATG CAC GAA GCA CTT CAC AAT CAT TAT ACC CAG AAG TCA CTG TCT CTT
TCC CTT GGG AAG

SEQ ID NO:133 - Codon optimized nucleic acid sequence encoding SEQ ID NO 113

GAG CCA AAG TCC AGC GAC AAG ACA CAT ACT TGT CCA CCC TGT CCA GCT
CCA GAG GCA GCC GGC GGT CCT TCC GTG TTC TTG TTTCCT CCC AAG CCA A
AG GAC ACA CTG ATG ATC TCT AGA ACT CCC GAG GTT ACA TGC GTT GTG G
TT GAC GTG TCT CAT GAG GAC CCTGAA GTG AAG TTT AAT TGG TAC GTC GA
C GGT GTC GAG GTA CAT AAT GCA AAA ACT AAG CCA CGC GAG GAA CAA TA
T AAT AGC ACATAC CGA GTG GTC AGC GTC TTG ACA GTG CTT CAC CAA GAC
TGG CTC AAT GGT AAG GAG TAT AAA TGC AAA GTA TCA AAC AAA GCCTTG
CCC GCA TCC ATC GAA AAA ACA ATA AGC AAG GCT AAG GGA CAA CCA CGG

GAG CCA CAA GTG TAT ACT CTC CCC CCT TCA AGAGAC GAG CTC ACA AAA A
AC CAA GTT TCA CTG ACT TGC CTG GTT AAA GGT TTT TAT CCC TCC GAT A
TA GCT GTT GAA TGG GAG AGTAAT GGA CAA CCA GAA AAT AAC TAT AAA AC
T ACT CCT CCC GTG CTT GAC AGT GAC GGG TCT TTT TTC TTG TAT TCT AA
A CTC ACCGTT GAT AAA AGT AGA TGG CAG CAG GGC AAT GTT TTC TCC TGC
TCA GTG ATG CAT GAA GCT CTG CAC AAT CAC TAC ACA CAA AAATCA CTG
TCC CTG TCT CCT GGT AAG GGT GGC GGT GGC AGC GTC AGG TCA AGT TCC
AGA ACA CCT AGT GAT AAA CCA GTA GCC CATGTA GTT GCT AAC CCC CAG G
CT GAG GGA CAA CTT CAG TGG CTT AAC CGC CGC GCT AAT GCT CTT CTT G
CT AAC GGA GTC GAA CTGAGA GAT AAC CAA CTT GTC GTG CCT AGT GAG GG
G TTG TAT CTC ATT TAC TCT CAG GTG CTG TTC AAG GGC CAG GGC TGT CC
A TCAACT CAC GTA CTG CTT ACA CAT ACT ATT AGC AGG ATA GCA GTG AGC
TAC CAA ACC AAA GTT AAC TTG TTG TCT GCC ATT AAA AGCCCT TGT CAG
AGG GAA ACC CCT GAG GGG GCA GAA GCT AAG CCA TGG TAC GAA CCT ATT
TAC CTT GGT GGG GTG TTT CAG TTG GAGAAA GGG GAT CGG CTT AGT GCT G
AA ATA AAT AGA CCC GAT TAT TTG AAC TTC CGG GAG AGT GGT CAG GTT T
AC TTC GGA ATC ATCGCC CTG GGA GGG GGG GGT TCT AGC TCA AGG ACA CC
A AGC GAT AAA CCA GTG GCA CAT GTG GTC GCT AAT CCC CAA GCA GAG GG
GCAA CTT CAG TGG TTG AAC CGC CGG GCT AAT GCA CTG CTC GCA AAC GGT
GTA GAG TTG AGG GAC AAT CAA CTC GTT GTA CCA AGTGAG GGC TTG TAT
CTC ATA TAC AGC CAG GTG CTT TTT AAA GGC CAG GGG TGT CCC AGT ACA
CAC GTG TTG CTC ACC CAC ACA ATATCA AGA ATA GCA GTC TCA TAC CAA A
CT AAG GTT AAT CTC CTC TCA GCA ATT AAA TCC CCT TGT CAG CGG GAG A
CC CCC GAA GGCGCT GAG GCT AAG CCC TGG TAC GAA CCC ATC TAT CTT GG
T GGG GTT TTT CAA CTG GAG AAA GGC GAT CGA TTG TCA GCC GAG ATTAAT
CGC CCA GAT TAC CTG AAC TTT CGC GAA TCC GGA CAG GTA TAC TTC GCC
ATT ATC GCA TTG GGT GGC GGT GGC AGC AGC AGTAGG ACT CCT AGC GAT
AAA CCC GTT GCT CAT GTT GTT GCA AAC CCA CAG GCA GAA GGG CAG CTC
CAA TGG CTC AAT CGG CGC GCAAAC GCA TTG CTG GCC AAC GGA GTA GAG C
TG CGG GAC AAC CAA CTT GTT GTT CCC AGC GAA GGT CTT TAC CTC ATT T
AT TCC CAAGTC CTT TTC AAG GGC CAA GGC TGT CCA AGT ACA CAC GTA CT
T CTT ACT CAC ACA ATA AGT CGC ATA GCA GTC TCT TAC CAA ACAAAA GTC
AAT CTC CTG TCT GCA ATT AAA TCC CCA TGT CAA AGA GAA ACC CCA GAA
GGG GCA GAG GCC AAG CCT TGG TAT GAG CCTATC TAT TTG GGC GGG GTT
TTC CAA CTT GAG AAG GGA GAC CGG CTT TCA GCT GAA ATC AAC AGG CCC
GAT TAT CTC AAC TTC AGGGAG AGT GGA CAA GTC TAC TTC GGA ATT ATA G
CC CTG

SEQ ID NO:134 - Codon optimized nucleic acid sequence encoding SEQ ID NO 114

GAA AGG AAG TCA AGC GTG GAA TGC CCT CCC TGT CCA CCA CCA CCC CTC
GCT GGA CCC AGC GTG TTC CTG TTC CCA CCC AAA CCCAAG GAT ACT CTC A
TG ATC AGC CGG ACA CCA GAG GTA ACT TGT GTA GTA GTA GAT GTT AGC C
AT GAG GAT CCT GAG GTG CAG TTTAAT TGG TAC GTT GAC GGG GTG GAG GT
A CAT AAC GCA AAA ACC AAA CCA CGA GAG GAG CAG TTC AAC AGC ACC TT

