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# DESCRIPTION

## BACKGROUND

### Technical Field

**[0001]** The present disclosure relates generally to conjugates, such as fusion polypeptides, of one or more histidyl-tRNA synthetase (HRS) polypeptide(s) and immunoglobulin Fc region(s), compositions comprising the same, and methods of using such polypeptides and compositions for treating or diagnosing a variety of conditions.

### Description of the Related Art

**[0002]** Physiocrines are generally small, naturally-occurring protein domains found in the aminoacyl-tRNA synthetases (AARSs) gene family of higher organisms, which are not required for the well-established role of aminoacyl-tRNA synthetases in protein synthesis. Until the Physiocrine paradigm was discovered, aminoacyl-tRNA synthetases, a family of about 20 enzymes, were known only for their ubiquitous expression in all living cells, and their essential role in the process of protein synthesis. More recent scientific findings however now suggest that aminoacyl-tRNA synthetases possess additional roles beyond protein synthesis and in fact have evolved in multicellular organisms to play important homeostatic roles in tissue physiology and disease.

**[0003]** Evidence for the existence of the non-canonical function of AARSs includes well defined sequence comparisons that establish that during the evolution from simple unicellular organisms to more complex life forms, AARSs have evolved to be more structurally complex through the addition of appended domains, without losing the ability to facilitate protein synthesis.

**[0004]** Consistent with this hypothesis, a rich and diverse set of expanded functions for AARSs have been found in higher eukaryotes, and in particular for human tRNA synthetases. This data, which is based both on the direct analysis of individual domains, as well as the discovery of mutations in genes for tRNA synthetases that are causally linked to disease, but do not affect aminoacylation or protein synthesis activity, suggests that these newly appended domains, or Physiocrines, are central to the newly acquired non-canonical functions of AARSs.

**[0005]** Additionally, there is increasing recognition that specific tRNA synthetases such as histidyl-tRNA synthetase (HRS) can be released or secreted from living cells and can provide important locally acting signals with immunomodulatory, chemotactic, and angiogenic properties. Direct confirmation of the role of AARS as extracellular signaling molecules has been obtained through studies showing the secretion and extracellular release of specific tRNA synthetases, as well as the direct demonstration that the addition of fragments of the tRNA synthetases comprising the newly appended domains (Physiocrines), but not other fragments lacking these domains, are active in a range of extracellular signaling pathways. These Physiocrines such as HRS represent a new and previously untapped opportunity to develop new first in class therapeutic proteins to treat human disease.

**[0006]** To best exploit these and other activities in therapeutic or diagnostic settings, there is a need in the art for HRS polypeptides having improved pharmacokinetic properties. These improved therapeutic forms of the HRS polypeptides enable the development of more effective therapeutic regimens for the treatment of various diseases and disorders, and require significantly less frequent administration than the unmodified proteins.



**[0007]** WO 2010/107825 relates to histidyl-tRNA synthetase splice variants that may be conjugated to a linker or other sequence such as an immunoglobulin Fc region.

**[0008]** WO 2011/072265 relates to histidyl- tRNA synthetases to treat inflammation and that may be fused with an Fc region to improve desired characteristics such as increased half-life in circulation and improved in vivo targeting.

**[0009]** WO2013/123432 relates to histidyl-tRNA synthetase polypeptides that may be fused to antibody Fc domains and used for the treatment of autoimmune diseases.

**[0010]** US 2014/066321 relates to histidyl-tRNA synthetase variant polypeptides that may be fused to Fc regions and that can be useful for therapy and drug discovery.

**[0011]** Carter et al. Experimental cell research, academic press 2011, 1261-1269 discloses the technique of fusing proteins and peptides to an Fc domain of an immunoglobulin with the purpose of improving its pharmacokinetic and biological properties.

**[0012]** Huang et al. Current opinion in biotechnology 2009, 692-699 relates to receptor-Fc fusion therapeutics and discloses the different approaches to designing Fc fusion proteins.

**[0013]** Haussermann et al. Pneumologie 2010, 496-503 discloses the relationship of histidyl-tRNA with certain diseases such as a specific form of Myositis with Interstitial Lung Disease.

**[0014]** Levine et al. Current opinion in rheumatology 2003, 708-713 relates to anti-aminoacyl tRNA synthetase immune responses and discloses the links between ARS molecules, inflammation and apoptosis.

**[0015]** US 2011/086058 relates to immunogenic compositions for preventing infection with influenza viruses and discloses the immunogenic compositions such as hemagglutinin of an influenza virus and an immunopotentiator such as an Fc fragment of human IgG.

## **BRIEF DESCRIPTION OF THE DRAWINGS**

### **[0016]**

**Figure 1** illustrates the structural make-up of an exemplary immunoglobulin, and provides an overview of antibody classes and subclasses.

**Figure 2** shows an alignment of Fc regions from human IgA1 (SEQ ID NO:156), IgA2 (SEQ ID NO:157), IgM (SEQ ID NO:158), IgG1 (SEQ ID NO:159), IgG2 (SEQ ID NO:160), IgG3 (SEQ ID NO:161), IgG4 (SEQ ID NO:162), and IgE (SEQ ID NO:163). The secondary structure of Fc $\alpha$  is shown above the sequences. Carets (^) and asterisks (\*) show residues that contribute respectively to 0-4% and 5-12% of the binding surface.

**Figure 3** shows the results of SDS-PAGE analysis under reducing and non reducing conditions of full length HRS and HRS(1-506). The results show that HRS(1-506) dramatically reduces the formation of disulfide mediated interchain bond formation compared to the full length protein. Samples (10  $\mu$ g) were loaded on a 4-12% Bis-Tris gel, using a MOPS-SDS running buffer.

**Figure 4** shows the anti-inflammatory properties of an exemplary HRS-derived polypeptide in a TNBS-induced mouse model of colitis. Studies were performed on male BDF-1 mice, with 12 mice/group. TNBS and budesonide were added at 5 mg/kg to the water. HRS(1-60) (Resokine, (HisRS<sup>N4</sup>)) was administered daily by



IV injection, starting 3 days prior to TNBS treatment, at a concentration of 1 or 5 mg/kg. This figure shows the percent (%) survival of treated and untreated mice over about 80 hours.

**Figure 5A** shows the dosing regimen used to evaluate the therapeutic utility of HRS(1-506) in the statin myopathy model. The treatment dosing groups included vehicle (n=11), 0.3mpk HRS(1-506) (n=8), 1.0mpk HRS(1-506) (n=8), 3.0mpk HRS(1-506) (n=8); **Figure 5B** shows the results of Troponin C measurements after 15 days of treatment with statins +/- HRS(1-506) at 0.3, 1.0, and 3.0 mg /Kg. The figure shows the positive effect of HRS(1-506) in reducing statin induced troponin C induction.

**Figure 6A** shows the results of CK measurements after 12 days of treatment with statins +/-HRS(1-506) at 0.3, 1.0, and 3.0 mg /Kg; **Figure 6B** shows the same data after 15 days of treatment. The figure shows the positive effect of HRS(1-506) in reducing statin induced CK levels.

**Figure 7** shows the levels of circulating HARS after 15 days of treatment with statins compared to the vehicle control. The figure shows that stains induce the release of extracellular HARS.

**Figure 8** shows representative H&E images of hamstring sections at 10x magnification after 15 days of treatment with statins +/- HRS(1-506) at 0.3, 1.0, and 3.0 mg /Kg.

**Figure 9** shows the results of gene expression profiling of statin treated rat hamstrings. The data depicts changes in the expression of 137 genes selected to track markers of muscle, and immune cell function, inflammation, metabolic status, tissue recovery, muscle growth and atrophy. Gene expression values were normalized to reference genes and represented as fold change vs. the vehicle treated group.

**Figure 10A** shows the results of gene expression profiling of statin treated rat hamstrings. The data depicts changes in the expression of 137 genes (as in Figure 7) to compare the relative changes in gene expression of statin treated animals compared to vehicle treated animals. **Figure 10B** shows the relative changes in gene expression of statin treated animals that were also treated with HRS(1-506) compared to animals treated with statin alone.

**Figure 11** shows the results of gene expression profiling of statin treated rat hamstrings of 10 diabetes/metabolic syndrome related genes after 15 days of treatment with statins +/- HRS(1-506) at 0.3, 1.0, and 3.0 mg /Kg.

**Figure 12** shows the results of gene expression profiling of statin treated rat hamstrings of 26 immune cell marker genes after 15 days of treatment with statins +/- HRS(1-506) at 0.3, 1.0, and 3.0 mg /Kg.

**Figures 13A-D** show the results of gene expression profiling of the CD11a, CD11b, CD8a, and CD8b genes in statin treated rat hamstrings after 15 days of treatment with statins +/- HRS(1-506) at 0.3, 1.0, and 3.0 mg /Kg.

**Figures 14A-C** show the results of gene expression profiling of the CD18, CCR5 and CD45R genes in statin treated rat hamstrings after 15 days of treatment with statins +/- HRS(1-506) at 0.3, 1.0, and 3.0 mg /Kg.

**Figure 15** shows the results of gene expression profiling of 17 inflammatory marker genes in statin treated rat hamstrings after 15 days of treatment with statins +/- HRS(1-506) at 0.3, 1.0, and 3.0 mg /Kg.

**Figures 16A-D** show the results of gene expression profiling of the inflammatory cytokines IL-6, MCP1, IL-10, and interferon-gamma (IFN- $\gamma$ ) in statin treated rat hamstrings after 15 days of treatment with statins +/- HRS(1-506) at 0.3, 1.0, and 3.0 mg /Kg.