T CGC GTA GTGTCA GTC CTG ACC GTA GTC CAC CAG GAC TGG TTG AAC GGT
AAG GAA TAC AAG TGT AAG GTT TCC AAC AAG GGT CTG CCT GCC TCTATC
GAG AAA ACA ATA AGC AAG ACA AAA GGC CAA CCT CGG GAA CCT CAG GTA
TAT ACA CTT CCC CCA AGT CGA GAG GAG ATG ACTAAG AAC CAG GTA AGC C
TT ACT TGC CTG GTA AAA GGT TTT TAT CCC AGC GAC ATC GCC GTC GAA T
GG GAA TCC AAT GGA CAG CCTGAG AAT AAC TAT AAG ACA ACC CCC CCT AT
G CTG GAT TCA GAC GGT AGC TTC TTT CTT TAT TCC AAA CTT ACC GTG GA
T AAA TCAAGG TGG CAG CAA GGG AAT GTT TTC TCT TGT AGT GTC ATG CAC
GAA GCC CTT CAC AAC CAT TAC ACT CAG AAA TCC CTC AGC TTGTCA CCT
GGA AAA GGG GGC GGC GGA AGT GTC CGA TCC TCC TCT CGG ACC CCA TCT
GAC AAG CCA GTT GCC CAT GTG GTG GCT AATCCA CAG GCT GAG GGG CAA C
TC CAG TGG CTG AAT AGG AGA GCT AAT GCT CTC CTT GCT AAT GGA GTT G
AA CTT AGA GAC AAT CAGCTT GTC GTC CCC TCT GAA GGG CTC TAT TTG AT
A TAC AGC CAG GTT CTT TTT AAG GGT CAG GGC TGT CCC TCC ACT CAT GT
G CTTCTC ACA CAC ACA ATC AGC CGC ATC GCA GTG AGT TAT CAA ACC AAA
GTT AAC CTG CTT TCC GCA ATC AAA AGC CCT TGT CAG AGAGAA ACC CCA
GAA GGA GCA GAA GCC AAA CCC TGG TAT GAG CCC ATC TAT CTC GGA GGA
GTA TTC CAA CTG GAA AAG GGT GAT AGGTTG AGC GCT GAG ATA AAT AGA C
CC GAC TAT CTG AAC TTC AGG GAG AGT GGT CAA GTA TAC TTT GGC ATT A
TT GCC CTC GGC GGCGGC GGC AGT TCC AGT CGG ACA CCC TCA GAT AAG CC
A GTT GCT CAC GTT GTG GCC AAC CCC CAA GCC GAA GGC CAG TTG CAG TG
GTTG AAT AGG CGG GCT AAT GCT CTG CTG GCA AAC GGT GTA GAA CTT CGA
GAT AAT CAA CTC GTT GTG CCC TCA GAG GGA CTC TATCTC ATT TAC AGC
CAG GTG CTT TTC AAA GGG CAG GGG TGT CCC TCT ACA CAT GTC CTT CTG
ACA CAT ACA ATC TCA CGA ATA GCTGTC TCC TAC CAA ACA AAA GTT AAT T
TG CTC AGT GCT ATA AAA TCC CCT TGC CAG CGG GAG ACA CCT GAG GGG G
CT GAG GCC AAACCT TGG TAC GAG CCT ATC TAT CTC GGC GGG GTA TTC CA
A CTT GAA AAA GGG GAC AGA CTT AGT GCC GAA ATA AAC CGC CCA GACTAC
CTT AAC TTC CGC GAG TCC GGG CAG GTT TAC TTT GGG ATA ATC GCA CTG
GGG GGA GGT GGA TCT TCA TCT AGA ACC CCA AGCGAC AAA CCA GTT GCT
CAT GTG GTC GCC AAT CCT CAA GCT GAA GGA CAG CTT CAA TGG CTT AAT
CGC CGG GCA AAC GCC CTT TTGGCA AAT GGC GTT GAG CTG CGG GAT AAT C
AA CTG GTA GTT CCA AGT GAG GGC TTG TAC TTG ATC TAT AGT CAA GTA C
TG TTC AAGGGC CAA GGC TGC CCA TCT ACA CAC GTT CTT TTG ACC CAC AC
T ATT TCA AGG ATT GCC GTC AGC TAT CAA ACT AAA GTG AAC CTCCTG TCT
GCT ATC AAG TCA CCC TGT CAA CGA GAA ACC CCT GAG GGT GCT GAA GCC
AAG CCC TGG TAT GAG CCC ATA TAT CTC GGCGGA GTC TTT CAA CTG GAG
AAG GGT GAC AGG CTG TCT GCC GAA ATC AAT CGG CCT GAC TAT CTG AAC
TTT CGG GAG AGC GGC CAGGTC TAC TTC GGC ATT ATT GCT CTC

SEQ ID NO:135 - Codon optimized nucleic acid sequence encoding SEQ ID NO 115

GTC AGG AGT AGC TCT AGG ACC CCA TCC GAT AAG CCC GTC GCA CAT GTG
GTG GCC AAC CCC CAG GCA GAA GGC CAA CTC CAG TGGCTT AAT AGA CGA G
CC AAT GCC CTT TTG GCT AAT GGC GTC GAG CTC AGG GAC AAT CAA CTT G

TG GTG CCT AGT GAG GGA CTC TATTTG ATT TAT AGC CAA GTA CTT TTC AA
G GGA CAG GGT TGT CCA TCT ACA CAC GTG CTT CTT ACC CAC ACT ATT TC
T CGG ATC GCAGTT TCT TAT CAA ACC AAA GTC AAC CTT TTG TCC GCT ATC
AAG AGT CCA TGT CAG AGA GAG ACA CCC GAG GGC GCT GAA GCT AAGCCC
TGG TAT GAG CCA ATC TAT CTT GGG GGA GTT TTC CAG CTC GAA AAA GGG
GAC CGG CTG TCT GCC GAA ATT AAC CGC CCT GACTAC CTC AAC TTT AGG G
AG AGT GGT CAG GTG TAT TTC GGA ATA ATC GCC TTG GGC GGT GGC GGG T
CA TCT AGC AGA ACC CCA TCCGAC AAG CCA GTC GCC CAT GTA GTG GCC AA
T CCA CAG GCA GAG GGA CAA TTG CAG TGG TTG AAT CGG CGA GCC AAT GC
A TTG CTCGCA AAC GGG GTG GAG CTC CGC GAT AAC CAG CTT GTA GTG CCA
TCC GAA GGA TTG TAT TTG ATT TAT TCT CAA GTG CTG TTC AAAGGA CAA
GGG TGC CCA TCT ACC CAT GTC TTG CTG ACA CAC ACA ATT TCC CGG ATC
GCT GTA TCC TAC CAA ACC AAG GTG AAT CTTTTG TCA GCA ATC AAA AGC C
CA TGT CAA CGC GAA ACA CCA GAG GGA GCA GAG GCC AAG CCT TGG TAC G
AG CCT ATT TAC CTG GGCGGT GTC TTT CAA CTT GAG AAG GGA GAT CGC TT
G AGC GCA GAA ATT AAT AGG CCT GAC TAC CTT AAC TTT AGG GAA AGT GG
A CAGGTA TAT TTT GGA ATA ATT GCA CTC GGT GGT GGG GGA TCA TCA AGC
CGC ACA CCT TCC GAT AAA CCC GTT GCC CAC GTA GTG GCAAAT CCC CAG
GCC GAA GGC CAA TTG CAA TGG CTG AAC CGA AGA GCC AAC GCT CTT CTC
GCA AAT GGT GTA GAA CTC CGG GAT AACCAG TTG GTG GTG CCC AGC GAG G
GC CTT TAT CTC ATA TAC TCT CAA GTC CTT TTC AAA GGG CAG GGA TGT C
CT AGT ACC CAT GTACTT CTC ACT CAC ACA ATC TCC AGG ATC GCC GTT TC
A TAT CAA ACA AAA GTT AAT TTG CTC AGC GCT ATA AAG AGT CCA TGC CA
ACGC GAA ACA CCT GAG GGG GCC GAA GCA AAA CCT TGG TAC GAG CCT ATT
TAT CTT GGT GGA GTA TTC CAA CTT GAA AAA GGT GACAGG TTG TCA GCT
GAG ATT AAT AGA CCA GAT TAT CTG AAT TTT CGC GAA TCT GGG CAC CTT
TAC TTC GGG ATA ATC GCT CTG GGAGGA GGA GGG AGT GAA CCA AAG TCC A
GC GAT AAA ACT CAT ACC TGT CCA CCT TGT CCA GCC CCC GAA GCT GCA G
GA GGC CCT AGCGTG TTC TTG TTT CCT CCC AAA CCC AAA GAC ACA TTG AT
G ATT AGT CGC ACT CCT GAA GTG ACA TGT GTT GTC GTA GAC GTA TCTCAT
GAA GAC CCC GAA GTC AAG TTT AAC TGG TAT GTC GAT GGA GTG GAG GTG
CAC AAT GCA AAG ACT AAG CCT AGG GAA GAA CAATAT AAC AGT ACC TAC
AGA GTT GTG TCA GTG CTT ACC GTC TTG CAT CAG GAT TGG CTC AAT GGA
AAA GAG TAT AAG TGT AAG GTAAGT AAC AAG GCA TTG CCC GCT AGC ATA G
AG AAA ACA ATA AGC AAG GCA AAG GGG CAG CCC AGG GAA CCC CAA GTC T
AT ACC CTTCCA CCA AGT CGG GAT GAA CTG ACT AAA AAT CAG GTG TCC TT
G ACT TGC CTT GTA AAG GGA TTC TAC CCC TCA GAT ATC GCA GTGGAG TGG
GAG AGC AAC GGA CAG CCA GAA AAC AAT TAC AAA ACC ACC CCC CCT GTC
CTG GAT TCA GAC GGT TCT TTC TTT TTG TACTCC AAA CTT ACA GTA GAC
AAG TCC AGG TGG CAA CAA GGC AAT GTC TTT AGC TGT TCT GTC ATG CAC
GAA GCC CTT CAC AAC CACTAT ACT CAA AAG TCA CTT TCT CTT TCC CCT G
GA AAA

SEQ ID NO:136 - Codon optimized nucleic acid sequence encoding SEQ ID NO 116

GTA AGA TCA TCT AGT CGG ACT CCA TCA GAC AAA CCA GTA GCC CAT GTT
GTT GCA AAC CCA CAA GCC GAG GGT CAA CTT CAG TGGCTC AAT AGG CGC G
CC AAT GCA CTG CTC GCT AAT GGA GTC GAA TTG CGC GAT AAC CAA TTG G
TG GTA CCT AGT GAG GGA CTT TATTTG ATC TAT AGT CAG GTG CTC TTT AA
A GGT CAG GGT TGC CCC TCC ACC CAC GTT CTC CTG ACA CAT ACC ATT AG
C AGG ATA GCTGTA AGT TAC CAG ACT AAA GTC AAC CTC CTT AGC GCT ATC
AAA AGT CCA TGT CAA AGA GAA ACT CCA GAA GGA GCA GAA GCC AAACCT
TGG TAC GAG CCT ATC TAC CTC GGA GGA GTA TTT CAG CTT GAA AAG GGG
GAT CGA CTG AGC GCC GAA ATC AAC AGA CCC GATTAC CTT AAC TTC CGA G
AA TCC GGC CAA GTA TAC TTC GGG ATT ATT GCC CTT GGG GGA GGT GGC T
CT TCA AGC AGA ACC CCA TCAGAC AAG CCA GTG GCT CAC GTC GTT GCC AA
T CCC CAA GCT GAA GGG CAA CTT CAA TGG CTT AAT CGA ACG GCT AAT GC
A CTT TTGGCC AAC GGT GTA GAA CTC CGA GAC AAC CAA TTG GTC GTG CCA
TCA GAA GGC CTT TAC CTC ATA TAC TCC CAG GTT CTT TTC AAGGGT CAG
GGA TGT CCT AGT ACA CAC GTA TTG TTG ACC CAT ACA ATT TCA AGG ATA
GCA GTA AGC TAC CAG ACT AAA GTT AAT CTGCTT AGT GCT ATA AAG TCT C
CT TGT CAG CGA GAG ACA CCC GAA GGC GCT GAG GCA AAA CCC TGG TAC G
AG CCC ATC TAC CTC GGGGGT GTT TTT CAA CTG GAG AAG GGA GAC CGA CT
G TCC GCC GAA ATT AAC CGG CCC GAC TAC CTC AAT TTT CGC GAA TCC GG
G CAAGTT TAT TTT GGT ATC ATT GCA TTG GGT GGT GGA GGC TCC AGT AGC
CGG ACT CCC TCC GAT AAA CCA GTG GCA CAT GTA GTC GCCAAC CCT CAA
GCA GAA GGG CAA TTG CAG TGG CTG AAT AGA CGC GCC AAT GCC CTC CTG
GCT AAT GGC GTA GAG CTT AGA GAT AATCAA TTG GTG GTG CCT AGT GAA G
GT CTG TAC CTC ATT TAC TCT CAG GTT CTC TTT AAG GGC CAA GGA TGT C
CC TCA ACT CAC GTACTG CTG ACT CAT ACT ATA TCA CGG ATA GCC GTC TC
T TAC CAG ACA AAA GTG AAT TTG CTG TCA GCC ATC AAG AGT CCA TGC CA
GCGA GAA ACC CCT GAG GGG GCT GAA GCT AAA CCA TGG TAT GAA CCA ATC
TAC CTT GGT GGC GTT TTC CAG CTC GAG AAG GGC GATAGA CTT AGC GCC
GAA ATT AAT CGA CCA GAC TAT CTC AAT TTT AGA GAG TCA GGA CAA GTG
TAC TTT GGT ATT ATA GCC TTG GGTGGG GGC GGT TCT GAA CGG AAA AGT T
CT GTT GAA TGC CCT CCA TGT CCT GCC CCC CCT GTG GCC GGT CCC TCA G
TC TTT CTC TTCCCA CCC AAG CCC AAA GAT ACA TTG ATG ATT AGT AGG AC
T CCC GAG GTG ACT TGC GTA GTT GTC GAT GTT TCC CAT GAA GAT CCAGAA
GTG CAA TTT AAC TGG TAT GTA GAC GGC GTC GAG GTC CAT AAT GCT AAA
ACT AAG CCC CGC GAG GAG CAG TTT AAT TCA ACCTTT AGA GTT GTG AGC
GTT CTG ACC GTT GTA CAC CAG GAT TGG CTT AAT GGT AAA GAG TAC AAG
TGC AAG GTG TCC AAC AAG GGACTT CCA GCA TCC ATT GAA AAG ACC ATT T
CC AAG ACT AAA GGG CAA CCA CGG GAA CCA CAA GTC TAC ACC CTC CCA C
CC AGC CGCGAA GAG ATG ACT AAA AAT CAG GTA TCA CTT ACT TGC CTG GT
T AAG GGT TTC TAC CCA TCT GAC ATT GCT GTC GAG TGG GAA TCTAAT GGG
CAA CCT GAA AAC AAT TAC AAG ACA ACA CCA CCT ATG CTG GAT TCC GAT
GGG AGT TTC TTC CTG TAC AGT AAA CTC ACTGTT GAC AAG TCC CGA TGG

CAG CAG GGA AAT GTC TTT TCA TGC TCC GTT ATG CAT GAG GCC CTC CAC AAC CAT TAT ACC CAA AAGTCT CTG TCC CTG TCA CCA GGA AAG

SEQ ID NO:137 - Codon optimized nucleic acid sequence encoding SEQ ID NO 117

GAA TCA AAG TAC GGT CCA CCT TGT CCT CCC TGT CCC GCC CCC GAG TTT CTG GGG GGT CCC TCT GTC TTT CTG TTT CCA CCA AAGCCC AAG GAC ACT C TG ATG ATT AGC AGA ACA CCA GAA GTA ACC TGT GTC GTC GTG GAT GTC T CA CAG GAG GAT CCC GAG GTA CAGTTC AAC TGG TAC GTG GAT GGT GTA GA G GTG CAT AAT GCA AAG ACT AAA CCA AGG GAA GAA CAA TTC AAT TCT AC T TAC CGG GTCGTA TCT GTC TTG ACC GTG CTT CAC CAA GAT TGG CTG AAC GGC AAG GAG TAT AAA TGT AAA GTT TCT AAT AAG GGG CTC CCA TCAAGT ATC GAG AAA ACC ATT TCA AAA GCA AAA GGG CAA CCT CGA GAG CCT CAA GTT TAC ACA CTC CCT CCA TCA CAA GAA GAA ATGACA AAG AAT CAA GTC A GC CTC ACC TGC CTT GTA AAG GGC TTC TAT CCC TCC GAC ATT GCA GTG G AA TGG GAG TCA AAC GGA CAACCT GAG AAT AAT TAT AAG ACC ACA CCT CC A GTG CTG GAC TCA GAT GGG TCA TTT TTC CTG TAC TCC CGC TTG ACC GT G GAC AAGTCT CGA TGG CAG GAA GGT AAT GTG TTC AGC TGT AGT GTG ATG CAC GAA GCA CTG CAC AAC CAT TAT ACC CAG AAA TCC CTG TCATTG TCC CTC GGT AAG GTG AGA TCC AGT AGC CGC ACA CCA AGT GAT AAA CCT GTA GCC CAC GTA GTG GCA AAT CCA CAA GCT GAAGGG CAG CTC CAG TGG CTG A AT CGC CGC GCA AAC GCA CTG CTG GCA AAT GGG GTA GAG CTT AGG GAC A AT CAG CTC GTA GTG CCCAGT GAA GGC CTC TAT CTC ATT TAT TCA CAA GT A CTT TTC AAA GGC CAG GGA TGC CCT AGT ACC CAT GTC CTT TTG ACA CA C ACCATC TCC CGA ATA GCC GTA AGC TAC CAA ACT AAG GTT AAT CTC CTT AGC GCA ATC AAA TCT CCT TGC CAA AGG GAA ACC CCC GAAGGC GCC GAA GCC AAG CCC TGG TAT GAA CCT ATA TAC CTT GGC GGG GTT TTT CAG CTG GAA AAG GGA GAC AGG TTG AGT GCC GAGATT AAT CGA CCA GAC TAC CTT A AT TTT AGA GAG TCC GGC CAG GTC TAT TTC GGG ATA ATC GCT CTG TCT T CT AGA ACT CCC AGTGAT AAA CCC GTT GCC CAC GTG GTG GCC AAC CCA CA G GCC GAA GGG CAA CTG CAG TGG CTG AAC AGA CGA GCA AAT GCA TTG TT GGCC AAC GGT GTT GAA CTG CGC GAC AAC CAA CTT GTG GTG CCT AGT GAG GGT CTC TAC TTG ATT TAT TCC CAA GTC CTC TTT AAAGGG CAA GGG TGT CCC TCT ACT CAT GTC CTG CTC ACT CAC ACC ATC TCC AGA ATT GCA GTA TCT TAT CAG ACA AAA GTA AAC TTGCTG TCA GCC ATT AAA TCA CCA TGT C AG AGG GAG ACA CCT GAA GGT GCA GAA GCT AAG CCT TGG TAT GAA CCT A TT TAT CTC GGCGGG GTG TTC CAA TTG GAG AAA GGG GAC CGA CTG AGC GC T GAA ATC AAT AGA CCC GAT TAT TTG AAC TTT AGA GAG AGT GGC CAGGTA TAC TTC GGT ATA ATA GCC CTG TCC AGT CGA ACT CCT TCT GAT AAG CCT GTC GCA CAT GTT GTG GCA AAT CCT CAA GCT GAGGGA CAG CTC CAA TGG TTG AAT AGA CGC GCC AAC GCA CTC CTC CCT AAC GGG GTT GAG CTC CGA GAC AAT CAG CTT GTC GTC CCAAGC GAG GGG CTG TAC CTT ATT TAC TCC C AG GTA TTG TTT AAG GGA CAG GGT TGC CCC TCC ACA CAT GTG CTC CTG A CC CAC ACTATC AGC CGA ATA GCC GTT AGC TAT CAA ACA AAG GTC AAT CT C CTG AGT GCA ATA AAG TCT CCT TGT CAG CGA GAA ACC CCC GAAGGC GCC