**Figure 17** shows the results of gene expression profiling of statin treated rat hamstrings of 14 adhesion, development, and fibrosis related genes after 15 days of treatment with statins +/- HRS(1-506) at 0.3, 1.0, and 3.0 mg /Kg.

**Figure 18** shows the results of gene expression profiling of statin treated rat hamstrings of 14 muscle wasting/atrophy related genes after 15 days of treatment with statins +/- HRS(1-506) at 0.3, 1.0, and 3.0 mg



/Kg.

**Figure 19A** shows the results of gene expression profiling of statin treated rat hamstrings of 14 muscle wasting/atrophy related genes after 15 days of treatment with statins +/- HRS(1-506) at 0.3, 1.0, and 3.0 mg /Kg. **Figure 19B** shows specific changes in MMP-3, and **Figure 19C** shows specific changes in MMP-9 gene expression under the same conditions.

**Figure 20** shows the results of gene expression profiling of statin treated rat hamstrings of 29 myogenesis related genes after 15 days of treatment with Statins +/- HRS(1-506) at 0.3, 1.0, and 3.0 mg /Kg.

**Figure 21** shows the results of SDS-PAGE analysis of purified Fc fusion proteins. Lane 1: See Blue Plus2 protein ladder (Life Technologies). Lane 2 and 6: Fc-HRS(2-60) lot# 472. Lane 3 and 7: HRS(1-60)-Fc lot#473. Lane 4 and 8: Fc-HRS(2-60) lot# 480. Lane 5 and 9: HRS(1-60)-Fc lot#482. Lanes 2-5 were run under non-reduced conditions, and lanes 6-9 reduced conditions.

**Figure 22.** Shows an analytical size-exclusion HPLC analysis of representative purified Fc-HRS(2-60) fusion after Protein A, cation exchange, and hydroxyapatite chromatography (overlay of duplicate injections). Purity is 99.2% main peak, and 0.8% high molecular weight (HMW) species.

**Figure 23A** shows the time versus concentration of HRS(1-60) following either intravenous or subcutaneous injection to mice. **Figure 23B** shows the time versus concentration of Fc-HRS(2-60) and HRS(1-60)-Fc following intravenous injection to mice. **Figure 23C** shows the time versus concentration of Fc-HRS(2-60) and HRS(1-60)-Fc following subcutaneous injection to mice.

**Figure 24A** shows disease activity index (DAI) scores at termination in mice treated with different HRS-Fc fusion proteins. Bars represent the mean DAI ( $\pm$  SEM) for each treatment group. The DAI incorporates information on bleeding and diarrhea together with a score for weight loss. **Figure 24B** shows colon weight: length ratio at termination in mice treated with compounds. Bars represent the mean ratio ( $\pm$  SEM) for each treatment group.

**Figure 25** shows an overview of transcriptional changes in TNBS study. Relative transcriptional changes in TNBS treated animals (group 2), animals treated with TNBS and budesonide (group3), TNBS and test article A (HRS(1-60); group 4), and TNBS and test article B (Fc-HRS(2-60); groups 5 and 6) are shown following normalization to naive animals (group 1). Each dot in the scatter plot represents a gene measured. 7 genes in group 2 were up-regulated more than 10-fold (IL6, IL1b, MCP-1, MMP3, MMP9, CD11b, and IL10).

**Figures 26A-26H** show the immune and inflammatory related genes up regulated by TNBS. Relative transcriptional changes of individual genes in TNBS treated animals (group 2), animals treated with TNBS and budesonide (group3), TNBS and test article A (HRS(1-60); group 4), and TNBS and test article B (Fc-HRS(2-60); groups 5 and 6) are shown following normalization to naive animals (group 1). Each dot in the scatter plot represents the abundance of the gene of interest in each animal within the group. Significance was calculated using a student's t-test where \* = p-value < 0.05 and \*\* = p-value < 0.01.

**Figures 27A-27D** shows the relative percentages of different T cell populations in the spleens of naive mice or mice treated intracolonicallly with TNBS to induce experimental colitis, treated with TNBS + 0.5mg/kg Fc-HRS(2-60). Shown are the percentage of live lymphocytes stained for (27A) CD3, (27B) CD8, (27C) CD4, and (27D) CD25 and FoxP3. Treg cells were additionally gated on CD4<sup>+</sup> cells.

**[0017] SUMMARY** Embodiments of the present disclosure relate generally to histidyl-tRNA synthetase (HRS) polypeptide conjugates having one or more immunoglobulin Fc regions covalently attached thereto, pharmaceutical compositions comprising such molecules, methods of manufacture, and methods for their therapeutic use. Among other advantages, the HRS-Fc conjugates of the present disclosure can possess



improved pharmacokinetic properties and/or improved therapeutically relevant biological activities, relative to corresponding, un-modified HRS polypeptides.

**[0018]** Certain embodiments of the present disclosure therefore include HRS fusion polypeptides, comprising a HRS polypeptide that comprises an amino acid sequence at least 80% identical to any one of SEQ ID NOS: 1-106, 170-181, or 185-191 or a sequence of any of **Tables D1, D3-D6, or D8**, and at least one Fc region fused to the C-terminus, the N-terminus, or both of the HRS polypeptide. In some embodiments of the present disclosure, the HRS polypeptide comprises, consists, or consists essentially of an amino acid sequence at least 90% identical to any of SEQ ID NOS: 1-106, 170-181, or 185-191 or a sequence of any of **Tables D1, D3-D6, or D8**. In particular embodiments of the present disclosure, the HRS polypeptide comprises, consists, or consists essentially of an amino acid sequence of any one of SEQ ID NOS: 1-106, 170-181, or 185-191 or a sequence of any of **Tables D1, D3-D6, or D8**.

**[0019]** In particular embodiments of the present disclosure, the HRS polypeptide comprises amino acid residues 2-40, 2-45, 2-50, 2-55, 2-60, 2-66, or 1-506 of SEQ ID NO: 1, or an amino acid sequence at least 90% identical to residues 2-40, 2-45, 2-50, 2-55, 2-60, 2-66, or 1-506 of SEQ ID NO:1. In some embodiments of the present disclosure, the HRS polypeptide is up to about 40-80 amino acids in length and comprises residues 2-45 of SEQ ID NO:1. In specific embodiments of the present disclosure, the HRS polypeptide consists or consists essentially of amino acid residues 2-40, 2-45, 2-50, 2-55, 2-60, 2-66, or 1-506 of SEQ ID NO:1.

**[0020]** In some embodiments of the present disclosure, at least one endogenous cysteine residue of the HRS polypeptide has been substituted with another amino acid or deleted. In certain embodiments of the present disclosure, the at least one endogenous cysteine residue is selected from Cys174, Cys191, Cys224, Cys235, Cys507, and Cys509. In particular embodiments of the present disclosure, the at least one endogenous cysteine residue is selected from Cys224, Cys235, Cys507, and Cys509. In specific embodiments of the present disclosure, the endogenous cysteine residues are Cys507 and Cys509. In some embodiments of the present disclosure, all endogenous surface exposed cysteine residues have been substituted with another amino acid or deleted.

**[0021]** In certain embodiments of the present disclosure, the HRS polypeptide is tandemly repeated. In particular embodiments of the present disclosure, the HRS polypeptide comprises a WHEP domain. In specific embodiments of the present disclosure, the HRS polypeptide lacks a functional aminoacylation domain. In some embodiments of the present disclosure, the HRS polypeptide consists essentially of a WHEP domain. In specific aspects of the present disclosure, the WHEP domain of an HRS polypeptide or variant or fragment thereof has the consensus sequence in **Table D5**.

**[0022]** In some embodiments of the present disclosure, the Fc region and the HRS polypeptide are separated by a peptide linker. In certain embodiments of the present disclosure, the peptide linker is about 1-200 amino acids, 1-150 amino acids, 1-100 amino acids, 1-90 amino acids, 1-80 amino acids, 1-70 amino acids, 1-60 amino acids, 1-50 amino acids, 1-40 amino acids, 1-30 amino acids, 1-20 amino acids, 1-10 amino acids, or 1-5 amino acids in length. In particular embodiments of the present disclosure, peptide linker is about 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, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 60, 70, 80, 90, or 100 amino acids in length. In certain embodiments of the present disclosure, the peptide linker consists of Gly and/or Ser residues. In some embodiments of the present disclosure, the peptide linker is a physiologically stable linker. In other embodiments of the present disclosure, the peptide linker is a releasable linker, optionally an enzymatically-cleavable linker. In specific embodiments of the present disclosure, the peptide linker comprises a sequence of any one of SEQ ID NOS:200-260, or other peptide linker described herein.

**[0023]** In some embodiments of the present disclosure, the Fc region is fused to the C-terminus of the HRS



polypeptide. In certain embodiments of the present disclosure, the Fc region is fused to the N-terminus of the HRS polypeptide.

**[0024]** In certain embodiments of the present disclosure, the Fc region comprises one or more of a hinge, CH<sub>2</sub>, CH<sub>3</sub>, and/or CH<sub>4</sub> domain from a mammalian IgA1, IgA2, IgD, IgE, IgG1, IgG2, IgG3, IgG4, and/or IgM. In some embodiments of the present disclosure, the Fc region comprises IgG1 hinge, CH<sub>2</sub>, and CH<sub>3</sub> domains. In some embodiments of the present disclosure, the Fc region comprises IgG2 hinge, CH<sub>2</sub>, and CH<sub>3</sub> domains. In some embodiments of the present disclosure, the Fc region comprises IgG3 hinge, CH<sub>2</sub>, and CH<sub>3</sub> domains. In particular embodiments of the present disclosure, the HRS fusion polypeptide does not comprise the CH<sub>1</sub>, C<sub>L</sub>, V<sub>L</sub>, and V<sub>H</sub> regions of an immunoglobulin.