GAG GCC AAG CCC TGG TAC GAG CCA ATT TAC CTC GGT GGA GTC TTT CAG
TTG GAG AAG GGG GAT AGA TTG AGC GCA GAAATT AAC CGA CCT GAC TAT
TTG AAC TTC AGA GAA AGC GGA CAA GTC TAT TTT GGT ATC ATC GCC CTG

SEQ ID NO:138 - Codon optimized nucleic acid sequence encoding SEQ ID NO 118

GAA TCA AAG TAC GGC CCT CCA TGT CCA CCC TGT CCT GCC CCT GAG TTT
CTC GGA GGA CCC AGT GTA TTC CTC TTC CCA CCA AAACCC AAG GAT ACC C
TC ATG ATC AGC AGG ACT CCC GAA GTT ACA TGC GTT GTC GTA GAC GTA T
CA CAG GAA GAT CCT GAG GTC CAATTT AAT TGG TAC GTC GAC GGA GTC GA
A GTT CAT AAC GCC AAA ACA AAA CCA CGA GAA GAG CAA TTT AAC AGT AC
A TAT CGC GTGGTC TCA GTG CTG ACC GTG CTC CAC CAG GAC TGG CTC AAT
GGG AAA GAA TAC AAA TGT AAG GTT TCC AAT AAG GGA CTC CCT AGCTCA
ATA GAA AAG ACC ATT TCA AAA GCT AAA GGC CAA CCC CGG GAG CCC CAA
GTC TAC ACC CTT CCC CCC TCT CAG GAA GAA ATGACC AAA AAT CAG GTG T
CC CTG ACC TGT CTT GTG AAA GGG TTT TAT CCC TCA GAC ATT GCC GTA G
AG TGG GAA TCA AAT GGA CAACCC GAG AAC AAC TAT AAA ACT ACT CCA CC
T GTT CTG GAC TCC GAT GGT TCC TTT TTC CTG TAC AGC CGC CTT ACC GT
T GAC AAATCA CGA TGG CAG GAA GGG AAT GTC TTC AGT TGT TCA GTA ATG
CAT GAA GCT CTC CAT AAC CAC TAT ACT CAG AAG TCC CTG TCCCTC TCT
CTG GGC AAG GGC GGC GGT GGT TCC GTC CGC AGT TCT TCT CGG ACT CCC
TCC GAC AAG CCA GTC GCA CAT GTA GTC GCCAAC CCA CAA GCA GAG GGA C
AG CTT CAG TGG CTC AAT CGA AGA GCA AAC GCC CTC CTT GCA AAC GGC G
TC GAA CTT CGC GAC AACCAA CTG GTT GTT CCA TCA GAA GGC TTG TAT CT
G ATC TAC TCT CAG GTG CTG TTT AAG GGA CAG GGA TGT CCT AGC ACA CA
T GTGCTC CTT ACT CAT ACA ATT TCA AGG ATC GCA GTA AGC TAC CAA ACT
AAA GTG AAC CTC CTT AGC GCC ATA AAG TCC CCA TGC CAAAGG GAG ACA
CCC GAG GGA GCA GAA GCA AAG CCA TGG TAT GAA CCT ATC TAT CTC GGT
GGA GTT TTC CAG TTG GAG AAA GGT GATAGA CTC TCT GCT GAG ATC AAT C
GC CCC GAC TAT CTG AAT TTC CGC GAA TCT GGG CAG GTC TAC TTT GGG A
TA ATA GCA CTG GGTGGC GGT GGA TCT CCC GTA GCT CAC GTG GTC GCT AA
C CCA CAG GCT GAG GGG CAA TTG CAA TGG TTG AAC CGG CGG GCT AAT GC
TTTG TTG GCA AAC GGC GTA GAA TTG AGA GAC AAC CAA TTG GTC GTT CCT
TCA GAA GGA TTG TAT CTC ATC TAC AGC CAA GTC TTGTTT AAA GGC CAA
GGC TGT CCA TCT ACA CAC GTG CTT CTT ACT CAC ACA ATC TCA CGA ATC
GCA GTA TCT TAT CAG ACC AAA GTGAAC TTG CTC TCT GCA ATA AAA AGC C
CT TGT CAA CGC GAA ACT CCA GAA GGG GCT GAA GCA AAG CCA TGG TAC G
AA CCT ATT TATCTC GGG GGG GTG TTC CAA CTC GAG AAA GGG GAC CGA CT
G TCC GCT GAA ATC AAC CGC CCT GAC TAT CTT AAT TTC CGG GAG TCTGGG
CAG GTA TAT TTC GGT ATA ATT GCA CTT GGA GGC GGG GGG TCA CCT GTG
GCA CAT GTA GTC GCC AAC CCC CAA GCT GAA GGACAA CTT CAA TGG CTC
AAT AGG CGC GCA AAT GCT CTG CTC GCA AAT GGA GTA GAA CTC CGG GAT
AAT CAA CTG GTT GTG CCT TCTGAA GGA CTG TAT CTG ATC TAT AGC CAA G
TT TTG TTC AAG GGC CAG GGG TGC CCA TCT ACA CAC GTA CTT CTT ACC C
AC ACA ATATCC CGC ATC GCC GTC AGT TAT CAG ACA AAA GTG AAC CTT TT

G TCC GCC ATC AAG AGC CCA TGT CAG CGC GAA ACT CCC GAG GGTGCT GAG
GCT AAA CCA TGG TAT GAG CCC ATC TAT TTG GGA GGC GTA TTT CAA CTG
GAA AAA GGG GAT CGA CTG AGC GCA GAG ATCAAT AGG CCC GAT TAT CTT
AAT TTC AGG GAG TCT GGT CAA GTG TAT TTT GGG ATA ATT GCT CTG