**[0025]** In specific embodiments of the present disclosure, the Fc region comprises any one of SEQ ID NOS:128-163 or 339-342, or a variant, or a fragment, or a combination thereof. In certain embodiments of the present disclosure, the hinge domain is a modified IgG1 hinge domain that comprises SEQ ID NO:341.

**[0026]** In particular embodiments of the present disclosure, the Fc region comprises an amino acid sequence at least 90% identical to  
 MSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD  
 GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG  
 QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSF  
 FLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:339) or  
 SDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV  
 EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP  
 REPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFL  
 YSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:340).

**[0027]** In certain embodiments of the present disclosure, the HRS-Fc fusion polypeptide comprises an amino acid sequence at least 90% identical to Fc-HRS(2-60) (SEQ ID NO:337), or HRS(1-60)-Fc (SEQ ID NO:338), or Fc-HRS(2-40) (SEQ ID NO:381), or HRS(1-40)-Fc (SEQ ID NO:386), or Fc-HRS(2-45) (SEQ ID NO: 382), or HRS(1-45)-Fc (SEQ ID NO: 387), or Fc-HRS(2-50) (SEQ ID NO: 383), or HRS(1-50)-Fc (SEQ ID NO: 388), or Fc-HRS(2-55) (SEQ ID NO: 384), or HRS(1-55)-Fc (SEQ ID NO: 389), or Fc-HRS(2-66) (SEQ ID NO:385), or HRS(1-66)-Fc (SEQ ID NO:390), or Fc-HRS(2-60) HRS(2-60) (SEQ ID NO:396).

**[0028]** In certain instances, the HRS fusion polypeptide has altered pharmacokinetics relative to a corresponding HRS polypeptide. Examples of said altered pharmacokinetics include increased serum half-life, increased bioavailability, increased exposure, and/or decreased clearance. In certain instances, the exposure is increased by at least 100 fold. In some instances, the HRS fusion polypeptide has a half life of at least 30 hours in mice. In certain instances, the bioavailability is subcutaneous bioavailability that is increased by at least about 30%. In some instances, the HRS fusion polypeptide has altered immune effector activity relative to a corresponding HRS polypeptide. Examples of such immune effector activities include one or more of complement activation, complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), or antibody-dependent cell-mediated phagocytosis (ADCP).

**[0029]** In certain embodiments of the present disclosure, the Fc region comprises a variant Fc region, relative to a wild-type Fc region. In some embodiments of the present disclosure, the variant Fc region comprises a sequence that is at least 90% identical to any one of SEQ ID NOS: 128-163 or 341, or a combination of said sequences. In certain embodiments of the present disclosure, the variant Fc region comprises a hybrid of one or more Fc regions from different species, different Ig classes, or different Ig subclasses. In particular embodiments of the present disclosure, the variant Fc region comprises a hybrid of one or more hinge, CH<sub>2</sub>, CH<sub>3</sub>, and/or CH<sub>4</sub> domains of Fc regions from different species, different Ig classes, and/or different Ig



subclasses.

**[0030]** In certain embodiments of the present disclosure, the variant Fc region is a modified glycoform, relative to a corresponding, wild-type Fc region. In particular embodiments of the present disclosure, the variant Fc region has altered pharmacokinetics relative to a corresponding, wild-type Fc region. Examples of such altered pharmacokinetics include serum half-life, bioavailability, and/or clearance. In some embodiments of the present disclosure, the variant Fc region has altered effector activity relative to a corresponding, wild-type Fc region. Examples of such effector activities include one or more of complement activation, complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), or antibody-dependent cell-mediated phagocytosis (ADCP).

**[0031]** In certain embodiments of the present disclosure, the variant Fc region has altered binding to one or more Fcγ receptors, relative to a corresponding, wild-type Fc region. Exemplary Fcγ receptors are described herein and known in the art.

**[0032]** In certain embodiments of the present disclosure, the variant Fc region has altered binding to one or more FcRn receptors, relative to a corresponding, wild-type Fc region. Exemplary FcRn receptors are described herein and known in the art.

**[0033]** In some embodiments of the present disclosure, the variant Fc region has altered (e.g., increased) solubility, relative to a corresponding, wild-type Fc region, and the HRS-Fc fusion polypeptide has altered solubility, relative to a corresponding, unmodified HRS polypeptide.

**[0034]** In specific embodiments of the present disclosure, the HRS-Fc fusion polypeptide is substantially in dimeric form in a physiological solution, or under other physiological conditions, such as *in vivo* conditions. In specific embodiments of the present disclosure, the HRS-Fc fusion polypeptide has substantially the same secondary structure a corresponding unmodified or differently modified HRS polypeptide, as determined via UV circular dichroism analysis.

**[0035]** In some embodiments of the present disclosure, the HRS-Fc fusion polypeptide has a plasma or sera pharmacokinetic AUC profile at least 5-fold greater than a corresponding, unmodified HRS polypeptide when administered to a mammal.

**[0036]** In certain embodiments of the present disclosure, the HRS-Fc fusion polypeptide has substantially the same activity of a corresponding unmodified or differently modified HRS polypeptide in an assay of anti-inflammatory activity.

**[0037]** In certain embodiments of the present disclosure, the HRS-Fc fusion polypeptide has greater than 2-fold the activity of a corresponding unmodified or differently modified HRS polypeptide in an assay of anti-inflammatory activity.

**[0038]** In certain embodiments of the present disclosure, the HRS-Fc fusion polypeptide has a stability which is at least 30% greater than a corresponding unmodified or differently modified HRS polypeptide when compared under similar conditions at room temperature, for 7 days in PBS at pH 7.4.

**[0039]** Specific examples of HRS-Fc fusion polypeptides may comprise at least one of SEQ ID NO:107-110 or 337-338 or 349-350 or 381-390 or 396, or an amino acid sequence at least 80%, 90%, 95%, 98% identical to SEQ ID NO:107-110 or 337-338 or 349-350 or 381-390 or 396. SEQ ID NOS:107 and 338 are the amino acid sequences of exemplary C-terminal Fc fusion polypeptides to residues 1-60 of SEQ ID NO:1 (HRS(1-60)\_Fc); SEQ ID NOS:108 and 337 are the amino acid sequences of exemplary N-terminal Fc fusion polypeptides to residues 1-60 of SEQ ID NO:1 (Fc\_HRS(1-60)); SEQ ID NO:109 is the amino acid sequence of an exemplary



C-terminal Fc fusion polypeptide to residues 1-506 of SEQ ID NO:1 (HRS(1-506)\_Fc); and SEQ ID NO:110 is the amino acid sequence of an exemplary N-terminal Fc fusion polypeptide to residues 1-506 of SEQ ID NO:1 (Fc\_HRS(1-506)).

**[0040]** In some embodiments of the present disclosure, the HRS-Fc fusion polypeptide has an anti-inflammatory activity, for example, in a cell-based assay or upon administration to a subject.

**[0041]** Also included are compositions, for example, pharmaceutical or therapeutic compositions, comprising a HRS-Fc fusion polypeptide described herein and a pharmaceutically acceptable or pharmaceutical grade carrier or excipient. In some compositions, the polypeptide as is at least about 95% pure and less than about 5% aggregated. In some embodiments of the present disclosure, the composition is formulated for delivery via oral, subcutaneous, intranasal, pulmonary or parental administration. In certain embodiments of the present disclosure, the composition comprises a delivery vehicle selected from the group consisting of liposomes, micelles, emulsions, and cells.

**[0042]** In some embodiments of the present disclosure, the composition is for use in a) treating an inflammatory or autoimmune disease, b) reducing muscle or lung inflammation optionally associated with an autoimmune or inflammatory disease, c) inducing tolerance to a histidyl-tRNA synthetase (HRS) autoantigen, d) eliminating a set or subset of T cells involved in an autoimmune response to a HRS autoantigen, e) reducing tissue inflammation in a subject, optionally muscle, lung, and/or skin tissue, f) treating a muscular dystrophy, g) treating rhabdomyolysis, muscle wasting, cachexia, muscle inflammation, or muscle injury, and/or h) treating a disease associated with an autoantibody.

**[0043]** Also included are dosing regimens which maintain an average steady-state concentration of an histidyl-tRNA synthetase (HRS)-Fc fusion polypeptide in a subject's plasma of between about 300 pM and about 1000 nM when using a dosing interval of 3 days or longer, comprising administering to the subject a therapeutic composition or HRS-Fc fusion polypeptide described herein.

**[0044]** Some embodiments of the present disclosure include methods for maintaining histidyl-tRNA synthetase (HRS)-Fc fusion polypeptide levels above the minimum effective therapeutic level in a subject in need thereof, comprising administering to the subject a therapeutic composition or HRS-Fc fusion polypeptide described herein.

**[0045]** Also included are methods for treating an inflammatory or autoimmune disease or condition in a subject in need thereof, comprising administering to the subject a therapeutic composition or HRS-Fc fusion polypeptide described herein.