SEQ ID NO:139 - Codon optimized nucleic acid sequence encoding SEQ ID NO 119

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CTG GGC GGA CCT TCC GTT TTC TTG TTC CCA CCA AAGCCA AAA GAT ACT C
TG ATG ATT TCC AGG ACC CCT GAA GTT ACC TGT GTG GTA GTG GAT GTC A
GC CAG GAG GAT CCA GAA GTT CAATTT AAT TGG TAT GTA GAC GGA GTC GA
A GTC CAT AAC GCT AAA ACT AAA CCT CGA GAA GAG CAG TTT AAT TCA AC
C TAC AGG GTTGTT TCC GTA CTG ACA GTT TTG CAT CAG GAC TGG CTG AAT
GGC AAG GAA TAC AAA TGC AAG GTC AGC AAC AAA GGA CTC CCA AGTTCA
ATA GAA AAG ACC ATT TCA AAA GCT AAA GGG CAA CCA CGA GAA CCT CAG
GTC TAC ACT CTC CCT CCC TCT CAG GAA GAG ATGACT AAA AAT CAG GTT T
CA CTT ACA TGC CTC GTG AAG GGC TTT TAC CCC AGC GAC ATT GCT GTT G
AG TGG GAG AGT AAC GGA CAACCT GAG AAC AAC TAC AAG ACT ACA CCT CC
T GTG CTG GAC TCA GAT GGT TCC TTC TTT TTG TAT AGC AGG CTT ACC GT
T GAT AAGTCC CGC TGG CAA GAA GGC AAC GTT TTC AGT TGT TCA GTA ATG
CAC GAA GCT CTC CAC AAT CAT TAT ACA CAG AAG AGT CTT AGCCTG TCC
CTG GGT AAG GGA GGC GGG GGG TCC GGG GGC GGG GGC TCA GTT CGC TCA
TCA AGC CGA ACA CCC TCA GAC AAG CCA GTTGCC CAC GTC GTA GCC AAC C
CC CAA GCT GAA GGA CAG TTG CAA TGG CTG AAT AGG CGA GCT AAT GCA T
TG TTG GCA AAT GGA GTAGAA CTG CGC GAT AAT CAA TTG GTT GTG CCC TC
A GAA GGG CTG TAC CTT ATT TAC TCC CAG GTG CTC TTC AAA GGG CAG GG
T TGCCCT TCA ACC CAC GTA CTT CTT ACC CAC ACA ATA AGC AGG ATT GCC
GTC TCC TAC CAA ACT AAA GTA AAC CTG TTG AGC GCT ATCAAG AGT CCT
TGC CAA CGG GAG ACC CCT GAA GGT GCA GAG GCA AAA CCA TGG TAC GAA
CCC ATT TAT CTC GGA GGG GTG TTC CAGTTG GAG AAG GGG GAC CGC CTG T
CT GCC GAA ATC AAT AGG CCA GAC TAC CTC AAC TTT CGC GAG TCC GGG C
AG GTG TAT TTT GGGATC ATA GCT TTG GGC GGT GGG GGA TCT CCT GTT GC
T CAT GTC GTT GCA AAC CCT CAG GCT GAA GGC CAA TTG CAA TGG CTC AA
CAGG AGA GCT AAC GCA TTG CTG GCC AAC GGG GTT GAG CTC CGC GAT AAC
CAG CTG GTA GTT CCC TCA GAG GGC TTG TAC CTT ATCTAT TCA CAG GTT
CTC TTC AAA GGA CAA GGA TGT CCT AGC ACA CAC GTC TTG CTT ACA CAT
ACC ATT AGC CGG ATA GCA GTT TCTTAT CAG ACT AAA GTT AAT CTC CTC T
CT GCC ATA AAG TCA CCC TGT CAG CGG GAA ACA CCT GAG GGT GCT GAA G
CA AAA CCT TGGTAT GAA CCA ATA TAC CTC GGT GGA GTT TTT CAA CTG GA
G AAG GGC GAC AGA CTG AGC GCT GAA ATA AAC AGA CCT GAC TAC CTTAAT
TTC CGA GAA TCA CCT CAA CTA TAC TTC GGG ATT ATA GCC TTG GGG GGT
GGA GGC TCC CCA GTG GCT CAT GTA GTC GCT AATCCC CAA GCT GAA GGC
CAA CTC CAA TGG CTT AAC AGG AGG GCC AAC GCA CTC CTC GCA AAT GGA
GTC GAG CTT AGG GAT AAT CAATTG GTG GTT CCC TCT GAG GGC TTG TAT C
TT ATT TAT TCA CAG GTC CTG TTT AAA GGC CAA GGC TGT CCT TCT ACA C

AT GTC CTGTTG ACT CAT ACC ATA AGT AGA ATA GCC GTG AGT TAC CAG AC
A AAG GTT AAC CTG CTT TCC GCA ATC AAA TCT CCA TGC CAA CGCGAG ACC
CCA GAA GGG GCA GAA GCA AAG CCT TGG TAC GAG CCC ATA TAT CTC GGT
GGG GTC TTT CAG CTC GAG AAA GGC GAC CGGCTT AGC GCT GAA ATC AAC
CGC CCA GAC TAT TTG AAC TTT CGG GAA AGT GGA CAA GTC TAC TTC GGT
ATC ATA GCA CTC

SEQ ID NO:140 - Codon optimized nucleic acid sequence encoding SEQ ID NO 120

GAA TCT AAG TAT GGA CCT CCT TGT CCA CCA TGT CCA GCT CCC GAG TTC
CTG GGA GGC CCA TCC GTG TTT TTG TTC CCC CCT AAGCCA AAA GAC ACA C
TT ATG ATA TCA AGA ACC CCA GAA GTT ACT TGT GTA GTC GTG GAC GTA T
CC CAG GAA GAC CCC GAG GTT CAATTT AAC TGG TAT GTA GAC GGC GTG GA
A GTC CAT AAT GCT AAG ACA AAG CCC CGG GAG GAA CAA TTC AAC TCC AC
A TAC CGA GTAGTA TCC GTA TTG ACC GTG CTC CAT CAG GAT TGG TTG AAT
GGA AAG GAA TAC AAG TGC AAA GTT TCT AAT AAG GGC CTG CCT TCTAGC
ATC GAG AAG ACC ATC AGC AAG GCT AAG GGA CAG CCT CGC GAA CCC CAA
GTT TAT ACC CTT CCT CCT AGC CAA GAG GAG ATGACT AAA AAT CAG GTG T
CA CTC ACC TGC CTC GTC AAA GGA TTC TAC CCA TCA GAT ATA GCA GTG G
AA TGG GAG TCC AAC GGG CAACCT GAG AAT AAC TAC AAA ACA ACT CCA CC
T GTC CTG GAC TCC GAC GGC TCC TTC TTT CTT TAT TCC AGA CTT ACC GT
G GAC AAAAGC AGA TGG CAA GAG GGG AAT GTG TTT AGC TGC AGT GTT ATG
CAT GAA GCT TTG CAT AAT CAT TAC ACC CAA AAA TCA CTT TCACTC TCT
CTT GGT AAG GGG GGT GGG GGA TCT GGT GGG GGA GGC TCC GTG CGA TCA
AGC TCT AGG ACA CCC TCT GAT AAA CCT GTTGCC CAC GTC GTC GCA AAT C
CC CAG GCC GAA GGA CAG TTC CAG TGG CTG AAT CGA AGA GCT AAC GCA C
TG TTG GCA AAC GGG GTGGAG CTC AGG GAT AAC CAG TTG GTG GTG CCT TC
A GAA GGG CTT TAT CTC ATT TAC TCA CAA GTA CTC TTT AAA GGG CAA GG
G TGCCCA TCT ACT CAC GTG TTG CTG ACT CAC ACT ATT TCT CGA ATC GCA
GTT AGC TAT CAA ACC AAG GTA AAC TTG CTC AGT GCC ATAAAA AGT CCT
TGT CAA AGG GAG ACA CCC GAA GGA GCA GAA GCA AAG CCC TGG TAC GAG
CCC ATT TAC CTC GGT GGT GTC TTC CAGCTG GAG AAA GGA GAC CGG CTC T
CT GCA GAG ATA AAC AGA CCT GAC TAT CTC AAC TTT AGA GAA TCA GGC C
AG GTT TAT TTC GGGATC ATC GCA CTC TCC AGC CGG ACC CCC TCA GAC AA
G CCC GTT GCA CAC GTC GTT GCT AAC CCA CAA GCT GAA GGG CAG TTG CA
GTGG TTG AAT CGA AGA GCA AAC GCT CTC TTG GCC AAC GGT GTA GAA CTC
CGC GAC AAC CAA CTG GTT GTA CCT TCA GAA GGG CTCTAT CTG ATT TAC
TCT CAG GTG CTT TTC AAG GGC CAA GGG TGC CCT AGT ACA CAT GTT CTG
CTT ACC CAC ACA ATT TCT AGA ATTGCA GTT AGC TAC CAG ACT AAA GTC A
AC CTG TTG AGT GCT ATC AAG TCC CCT TGT CAG AGA GAA ACC CCA GAG G
GA GCT GAG GCTAAA CCT TGG TAT GAG CCC ATA TAC CTC GGT GGT GTA TT
C CAA TTG GAG AAA GGT GAT CGA TTG TCA GCT GAA ATC AAC AGA CCAGAC
TAT CTG AAT TTC AGA GAG TCA GGA CAA GTT TAC TTC GGC ATA ATC GCA
TTG AGT AGT CGG ACA CCC TCC GAT AAA CCT GTGGCA CAT GTT GTA GCT
AAC CCT CAA GCA GAG GGG CAG CTC CAA TGG CTG AAC CGG CGC GCT AAT

GCC CTG TTG GCT AAC GGC GTTGAG TTG CGA GAT AAC CAG CTG GTT GTG C
CC TCT GAA GGT CTG TAC TTG ATC TAC TCC CAA GTC CTG TTT AAG GGT C
AA GGC TGTCCC AGC ACA CAC GTG TTG CTC ACC CAC ACT ATC AGC CGG AT
T GCC GTA AGC TAT CAA ACT AAA GTC AAT CTT CTG TCC GCC ATCAAA AGT
CCA TGT CAG CGC GAA ACC CCT GAG GGT GCC GAA GCC AAG CCT TGG TAC
GAG CCA ATC TAC CTG GGT GGC GTC TTT CAGCTC GAA AAG GGG GAC CGG
CTC TCT GCA GAG ATA AAT CGC CCT GAT TAT CTT AAC TTT CGC GAG TCC
GGG CAG GTA TAC TTT GGGATT ATA GCT CTT

CLAIMS

1. A fusion protein comprising:

   a. a first TNF homology domain (THD) comprising D143N/A145R mutations, wherein the THD has at least 95% identity to SEQ ID NO: 3;

   b. a second THD comprising D143N/A145R mutations, wherein the THD has at least 95% identity to SEQ ID NO: 3;

   c. a third THD comprising D143N/A145R mutations, wherein the THD has at least 95% identity to SEQ ID NO: 3;

   d. an immunoglobulin Fc domain; and

   e. a first linker peptide covalently linking the first and second THDs and a second linker covalently linking the second and third THDs.