**[0046]** Some embodiments of the present disclosure include methods of reducing muscle or lung inflammation associated with an autoimmune or inflammatory disease in a subject in need thereof, comprising administering to the subject a therapeutic composition or HRS-Fc fusion polypeptide described herein.

**[0047]** Certain embodiments of the present disclosure include methods of inducing tolerance to a histidyl-tRNA synthetase (HRS) autoantigen in a subject in need thereof, comprising administering to the subject a therapeutic composition or HRS-Fc fusion polypeptide described herein.

**[0048]** Some embodiments of the present disclosure include methods for eliminating a set or subset of T cells involved in an autoimmune response to a histidyl-tRNA synthetase (HRS) autoantigen in a subject in need thereof, comprising administering to the subject a therapeutic composition or HRS-Fc fusion polypeptide described herein.

**[0049]** Also included are methods of reducing tissue inflammation in a subject in need thereof, comprising



administering to the subject a therapeutic composition or HRS-Fc fusion polypeptide described herein. In certain embodiments of the present disclosure, the tissue is selected from muscle, gut, brain, lung, and skin.

**[0050]** Some embodiments of the present disclosure include methods of treating a muscular dystrophy in a subject in need thereof, comprising administering to the subject a therapeutic composition or HRS-Fc fusion polypeptide described herein. In particular embodiments of the present disclosure, the muscular dystrophy is selected from Duchenne muscular dystrophy, Becker muscular dystrophy, Emery-Dreifuss muscular dystrophy, Limb-girdle muscular dystrophy, facioscapulohumeral muscular dystrophy, myotonic dystrophy, oculopharyngeal muscular dystrophy, distal muscular dystrophy, and congenital muscular dystrophy.

**[0051]** Certain embodiments of the present disclosure include methods of treating rhabdomyolysis, muscle wasting, cachexia, muscle inflammation, or muscle injury in a subject in need thereof, comprising administering to the subject a therapeutic composition or HRS-Fc fusion polypeptide described herein.

**[0052]** Some embodiments of the present disclosure include methods of treating a disease associated with an autoantibody, comprising administering to a subject in need thereof a composition or AARS/HRS polypeptide described herein. In some embodiments of the present disclosure, the disease is selected from the group consisting of inflammatory myopathies, including inflammatory myopathies, polymyositis, dermatomyositis and related disorders, polymyositis-scleroderma overlap, inclusion body myositis (IBM), anti-synthetase syndrome, interstitial lung disease, arthritis, and Reynaud's phenomenon. In some embodiments of the present disclosure, the composition is administered to the subject prior to the appearance of disease symptoms. In some embodiments of the present disclosure, the autoantibody is specific for histidyl-tRNA synthetase. In some embodiments of the present disclosure, the HRS polypeptide comprises at least one epitope of the histidyl-tRNA synthetase recognized by the disease specific autoantibody. In some embodiments of the present disclosure, the epitope is an immunodominant epitope recognized by antibodies in sera from the subject. In some embodiments of the present disclosure, the HRS polypeptide blocks the binding of the autoantibody to native histidyl-tRNA synthetase. In some embodiments of the present disclosure, the HRS polypeptide causes clonal deletion of auto-reactive T-cells. In some embodiments of the present disclosure, the HRS polypeptide causes functional inactivation of the T cells involved in the autoimmune response. In some embodiments of the present disclosure, administration of the HRS polypeptide results in reduced muscle or lung inflammation. In some embodiments of the present disclosure, the HRS polypeptide induces tolerance to an auto-antigen.

**[0053]** In certain embodiments of the present disclosure, the composition is formulated for delivery via oral, intranasal, pulmonary, intramuscular, or parental administration.

**[0054]** Also included are isolated polynucleotides, comprising a nucleotide sequence that encodes a HRS-Fc conjugate or fusion polypeptide described herein, including vectors that comprise such polynucleotides, and host cells that comprise said polynucleotides and/or vectors.

**[0055]** Some embodiments of the present disclosure include methods for manufacturing a HRS-Fc fusion polypeptide described herein, comprising a) culturing a host cell (e.g., E. coli K-12 host cell) to express a HRS-Fc fusion polypeptide, wherein the host cell comprises a polynucleotide that encodes a HRS-Fc fusion polypeptide described herein, which is operably linked to a regulatory element; and b) isolating the HRS-Fc fusion polypeptide from the host cell. In specific embodiments of the present disclosure, E. coli K-12 strain is selected from W3110 and UT5600.

#### **DETAILED DESCRIPTION**

**[0056]** The invention is defined by the appended claims. Any embodiment not falling under the scope of the



appended claims does not form part of the invention.

**[0057]** The practice of the present disclosure will employ, unless indicated specifically to the contrary, conventional methods of molecular biology and recombinant DNA techniques within the skill of the art, many of which are described below for the purpose of illustration. Such techniques are explained fully in the literature. See, e.g., Sambrook, et al., *Molecular Cloning: A Laboratory Manual* (3rd Edition, 2000); *DNA Cloning: A Practical Approach*, vol. I & II (D. Glover, ed.); *Oligonucleotide Synthesis* (N. Gait, ed., 1984); *Oligonucleotide Synthesis: Methods and Applications* (P. Herdewijn, ed., 2004); *Nucleic Acid Hybridization* (B. Hames & S. Higgins, eds., 1985); *Nucleic Acid Hybridization: Modern Applications* (Buzdin and Lukyanov, eds., 2009); *Transcription and Translation* (B. Hames & S. Higgins, eds., 1984); *Animal Cell Culture* (R. Freshney, ed., 1986); Freshney, R.I. (2005) *Culture of Animal Cells, a Manual of Basic Technique*, 5th Ed. Hoboken NJ, John Wiley & Sons; B. Perbal, *A Practical Guide to Molecular Cloning* (3rd Edition 2010); Farrell, R., *RNA Methodologies: A Laboratory Guide for Isolation and Characterization* (3rd Edition 2005). Poly(ethylene glycol), *Chemistry and Biological Applications*, ACS, Washington, 1997; Veronese, F., and J.M. Harris, Eds., *Peptide and protein PEGylation*, *Advanced Drug Delivery Reviews*, 54(4) 453-609 (2002); Zalipsky, S., et al., "Use of functionalized Poly(Ethylene Glycols) for modification of polypeptides" in *Polyethylene Glycol Chemistry: Biotechnical and Biomedical Applications*.

### **Definitions**

**[0058]** Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the disclosure belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, preferred methods and materials are described. For the purposes of the present disclosure, the following terms are defined below.

**[0059]** The articles "a" and "an" are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

**[0060]** By "about" is meant a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that varies by as much as 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1% to a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length.

**[0061]** As used herein, the term "amino acid" is intended to mean both naturally occurring and non-naturally occurring amino acids as well as amino acid analogs and mimetics. Naturally occurring amino acids include the 20 (L)-amino acids utilized during protein biosynthesis as well as others such as 4-hydroxyproline, hydroxylysine, desmosine, isodesmosine, homocysteine, citrulline and ornithine, for example. Non-naturally occurring amino acids include, for example, (D)-amino acids, norleucine, norvaline, p-fluorophenylalanine, ethionine and the like, which are known to a person skilled in the art. Amino acid analogs include modified forms of naturally and non-naturally occurring amino acids. Such modifications can include, for example, substitution or replacement of chemical groups and moieties on the amino acid or by derivatization of the amino acid. Amino acid mimetics include, for example, organic structures which exhibit functionally similar properties such as charge and charge spacing characteristic of the reference amino acid. For example, an organic structure which mimics arginine (Arg or R) would have a positive charge moiety located in similar molecular space and having the same degree of mobility as the e-amino group of the side chain of the naturally occurring Arg amino acid. Mimetics also include constrained structures so as to maintain optimal spacing and charge interactions of the amino acid or of the amino acid functional groups. Those skilled in the art know or can determine what structures constitute functionally equivalent amino acid analogs and amino acid mimetics.



[0062] As used herein, a subject "**at risk**" of developing a disease or adverse reaction may or may not have detectable disease, or symptoms of disease, and may or may not have displayed detectable disease or symptoms of disease prior to the treatment methods described herein. "At risk" denotes that a subject has one or more risk factors, which are measurable parameters that correlate with development of a disease, as described herein and known in the art. A subject having one or more of these risk factors has a higher probability of developing disease, or an adverse reaction than a subject without one or more of these risk factor(s).

[0063] An "**autoimmune disease**" as used herein is a disease or disorder arising from and directed against an individual's own tissues. Examples of autoimmune diseases or disorders include, but are not limited to, inflammatory responses such as inflammatory skin diseases including psoriasis and dermatitis (e.g., atopic dermatitis); systemic scleroderma and sclerosis; responses associated with inflammatory bowel disease (such as Crohn's disease and ulcerative colitis); respiratory distress syndrome (including adult respiratory distress syndrome; ARDS); dermatitis; meningitis; encephalitis; uveitis; colitis; glomerulonephritis; allergic conditions such as eczema and asthma and other conditions involving infiltration of T cells and chronic inflammatory responses; atherosclerosis; leukocyte adhesion deficiency; rheumatoid arthritis; systemic lupus erythematosus (SLE); diabetes mellitus (e.g., Type I diabetes mellitus or insulin dependent diabetes mellitus); multiple sclerosis; Reynaud's syndrome; autoimmune thyroiditis; allergic encephalomyelitis; Sjorgen's syndrome; juvenile onset diabetes; and immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes typically found in tuberculosis, sarcoidosis, polymyositis, inflammatory myopathies, interstitial lung disease, granulomatosis and vasculitis; pernicious anemia (Addison's disease); diseases involving leukocyte diapedesis; central nervous system (CNS) inflammatory disorder; multiple organ injury syndrome; hemolytic anemia (including, but not limited to cryoglobulinemia or Coombs positive anemia); myasthenia gravis; antigen-antibody complex mediated diseases; anti-glomerular basement membrane disease; antiphospholipid syndrome; allergic neuritis; Graves' disease; Lambert-Eaton myasthenic syndrome; pemphigoid bullous; pemphigus; autoimmune polyendocrinopathies; Reiter's disease; stiff-man syndrome; Behcet disease; giant cell arteritis; immune complex nephritis; IgA nephropathy; IgM polyneuropathies; immune thrombocytopenic purpura (ITP) or autoimmune thrombocytopenia, etc.