2. The fusion protein according to claim 1, wherein each of said linker peptides is from 1 to 31 amino acids in length.

3. The fusion protein according to claim 1 or 2, wherein at least one of said THDs comprises SEQ ID NO: 3.

4. The fusion protein according to claim 3, wherein each of said THDs comprises SEQ ID NO: 3.

5. The fusion protein according to claim 1, 2, 3 or 4, wherein said Fc domain is covalently linked to the N-terminus of the N-terminal THD or the C-terminus of the C-terminal THD.

6. The fusion protein according to claim 1, 2, 3, 4 or 5, wherein said Fc domain is covalently linked to said THD by a third peptide linker.

7. The fusion protein according to claim 1, 2, 3, 4, 5 or 6, wherein said third peptide linker is from 1-31 amino acids in length.

8. The fusion protein according to claim 1, 2, 3, 4, 5, 6 or 7, wherein said first, second or third linker is comprised of serine or glycine amino acids.

9. The fusion protein according to claim 1, 2, 3, 4, 5, 6, 7 or 8, wherein said first, second and third linkers are comprised of serine and glycine amino acids.

10. The fusion protein of claim 1, 2, 3, 4, 5, 6, 7, 8 or 9, wherein the immunoglobulin Fc domain is an IgG1, IgG2 or IgG4 immunoglobulin Fc domain.

11. The fusion protein of claim 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, wherein the IgG1 immunoglobulin Fc domain comprises an N297A mutation.

12. The fusion protein of claim 10, wherein the immunoglobulin Fc domain comprises is selected from the group consisting of SEQ ID NO:6, SEQ ID NO: 12 and SEQ ID NO:18.

13. The fusion protein of claim 10, wherein the immunoglobulin Fc domain comprises SEQ ID NO: 6.

14. The fusion protein of claim 10, wherein the fusion protein comprises SEQ ID NO: 31, SEQ ID NO:32, SEQ ID NO:34 or SEQ ID NO:35.

15. The fusion protein according to claim 10, wherein said Fc is an IgG4 Fc domain.

16. The fusion protein according to claim 15, wherein said Fc IgG4 Fc domain comprises the sequence as shown in SEQ ID NO:12.

17. The fusion protein according to claim 16, wherein said fusion protein comprises a sequence selected from the group consisting of 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 118, 119 and 120.

18. The fusion protein of claim 1, wherein the fusion protein selectively activates TNFR2 over TNFR1.

19. The fusion protein of claim 1, wherein upon administration to a subject, the fusion protein selectively activates a TNFR2 in the subject over TNFR1 in the subject.

20. The fusion protein of claim 1, wherein upon administration to a subject, the fusion protein preferentially activates T regulatory cells in the subject relative to conventional T cells in the subject.

21. The fusion protein of claim 1, wherein upon administration to a subject, the fusion protein increases myelination in said subject compared to control administration.

22. A pharmaceutical composition comprising the fusion protein of claim 1.

23. A nucleic acid encoding a fusion protein according to claim 1.

24. The nucleic acid according to claim 22, encoding the fusion protein according to claim 10.

25. The nucleic acid according to claim 23, comprising the sequence of SEQ ID NO: 36, SEQ ID NO:37, SEQ ID NO:38 or SEQ ID NO:39.

26. A cell comprising the nucleic acid according to claim 22.

27. A method of making a scTNFR2 agonist comprising culturing a cell according to claim 25 under conditions that express said nucleic acid and isolating said scTNFR2 agonist.

28. A method of increasing myelin deposition in a patient in need thereof comprising administering a fusion protein according to claim 1 to said patient.

29. A method of treating demyelinating disease in a patient in need thereof comprising administering a fusion protein according to claim 1 to said patient.

30. The method according to claim 28, wherein said demyelinating disease is optic neuritis or multiple sclerosis.

31. A method of treating pain in a patient in need thereof comprising administering a fusion protein according to claim 1 to said patient.

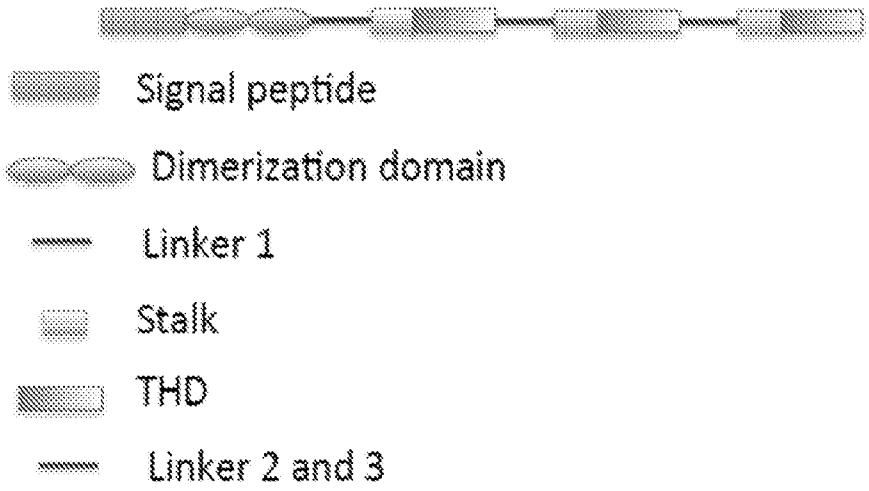

Figure 1A

Figure 1B

Figure 1C

Figure 1D

Figure 1E

Figure 1F

Figure 1G

MSTESMIRDVELAEEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTLFCLLHFGVIGPQR
EEFPRDLSLISPLAQAVRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLDFAESGQVYFGIIAL

Figure 2

VRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLDFAESGQVYFGIIAL

Figure 3

PVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLNFRESGQVYFGIIAL

Figure 4

VRSS SRTPSDK

Figure 5

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Human IgG1 Fc fusions with FcgR & C1Q knock-out
trimeric TNF at Fc N-terminus

Version 1
v1g1 EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
v1g1 KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELT
v1g1 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
v1g1 LHNHYTQKSLSLSPGK
C => S
L234A L235A P331S
v1g1 EPKSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
v1g1 KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAKGQPREPQVYTLPPSRDELT
v1g1 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
v1g1 LHNHYTQKSLSLSPGK

Version 2  with GGGGS between Fc and trimeric TNF
v2g1 GGGGSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
v2g1 KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELT
v2g1 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
v2g1 LHNHYTQKSLSLSPGK
C => S
L234A L235A P331S
v2g1 GGGGSEPKSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
v2g1 KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAKGQPREPQVYTLPPSRDELT
v2g1 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
v2g1 LHNHYTQKSLSLSPGK
```

Figure 6A

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Human IgG1 Fc fusions with FcgR & C1Q knock-out.
trimeric TNF at Fc C-terminus

Version 3
v3g1 EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
v3g1 KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELT
v3g1 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
v3g1 LHNHYTQKSLSLSPGKGGGGS) n=1-5
c =< 5
L234A L235A P329G
v3g1 EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
v3g1 KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELT
v3g1 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
v3g1 LHNHYTQKSLSLSPGKGGGGS) n=1-5
```

Figure 6B

```
Human IgG4 Fc fusions
Chimeric TNF at Fc N-terminus

Version 1
v104 ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMT
v104 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK
v104 LIMILTQKSLSLSLGK
v104 S => P
v104 ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMT
v104 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK
v104 LIMILTQKSLSLSLGK

Version 2 with GGGGS between Fc and trimeric TNF
v204 GGGGSESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMT
v204 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK
v204 LIMILTQKSLSLSLGK
v204 S => P
v204 GGGGSESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMT
v204 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK
v204 LIMILTQKSLSLSLGK
```