[0064] Throughout this specification, unless the context requires otherwise, the words "**comprise**," "**comprises**," and "**comprising**" will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements. By "**consisting of**" is meant including, and limited to, whatever follows the phrase "consisting of." Thus, the phrase "consisting of" indicates that the listed elements are required or mandatory, and that no other elements may be present. By "**consisting essentially of**" is meant including any elements listed after the phrase, and limited to other elements that do not interfere with or contribute to the activity or action specified in the disclosure for the listed elements. Thus, the phrase "consisting essentially of" indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present depending upon whether or not they materially affect the activity or action of the listed elements.

[0065] The term "**clonal deletion**" refers to the deletion (e.g., loss, or death) of auto-reactive T-cells. Clonal deletion can be achieved centrally in the thymus, or in the periphery, or both.

[0066] The term "**conjugate**" is intended to refer to the entity formed as a result of covalent attachment of a molecule, e.g., a biologically active molecule (e.g., HRS polypeptide), to an immunoglobulin Fc region. One example of a conjugate polypeptide is a "**fusion protein**" or "**fusion polypeptide**," that is, a polypeptide that is created through the joining of two or more coding sequences, which originally coded for separate polypeptides; translation of the joined coding sequences results in a single, fusion polypeptide, typically with functional properties derived from each of the separate polypeptides.



[0067] The recitation "**endotoxin free**" or "**substantially endotoxin free**" relates generally to compositions, solvents, and/or vessels that contain at most trace amounts (e.g., amounts having no clinically adverse physiological effects to a subject) of endotoxin, and preferably undetectable amounts of endotoxin. Endotoxins are toxins associated with certain bacteria, typically gram-negative bacteria, although endotoxins may be found in gram-positive bacteria, such as *Listeria monocytogenes*. The most prevalent endotoxins are lipopolysaccharides (LPS) or lipo-oligosaccharides (LOS) found in the outer membrane of various Gram-negative bacteria, and which represent a central pathogenic feature in the ability of these bacteria to cause disease. Small amounts of endotoxin in humans may produce fever, a lowering of the blood pressure, and activation of inflammation and coagulation, among other adverse physiological effects.

[0068] Therefore, in pharmaceutical production, it is often desirable to remove most or all traces of endotoxin from drug products and/or drug containers, because even small amounts may cause adverse effects in humans. A depyrogenation oven may be used for this purpose, as temperatures in excess of 300°C are typically required to break down most endotoxins. For instance, based on primary packaging material such as syringes or vials, the combination of a glass temperature of 250°C and a holding time of 30 minutes is often sufficient to achieve a 3 log reduction in endotoxin levels. Other methods of removing endotoxins are contemplated, including, for example, chromatography and filtration methods, as described herein and known in the art. Also included are methods of producing HRS-Fc conjugates in and isolating them from eukaryotic cells such as mammalian cells to reduce, if not eliminate, the risk of endotoxins being present in a composition of the disclosure. Preferred are methods of producing HRS-Fc conjugates in and isolating them from serum free cells.

[0069] Endotoxins can be detected using routine techniques known in the art. For example, the Limulus Amoebocyte Lysate assay, which utilizes blood from the horseshoe crab, is a very sensitive assay for detecting presence of endotoxin. In this test, very low levels of LPS can cause detectable coagulation of the limulus lysate due a powerful enzymatic cascade that amplifies this reaction. Endotoxins can also be quantitated by enzyme-linked immunosorbent assay (ELISA). To be substantially endotoxin free, endotoxin levels may be less than about 0.001, 0.005, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.08, 0.09, 0.1, 0.5, 1.0, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, or 10 EU/ml. Typically, 1 ng lipopolysaccharide (LPS) corresponds to about 1-10 EU.

[0070] As used herein, the terms "**function**" and "**functional**" and the like refer to a biological, enzymatic, or therapeutic function.

[0071] "**Homology**" refers to the percentage number of amino acids that are identical or constitute conservative substitutions. Homology may be determined using sequence comparison programs such as GAP (Deveraux et al., Nucleic Acids Research. 12, 387-395, 1984). In this way sequences of a similar or substantially different length to those cited herein could be compared by insertion of gaps into the alignment, such gaps being determined, for example, by the comparison algorithm used by GAP.

[0072] A "**physiologically stable**" linker refers to a linker that is substantially stable in water or under physiological conditions (e.g., *in vivo*, *in vitro* culture conditions, for example, in the presence of one or more proteases), that is to say, it does not undergo a degradation reaction (e.g., enzymatically degradable reaction) under physiological conditions to any appreciable extent over an extended period of time. Generally, a physiologically stable linker is one that exhibits a rate of degradation of less than about 0.5%, about 1%, about 2%, about 3%, about 4%, or about 5% per day under physiological conditions.

[0073] By "**isolated**" is meant material that is substantially or essentially free from components that normally accompany it in its native state. For example, an "isolated peptide" or an "isolated polypeptide" and the like, as used herein, includes the *in vitro* isolation and/or purification of a peptide or polypeptide molecule from its natural cellular environment, and from association with other components of the cell; *i.e.*, it is not significantly associated with *in vivo* substances.



[0074] The term "**half maximal effective concentration**" or "**EC<sub>50</sub>**" refers to the concentration of a HRS-Fc conjugate described herein at which it induces a response halfway between the baseline and maximum after some specified exposure time; the EC<sub>50</sub> of a graded dose response curve therefore represents the concentration of a compound at which 50% of its maximal effect is observed. In certain embodiments of the present disclosure, the EC<sub>50</sub> of an agent provided herein is indicated in relation to a "non-canonical" activity, as noted above. EC<sub>50</sub> also represents the plasma concentration required for obtaining 50% of a maximum effect *in vivo*. Similarly, the "EC<sub>90</sub>" refers to the concentration of an agent or composition at which 90% of its maximal effect is observed. The "EC<sub>90</sub>" can be calculated from the "EC<sub>50</sub>" and the Hill slope, or it can be determined from the data directly, using routine knowledge in the art. In some embodiments of the present disclosure, the EC<sub>50</sub> of a HRS-Fc conjugate is less than about 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 60, 70, 80, 90, or 100 nM. Preferably, biotherapeutic composition will have an EC<sub>50</sub> value of about nM or less.

[0075] The "**half-life**" of a HRS-Fc conjugate can refer to the time it takes for the conjugate to lose half of its pharmacologic, physiologic, or other activity, relative to such activity at the time of administration into the serum or tissue of an organism, or relative to any other defined time-point. "Half-life" can also refer to the time it takes for the amount or concentration of a HRS-Fc conjugate to be reduced by half of a starting amount administered into the serum or tissue of an organism, relative to such amount or concentration at the time of administration into the serum or tissue of an organism, or relative to any other defined time-point. The half-life can be measured in serum and/or any one or more selected tissues.

[0076] The term "**linkage**," "**linker**," "**linker moiety**," or "**L**" is used herein to refer to a linker that can be used to separate a HRS polypeptides from another HRS polypeptide and/or from one or more Fc regions. The linker may be physiologically stable or may include a releasable linker such as an enzymatically degradable linker (e.g., proteolytically cleavable linkers). In certain aspects of the present disclosure, the linker may be a peptide linker, for instance, as part of a HRS-Fc fusion protein. In some aspects of the present disclosure, the linker may be a non-peptide linker.

[0077] The terms "**modulating**" and "**altering**" include "increasing," "enhancing" or "stimulating," as well as "decreasing" or "reducing," typically in a statistically significant or a physiologically significant amount or degree relative to a control. An "**increased**," "**stimulated**" or "**enhanced**" amount is typically a "statistically significant" amount, and may include an increase that is 1.1, 1.2, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30 or more times (e.g., 500, 1000 times) (including all integers and decimal points in between and above 1, e.g., 1.5, 1.6, 1.7, 1.8, etc.) the amount produced by no composition (e.g., in the absence of any of the HRS-Fc conjugates of the disclosure) or a control composition, sample or test subject. A "**decreased**" or "**reduced**" amount is typically a "**statistically significant**" amount, and may include a 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% decrease in the amount produced by no composition (the absence of an agent or compound) or a control composition, including all integers in between. As one non-limiting example, a control in comparing canonical and non-canonical activities could include the HRS-Fc conjugate of interest compared to a corresponding (sequence-wise), unmodified or differently modified HRS polypeptide. Other examples of comparisons and "statistically significant" amounts are described herein.

[0078] "**Non-canonical**" activity as used herein, refers generally to either i) a new, non-aminoacylation activity possessed by HRS polypeptide of the disclosure that is not possessed to any significant degree by the intact native full length parental protein, or ii) an activity that was possessed by the by the intact native full length parental protein, where the HRS polypeptide either exhibits a significantly higher (e.g., at least 20% greater) specific activity with respect to the non-canonical activity compared to the intact native full length parental protein, or exhibits the activity in a new context; for example by isolating the activity from other activities



possessed by the intact native full length parental protein. In the case of HRS polypeptides, non-limiting examples of non-canonical activities include extracellular signaling including the modulation of cell proliferation, modulation of cell migration, modulation of cell differentiation (e.g., hematopoiesis, neurogenesis, myogenesis, osteogenesis, and adipogenesis), modulation of gene transcription, modulation of apoptosis or other forms of cell death, modulation of cell signaling, modulation of cellular uptake, or secretion, modulation of angiogenesis, modulation of cell binding, modulation of cellular metabolism, modulation of cytokine production or activity, modulation of cytokine receptor activity, modulation of inflammation, immunogenicity, and the like.

**[0079]** In certain embodiments of the present disclosure, the "**purity**" of any given agent (e.g., HRS-Fc conjugate such as a fusion protein) in a composition may be specifically defined. For instance, certain compositions may comprise an agent that is at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% pure, including all decimals in between, as measured, for example and by no means limiting, by high pressure liquid chromatography (HPLC), a well-known form of column chromatography used frequently in biochemistry and analytical chemistry to separate, identify, and quantify compounds.

**[0080]** Without wishing to be bound to any particular theory, an "**enzymatically degradable linker**" means a linker, e.g., amino acid sequence that is subject to degradation by one or more enzymes, e.g., peptidases or proteases.

**[0081]** The terms "**polypeptide**" and "**protein**" are used interchangeably herein to refer to a polymer of amino acid residues and to variants and synthetic analogues of the same. Thus, these terms apply to amino acid polymers in which one or more amino acid residues are synthetic non-naturally occurring amino acids, such as a chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally-occurring amino acid polymers.

**[0082]** A "**releasable linker**" includes, but is not limited to, a physiologically cleavable linker and an enzymatically degradable linker. Thus, a "releasable linker" is a linker that may undergo either spontaneous hydrolysis, or cleavage by some other mechanism (e.g., enzyme-catalyzed, acid-catalyzed, base-catalyzed, and so forth) under physiological conditions. For example, a "releasable linker" can involve an elimination reaction that has a base abstraction of a proton, (e.g., an ionizable hydrogen atom, H $\alpha$ ), as the driving force. For purposes herein, a "releasable linker" is synonymous with a "degradable linker." In particular embodiments of the present disclosure, a releasable linker has a half life at pH 7.4, 25°C, e.g., a physiological pH, human body temperature (e.g., *in vivo*), of about 30 minutes, about 1 hour, about 2 hour, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 12 hours, about 18 hours, about 24 hours, about 36 hours, about 48 hours, about 72 hours, or about 96 hours or more.

**[0083]** By "**statistically significant**," it is meant that the result was unlikely to have occurred by chance. Statistical significance can be determined by any method known in the art. Commonly used measures of significance include the p-value, which is the frequency or probability with which the observed event would occur, if the null hypothesis were true. If the obtained p-value is smaller than the significance level, then the null hypothesis is rejected. In simple cases, the significance level is defined at a p-value of 0.05 or less.

**[0084]** The term "**solubility**" refers to the property of a HRS-Fc conjugate polypeptide provided herein to dissolve in a liquid solvent and form a homogeneous solution. Solubility is typically expressed as a concentration, either by mass of solute per unit volume of solvent (g of solute per kg of solvent, g per dL (100 mL), mg/ml, etc.), molarity, molality, mole fraction or other similar descriptions of concentration. The maximum equilibrium amount of solute that can dissolve per amount of solvent is the solubility of that solute in that solvent under the specified conditions, including temperature, pressure, pH, and the nature of the solvent. In certain embodiments of the present disclosure, solubility is measured at physiological pH, or other pH, for example, at pH 5.0, pH 6.0, pH 7.0, or pH 7.4. In certain embodiments of the present disclosure, solubility is



measured in water or a physiological buffer such as PBS or NaCl (with or without NaP). In specific embodiments of the present disclosure, solubility is measured at relatively lower pH (e.g., pH 6.0) and relatively higher salt (e.g., 500mM NaCl and 10mM NaP). In certain embodiments of the present disclosure, solubility is measured in a biological fluid (solvent) such as blood or serum. In certain embodiments of the present disclosure, the temperature can be about room temperature (e.g., about 20, 21, 22, 23, 24, 25°C) or about body temperature (37°C). In certain embodiments of the present disclosure, a HRS-Fc conjugate polypeptide has a solubility of at least about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, or 30 mg/ml at room temperature or at 37°C.

**[0085]** A "**subject**," as used herein, includes any animal that exhibits a symptom, or is at risk for exhibiting a symptom, which can be treated or diagnosed with a HRS-Fc conjugate polypeptide of the disclosure. Suitable subjects (patients) include laboratory animals (such as mouse, rat, rabbit, or guinea pig), farm animals, and domestic animals or pets (such as a cat or dog). Non-human primates and, preferably, human patients, are included.

**[0086]** "**Substantially**" or "**essentially**" means nearly totally or completely, for instance, 95%, 96%, 97%, 98%, 99% or greater of some given quantity.

**[0087]** "**Treatment**" or "**treating**," as used herein, includes any desirable effect on the symptoms or pathology of a disease or condition, and may include even minimal changes or improvements in one or more measurable markers of the disease or condition being treated. "Treatment" or "treating" does not necessarily indicate complete eradication or cure of the disease or condition, or associated symptoms thereof. The subject receiving this treatment is any subject in need thereof. Exemplary markers of clinical improvement will be apparent to persons skilled in the art.

#### ***Histidyl-tRNA synthetase derived polypeptides***

**[0088]** Embodiments of the present disclosure relate to histidyl-tRNA synthetase polypeptide ("HRS or HisRS polypeptides")-Fc conjugates, including HRS-Fc conjugates that comprise wild-type HRS sequences, naturally-occurring sequences, non-naturally occurring sequences, and/or variants and fragments thereof. Specific examples of HRS derived polypeptides include those with altered cysteine content. Histidyl-tRNA synthetases belong to the class II tRNA synthetase family, which has three highly conserved sequence motifs. Class I and II tRNA synthetases are widely recognized as being responsible for the specific attachment of an amino acid to its cognate tRNA in a two-step reaction: the amino acid (AA) is first activated by ATP to form AA-AMP and then transferred to the acceptor end of the tRNA. The full length histidyl-tRNA synthetases typically exist either as a cytosolic homodimer, or an alternatively spliced mitochondrial form.

**[0089]** More recently it has been established that some biological fragments, or alternatively spliced isoforms of eukaryotic histidyl-tRNA synthetases (Physiocrines, or HRS polypeptides), or in some contexts the intact synthetase, modulate certain cell-signaling pathways, or have anti-inflammatory properties. These activities, which are distinct from the classical role of tRNA synthetases in protein synthesis, are collectively referred to herein as "non-canonical activities." These Physiocrines may be produced naturally by either alternative splicing or proteolysis, and can act in a cell autonomous fashion (*i.e.*, within the host cell) or a non-cell autonomous fashion (*i.e.*, outside the host cell) to regulate a variety of homeostatic mechanisms. For example, as provided in the present disclosure, HRS polypeptides such as the N-terminal fragment of histidyl-tRNA synthetase (e.g., HRS 1-48, HRS 1-60) are capable, *inter alia*, of exerting an anti-inflammatory signal by blocking the migration, activation, or differentiation of inflammatory cells (e.g., monocytes, macrophages, T cells, B cells) associated with the sites of active inflammation *in vivo*. In addition, certain mutations or deletions (e.g., HRS 1-506, HRS 1-60) relative to the full-length HRS polypeptide sequence confer increased activities and/or improved pharmacological properties. The sequences of certain exemplary HRS polypeptides are



provided in Table D1.