Figure 7A

```
Human IgG4 Fc fusions
trimeric TNF at Fc C-terminus

Version 3
v3g4    ESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNA
v3g4 KTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMT
v3g4 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEA
v3g4 LHNHYTQKSLSLSL(GGGGS) n=1~5
S => P
v3g4    ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNA
v3g4 KTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMT
v3g4 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEA
v3g4 LHNHYTQKSLSLSL(GGGGS) n=1~5
```

Figure 7B

```
Human IgG2 Fc fusions with C1q knock-out
trimeric TNF at Fc N-terminus

Version 1
v1g2      ERKSCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNA
v1g2 KTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEM
v1g2 TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
v1g2 EALHNHYTQKSLSLSPGK
C =>  S
P338
v1g2      ERKSVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNA
v1g2 KTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEM
v1g2 TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
v1g2 EALHNHYTQKSLSLSPGK

Version 2  with GGGGS between Fc and trimeric TNF
v1g2 GGGGSERKSCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNA
v1g2 KTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEM
v1g2 TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
v1g2 EALHNHYTQKSLSLSPGK
C =>  S
P338
v1g2 GGGGSERKSSVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNA
v1g2 KTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEM
v1g2 TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
v1g2 EALHNHYTQKSLSLSPGK
```

Figure 8A

```
Human IgG2 Fc fusions
trimeric TNF at Fc C-terminus

Version 3
vlg2    ERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNA
vlg2 KTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEM
vlg2 TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
vlg2 EALHNHYTQKSLSLSP(GGGGS) n=1-5
C => S
P331S
vlg2    ERKSSVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNA
vlg2 KTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPASIEKTISKTKGQPREPQVYTLPPSREEM
vlg2 TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
vlg2 EALHNHYTQKSLSLSP(GGGGS) n=1-5
```

Figure 8B

```
v3g1 EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
v3g1 KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELT
v3g1 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
v3g1 LHNHYTQKSLSLSP(ELAQLEESSAEAQDGELDG)
```