| Table D1                   |                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |            |
|----------------------------|--------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| Exemplary HRS polypeptides |                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |            |
| Name                       | Type / species /Residues | Amino acid and Nucleic Acid Sequences                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | SEQ ID NO: |
| N-terminal Physiocrines    |                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |            |
| FL cytosolic wild type     | Protein / Human /        | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSRQMAVREKVFDVII<br>RCFKRHGAEVIDTPVFELKETLMGKYGEDSKLIYDLKDQGG<br>ELLSLRYDLTVPFARYLAMNKL TNIKRYHIAKVYRRDNPAM<br>TRGRYREFYQCDFDIAGNFDPMIPDAECLKIMCEILSSLQIGD<br>FLVKVNDRRILDGMFAICGVSDSKFRTICSSVDKLDKVSWE<br>EVKNEMVGEKGLAPEVADRIGDYVQQHGGVSLVEQLLQDP<br>KLSQNKQALEGLGDLKLLFEYLT LFGIDDKISFDLSLARGLD<br>YYTGVIYEAVLLQTPAQAGEEPLGVGSVAAGGRYDGLVGM<br>FDPKGRKVPCVGLSIGVERIFSIVEQRLEALEEKIRTTEQVL<br>VASAQKKLLEERLKL VSELWDAGIKAELLYKKNPKLLNQL<br>QYCEEAGIPLVAIIGEQLKDGVIKLSVTSREEVDVRREDL<br>VEEIKRRTGQPLCIC | 1          |
| FL mitochondrial wild type | Protein / Human /        | MPLLGLLPRAWASLLSQLLRPPCASCTGAVRCQSQVAEAV<br>LTSQLKAHQEKPFIKTPKGTRDLSPQHMVVREKILDLVISC<br>FKRHGAKGMDTPAFELKETL TEKYGEDSGLMYDLKDQGG<br>ELLSLRYDLTVPFARYLAMNKVKMKRYHVGKVWRRESPTI<br>VQGRYREFCQCDFDIAGQFDPMPDAECLKIMCEILSGLQLG<br>DFLIKVNDRRIVDGMFAVCGVPESKFRAICSSIDKLDKMAW<br>KDVRHEMVVKKGLAPEVADRIGDYVQCHGGVSLVEQMFG<br>DPRLSQNKQALEGLGDLKLLFEYLT LFGIADKISFDLSLARG<br>LDYYTGVIYEAVLLQTPTQAGEEPLNVGSVAAGGRYDGLV<br>GMFDPKGHKVPCVGLSIGVERIFYIVEQRMKTKGEKVRTTE<br>TQVFVATPQKNFLQERLKLIAELWDSGIKAEMLYKNNPKLL<br>TQLHYCESTGIPLVVIIGEQLKEGVKIRSVASREEVAIKREN<br>FVAEIQRLSSES        | 2          |
| HisRS 1 <sup>N1</sup>      | Protein / Human / 1-141  | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSRQMAVREKVFDVII<br>RCFKRHGAEVIDTPVFELKETLMGKYGEDSKLIYDLKDQGG<br>ELLSLRYDLTVPFARYLAM                                                                                                                                                                                                                                                                                                                                                                                                                     | 3          |
| HisRS 1 <sup>N2</sup>      | Protein / Human / 1-408  | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSRQMAVREKVFDVII<br>RCFKRHGAEVIDTPVFELKETLMGKYGEDSKLIYDLKDQGG<br>ELLSLRYDLTVPFARYLAMNKL TNIKRYHIAKVYRRDNPAM<br>TRGRYREFYQCDFDIAGNFDPMIPDAECLKIMCEILSSLQIGD<br>FLVKVNDRRILDGMFAICGVSDSKFRTICSSVDKLDKVSWE<br>EVKNEMVGEKGLAPEVADRIGDYVQQHGGVSLVEQLLQDP<br>KLSQNKQALEGLGDLKLLFEYLT LFGIDDKISFDLSLARGLD<br><br>YYTGVIYEAVLLQTPAQAGEEPLGVGSVAAGGRYDGLVGM<br>FDPKGRKVPCVGLSIGVERIFSIVEQRLEALEEKIRTTE                                                                                                            | 4          |
| HisRS 1 <sup>N3</sup>      | Protein / Human / 1-113  | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSRQMAVREKVFDVII<br>RCFKRHGAEVIDTPVFELKETLMGKYGEDSKL                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 5          |
| HisRS 1 <sup>N4</sup>      | Protein / Human / 1-60   | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPK                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | 6          |



| Table D1                   |                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                  |            |
|----------------------------|-----------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| Exemplary HRS polypeptides |                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                  |            |
| Name                       | Type / species /Residues          | Amino acid and Nucleic Acid Sequences                                                                                                                                                                                                                                                                                                                                                                                                            | SEQ ID NO: |
| HisRS 1 <sup>N5</sup>      | Protein / Human / 1-243 + 27aa    | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVREKVFDVII<br>RCFKRHGAVIDTPVFELKETLMGKYGEDSKLIYDLKDQGG<br>ELLSLRYDLTVPFARYLAMNKLNIKRYHIAKVYRRDNPAM<br>TRGRYREFYQCDFDIAGNFDPMIPDAECLKIMCEILSSLQIGD<br>FLVKVNDRRILDGMFAICGVSDSKFRTICSSVDKLDKVGYP<br>WWNSCSRILNYPKTSRPWRAWET                                                                                                                                             | 7          |
| C-terminal Physiocrines    |                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                  |            |
| Name                       | Type / species /Residues          | Amino acid and Nucleic Acid Sequences                                                                                                                                                                                                                                                                                                                                                                                                            | SEQ ID NO. |
| HisRS1 <sup>C1</sup>       | Protein / Human / 405-509         | RTTETQVLVASAQKKLEERLKLVSELWDAGIKAELLYKK<br>NPKLLNQLQYCEEAGIPLVAIIGEQLKDGVIKLRSVTSREE<br>VDVRREDLVEEIKRRTGQPLCIC                                                                                                                                                                                                                                                                                                                                  | 8          |
| HisRS1 <sup>C2</sup>       | Protein / Human / 1-60 + 175-509  | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKDFDIAGNFDPMIPDAECLKIM<br>CEILSSLQIGDFLVKVNDRRILDGMFAICGVSDSKFRTICSSV<br>DKLDKVSWEVKNEMVGEKGLAPEVADRIGDYVQQHGG<br>VSLVEQLLQDPKLSQNKQALEGLGDLKLLFEYLT LFGIDDK<br>ISFDLSLARGLDYYTGVIYEAVLLQTPAQAGEEPLGVGSVA<br>AGGRYDGLVGMFDPKGRKVPCVGLSIGVERIFSIVEQRLEA<br>LEEKIRTTETQVLVASAQKKLEERLKLVSELWDAGIKAEL<br>LYKKNPKLLNQLQYCEEAGIPLVAIIGEQLKDGVIKLRSV<br>TSREEVDVRREDLVEEIKRRTGQPLCIC    | 9          |
| HisRS 1 <sup>C3</sup>      | Protein / Human / 1-60 + 211-509  | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKVNDRRILDGMFAICGVSDSK<br>FRTICSSVDKLDKVSWEVKNEMVGEKGLAPEVADRIGDY<br>VQQHGGVSLVEQLLQDPKLSQNKQALEGLGDLKLLFEYLT<br>LFGIDDKISFDLSLARGLDYYTGVIYEAVLLQTPAQAGEEPL<br>GVGSVAAGGRYDGLVGMFDPKGRKVPCVGLSIGVERIFSI<br>VEQRLEALEEKIRTTETQVLVASAQKKLEERLKLVSELW<br>DAGIKAELLYKKNPKLLNQLQYCEEAGIPLVAIIGEQLKD<br>GVIKLRSVTSREEVDVRREDLVEEIKRRTGQPLCIC                                             | 10         |
| HisRS 1 <sup>C4</sup>      | Protein / Human / 1-100 + 211-509 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVREKVFDVI<br>IRCFKRHGAVIDTPVFELKVNDRRILDGMFAICGVSDSKF<br>RTICSSVDKLDKVSWEVKNEMVGEKGLAPEVADRIGDYV<br>QQHGGVSLVEQLLQDPKLSQNKQALEGLGDLKLLFEYLT L<br>FGIDDKISFDLSLARGLDYYTGVIYEAVLLQTPAQAGEEPL<br>GVGSVAAGGRYDGLVGMFDPKGRKVPCVGLSIGVERIFSI<br>VEQRLEALEEKIRTTETQVLVASAQKKLEERLKLVSELW<br>DAGIKAELLYKKNPKLLNQLQYCEEAGIPLVAIIGEQLKD<br>GVIKLRSVTSREEVDVRREDLVEEIKRRTGQPLCIC | 11         |
| HisRS1 <sup>C5</sup>       | Protein / Human / 1-174 + 211-509 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVREKVFDVI<br>IRCFKRHGAVIDTPVFELKETLMGKYGEDSKLIYDLKDQG<br>GELLSLRYDLTVPFARYLAMNKLNIKRYHIAKVYRRDNP<br>AMTRGRYREFYQCVNDRILDGMFAICGVSDSKFRTICSSV<br>DKLDKVSWEVKNEMVGEKGLAPEVADRIGDYVQQHGG<br>VSLVEQLLQDPKLSQNKQALEGLGDLKLLFEYLT LFGIDDK                                                                                                                                   | 12         |