Figure 9

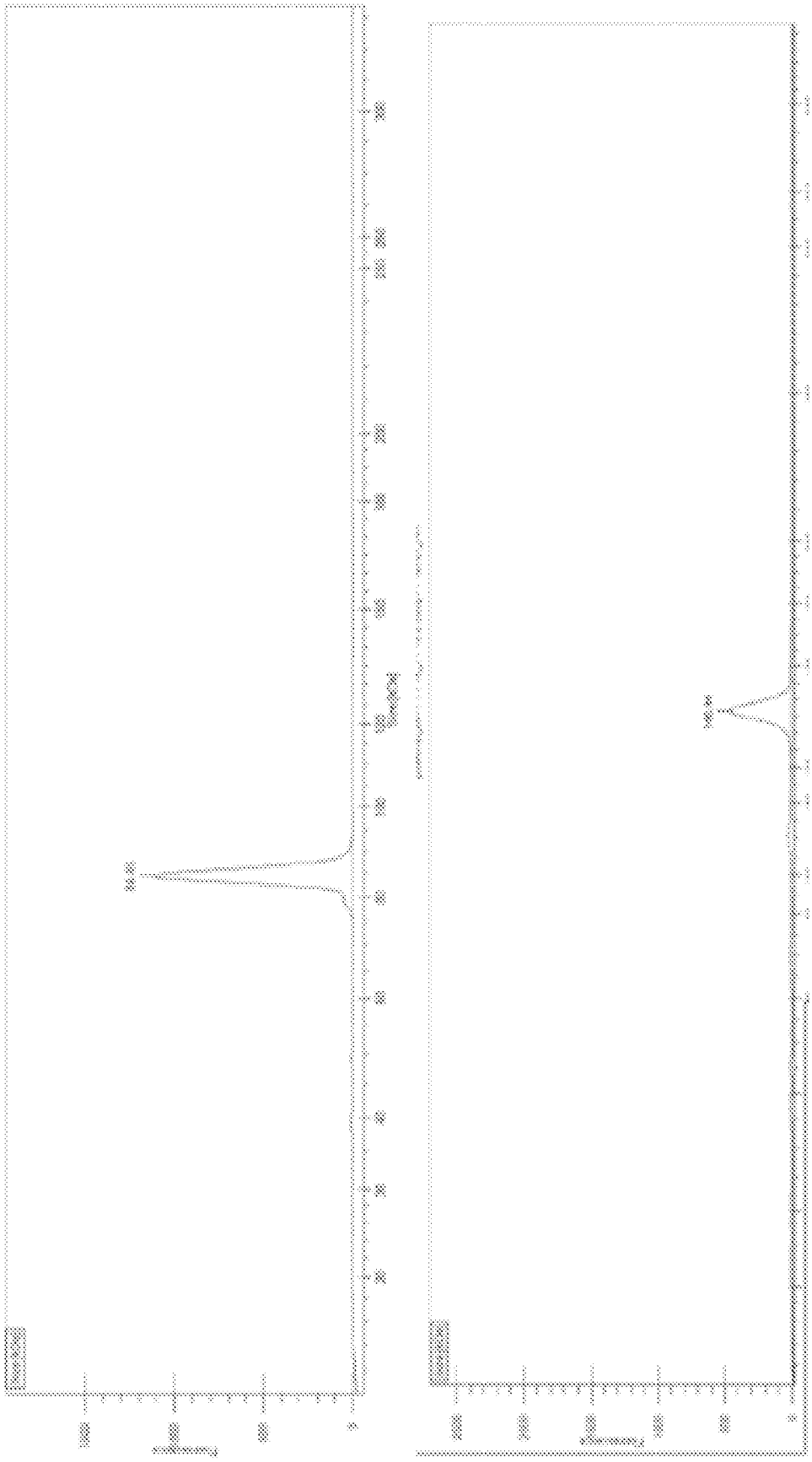

Figure 10A Figure 10B

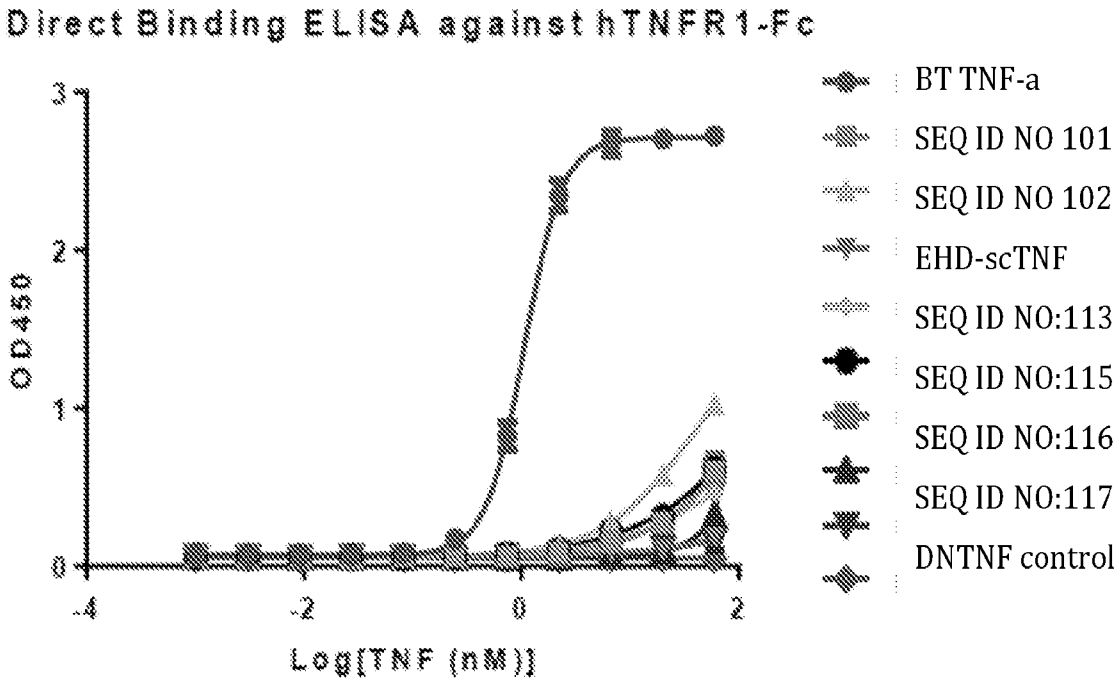

Figure 11A Binding of TNF Variants to immobilized TNFR1

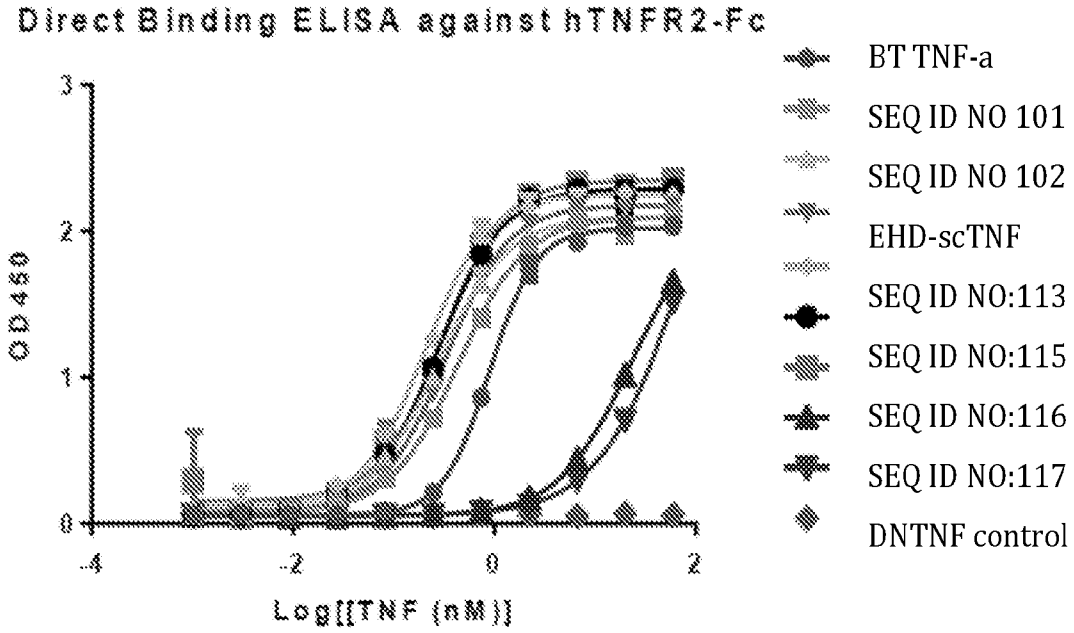

Figure 11B Binding of TNF Variants to immobilized TNFR2

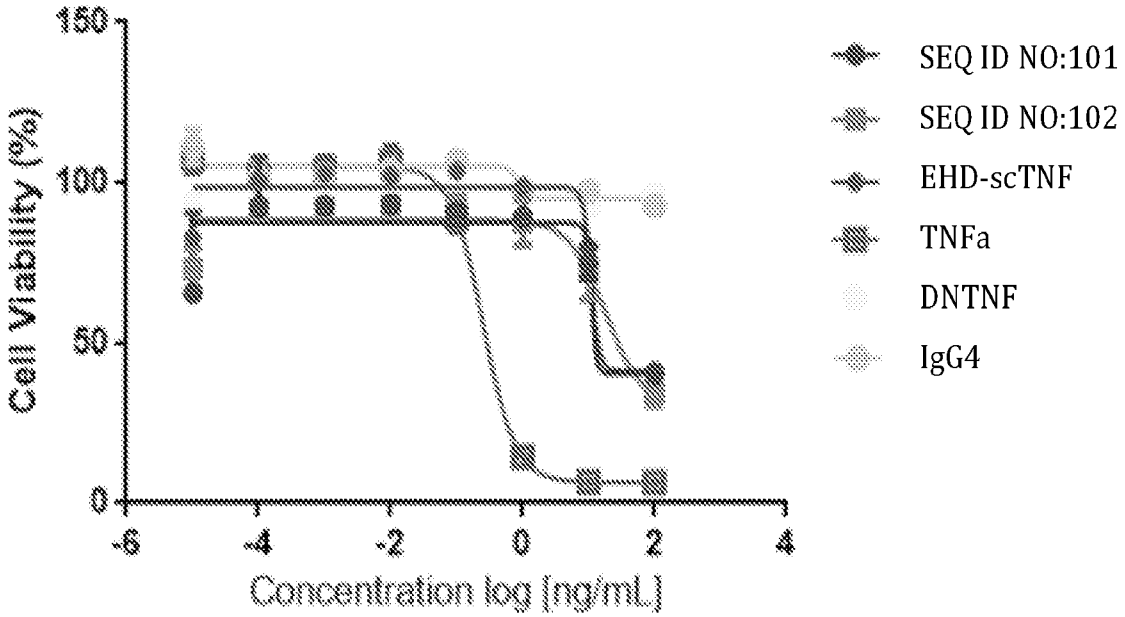

Figure 12a Kym-1 Cell Viability assay in the presence of TNF variants

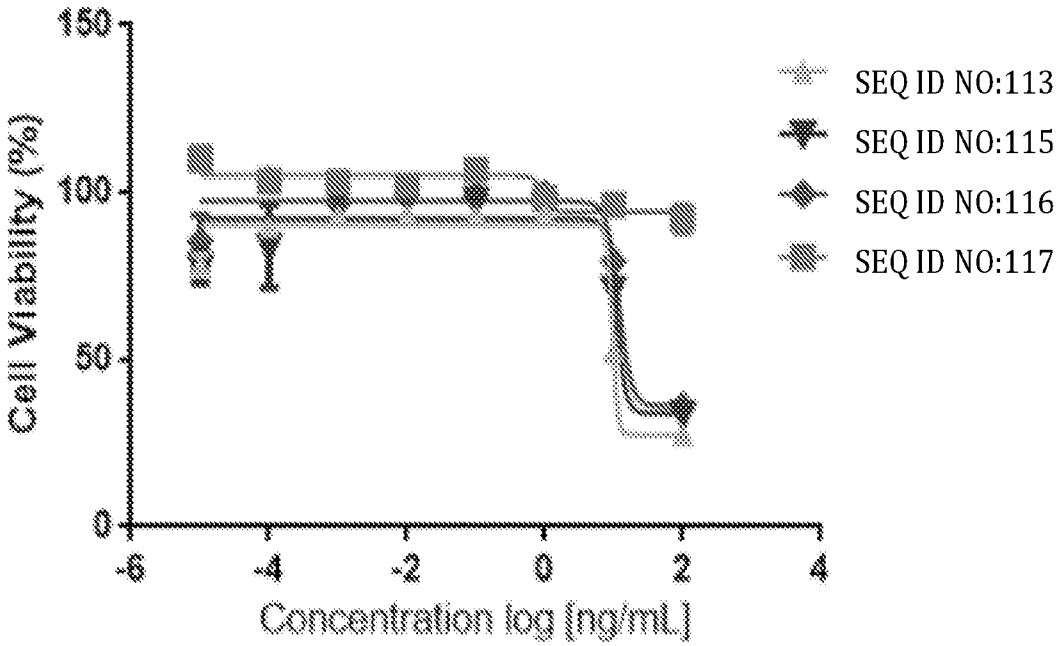

Figure 12b Kym-1 Cell Viability assay in the presence of TNF variants.

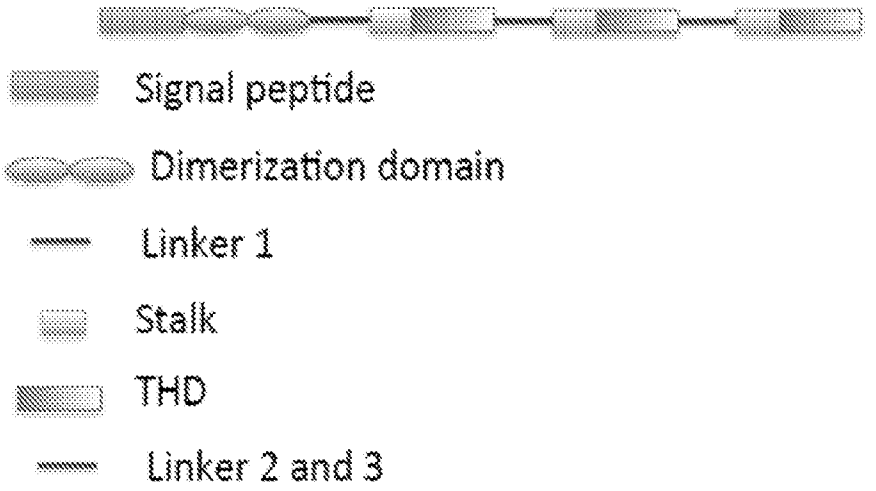
Signal peptide
Dimerization domain
Linker 1
Stalk
THD
Linker 2 and 3

Figure 1A