| C-terminal Physiocrines |                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |            |
|-------------------------|-----------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| Name                    | Type / species /Residues          | Amino acid and Nucleic Acid Sequences                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | SEQ ID NO. |
|                         |                                   | ISFDLSLARGLDYYTGVIYEAVLLQTPAQAGEEPLGVGSVA<br>AGGRYDGLVGMFDPKGRKVPCVGLSIGVERIFSIVEQRLEA<br>LEEKIRTTETQVLVASAQKKLLEERLKLVSELWDAGIKAEL<br>LYKKNPKLLNQLQYCEEAGIPLVAIIGEQLKDGVIKLRSV<br>TSREEVDVRREDLVEEIKRRTGQPLCIC                                                                                                                                                                                                                                                                                                                |            |
| HisRS1 <sup>C6</sup>    | Protein / Human / 1-60 + 101-509  | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKETLMGKYGEDSKLIYDLKDQ<br>GGELLSLRYDLTVPFARYLAMNKLTNIKRYHIKVYRRDN<br>PAMTRGRYREFYQCDFDIAGNFDPMIPDAECLKIMCEILSS<br>LQIGDFLVKVNDRRILDGMFAICGVSDSKFRTICSSVDKLD<br>KVSWEVKNEMVGEKGLAPEVADRIGDYVQQHGGVSLVE<br>QLLQDPKLSQNKQALEGLGDLKLLFEYLTFLGIDDKISFDL<br>SLARGLDYYTGVIYEAVLLQTPAQAGEEPLGVGSVAAGGR<br>YDGLVGMFDPKGRKVPCVGLSIGVERIFSIVEQRLEALEEKI<br>RTTETQVLVASAQKKLLEERLKLVSELWDAGIKAELLYKK<br>NPKLLNQLQYCEEAGIPLVAIIGEQLKDGVIKLRSVTSREE<br>VDVRREDLVEEIKRRTGQPLCIC | 13         |
| HisRS 1 <sup>C7</sup>   | Protein / Human / 1-100 + 175-509 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPQMAVREKVFDVI<br>IRCFKRHGAVIDTPVFELKDFDIAGNFDPMIPDAECLKIMC<br>EILSSLQIGDFLVKVNDRRILDGMFAICGVSDSKFRTICSSV<br>DKLDKVSWEVKNEMVGEKGLAPEVADRIGDYVQQHGG<br>VSLVEQLLQDPKLSQNKQALEGLGDLKLLFEYLTFLGIDDK<br>ISFDLSLARGLDYYTGVIYEAVLLQTPAQAGEEPLGVGSVA<br>AGGRYDGLVGMFDPKGRKVPCVGLSIGVERIFSIVEQRLEA<br>LEEKIRTTETQVLVASAQKKLLEERLKLVSELWDAGIKAEL<br>LYKKNPKLLNQLQYCEEAGIPLVAIIGEQLKDGVIKLRSV<br>TSREEVDVRREDLVEEIKRRTGQPLCIC                                        | 14         |
| HisRS 1 <sup>C8</sup>   | Protein / Human / 1-60 + 399-509  | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKALEEKIRTTETQVLVASAQK<br>KLEERLKLVSELWDAGIKAELLYKKNPKLLNQLQYCEEA<br>GIPLVAIIGEQLKDGVIKLRSVTSREEVDVRREDLVEEIKR<br>RTGQPLCIC                                                                                                                                                                                                                                                                                                                                      | 15         |
| HisRS 1 <sup>C9</sup>   | Protein / Human / 1-100 + 399-509 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPQMAVREKVFDVI<br>IRCFKRHGAVIDTPVFELKALEEKIRTTETQVLVASAQKK<br>LLEERLKLVSELWDAGIKAELLYKKNPKLLNQLQYCEEAG<br>IPLVAIIGEQLKDGVIKLRSVTSREEVDVRREDLVEEIKRR<br>TGQPLCIC                                                                                                                                                                                                                                                                                           | 16         |
| HisRS1 <sup>C10</sup>   | Protein / Human / 369-509         | MFDPKGRKVPCVGLSIGVERIFSIVEQRLEALEEKIRTTETQ<br>VLVASAQKKLLEERLKLVSELWDAGIKAELLYKKNPKLLN<br>QLQYCEEAGIPLVAIIGEQLKDGVIKLRSVTSREEVDVRRE<br>DLVEEIKRRTGQPLCIC                                                                                                                                                                                                                                                                                                                                                                       | 17         |



| Internal Physiocrines |                           |                                                                                                                                                           |            |
|-----------------------|---------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| Name                  | Type / species /Residues  | Amino acid and Nucleic Acid Sequences                                                                                                                     | SEQ ID NO. |
| HisRS1 <sup>11</sup>  | Protein / Human / 191-333 | CLKIMCEILSSLQIGDFLVKVNDRRILDGMFAICGVSDSKFR<br>TICSSVDKLDKVSWEVKNEVMGEKGLAPEVADRIGDYV<br>QQHGGVSLVEQLLQDPKLSQNKQALEGLGDLKLLFEYLT<br>FGIDDKISFDLSLARGLDYYTG | 18         |

[0090] A number of naturally occurring histidyl-tRNA synthetase single nucleotide polymorphisms (SNPs) and naturally occurring variants of the human gene have been sequenced, and are known in the art to be at least partially functionally interchangeable. Several such variants of histidyl-tRNA synthetase (*i.e.*, representative histidyl-tRNA synthetase SNPs) are shown in Table D2.

| Table D2                            |                   |                            |                   |
|-------------------------------------|-------------------|----------------------------|-------------------|
| Human Histidyl tRNA synthetase SNPs |                   |                            |                   |
| Gene Bank Accession Number          | Nucleotide Change | Gene Bank Accession Number | Nucleotide Change |
| rs193103291                         | A/G               | rs186312047                | A/G               |
| rs192923161                         | C/T               | rs186176857                | C/T               |
| rs192784934                         | A/G               | rs186043734                | C/G               |
| rs192164884                         | A/G               | rs185867584                | C/T               |
| rs192090865                         | A/C               | rs185828130                | A/G               |
| rs192015101                         | A/T               | rs185537686                | A/G               |
| rs191999492                         | A/G               | rs185440931                | C/T               |
| rs191852363                         | C/T               | rs185100584                | A/C               |
| rs191532032                         | A/T               | rs185077558                | C/T               |
| rs191391414                         | C/T               | rs184748736                | C/G               |
| rs191385862                         | A/G               | rs184591417                | C/T               |
| rs191205977                         | A/G               | rs184400035                | C/G               |
| rs191104160                         | A/G               | rs184098206                | C/T               |
| rs190989313                         | C/G               | rs183982931                | C/T               |
| rs190818970                         | A/T               | rs183942045                | A/G               |
| rs190476138                         | C/T               | rs183854085                | A/G               |
| rs190289555                         | C/T               | rs183430882                | G/T               |
| rs190065567                         | A/G               | rs183419967                | A/C               |
| rs189624055                         | C/T               | rs183366286                | A/G               |
| rs189563577                         | G/T               | rs183084050                | C/T               |
| rs189404434                         | A/G               | rs182948878                | C/T               |
| rs189268935                         | A/G               | rs182813126                | A/G               |
| rs189103453                         | A/T               | rs182498374                | A/G               |
| rs188839103                         | A/G               | rs182161259                | A/T               |
| rs188766717                         | A/G               | rs182119902                | C/T               |
| rs188705391                         | A/G               | rs182106891                | C/T               |



| Table D2                            |                   |                            |                   |
|-------------------------------------|-------------------|----------------------------|-------------------|
| Human Histidyl tRNA synthetase SNPs |                   |                            |                   |
| Gene Bank Accession Number          | Nucleotide Change | Gene Bank Accession Number | Nucleotide Change |
| rs188490030                         | A/G               | rs181930530                | A/G               |
| rs188345926                         | C/T               | rs181819577                | A/G               |
| rs188174426                         | A/G               | rs181706697                | C/T               |
| rs187897435                         | C/T               | rs181400061                | G/T               |
| rs187880261                         | A/G               | rs181240610                | G/T               |
| rs187729939                         | G/T               | rs181150977                | A/C               |
| rs187617985                         | A/T               | rs180848617                | A/G               |
| rs187344319                         | C/T               | rs180765564                | A/G               |
| rs187136933                         | C/T               | rs151330569                | C/G               |
| rs186823043                         | C/G               | rs151258227                | C/T               |
| rs186764765                         | C/T               | rs151174822                | C/T               |
| rs186663247                         | A/G               | rs150874684                | C/T               |
| rs186526524                         | A/G               | rs150589670                | A/G               |
| rs150274370                         | C/T               | rs145059663                | C/T               |
| rs150090766                         | A/G               | rs144588417                | C/T               |
| rs149977222                         | A/G               | rs144457474                | A/G               |
| rs149821411                         | C/T               | rs144322728                | C/T               |
| rs149542384                         | A/G               | rs143897456                | -/C               |
| rs149336018                         | C/G               | rs143569397                | G/T               |
| rs149283940                         | C/T               | rs143476664                | C/T               |
| rs149259830                         | C/T               | rs143473232                | C/G               |
| rs149241235                         | C/T               | rs143436373                | G/T               |
| rs149018062                         | C/T               | rs143166254                | A/G               |
| rs148935291                         | C/T               | rs143011702                | C/G               |
| rs148921342                         | -/A               | rs142994969                | A/G               |
| rs148614030                         | C/T               | rs142880704                | A/G               |
| rs148584540                         | C/T               | rs142630342                | A/G               |
| rs148532075                         | A/C               | rs142522782                | -/AAAC            |
| rs148516171                         | C/T               | rs142443502                | C/T               |
| rs148394305                         | -/AA              | rs142305093                | C/T               |
| rs148267541                         | C/T               | rs142289599                | A/G               |
| rs148213958                         | C/T               | rs142088963                | A/C               |
| rs147637634                         | A/G               | rs141765732                | A/C               |
| rs147372931                         | A/C/G             | rs141386881                | A/T               |
| rs147350096                         | A/C               | rs141291994                | A/G               |
| rs147288996                         | C/T               | rs141285041                | C/T               |
| rs147194882                         | G/T               | rs141220649                | C/T               |



| Table D2                            |                   |                            |                   |
|-------------------------------------|-------------------|----------------------------|-------------------|
| Human Histidyl tRNA synthetase SNPs |                   |                            |                   |
| Gene Bank Accession Number          | Nucleotide Change | Gene Bank Accession Number | Nucleotide Change |
| rs147185134                         | C/T               | rs141147961                | -/C               |
| rs147172925                         | A/G               | rs141123446                | -/A               |
| rs147011612                         | C/T               | rs140516034                | A/G               |
| rs147001782                         | A/G               | rs140169815                | C/T               |
| rs146922029                         | C/T               | rs140005970                | G/T               |
| rs146835587                         | A/G               | rs139699964                | C/T               |
| rs146820726                         | C/T               | rs139555499                | A/G               |
| rs146801682                         | C/T               | rs139447495                | C/T               |
| rs146571500                         | G/T               | rs139364834                | -/A               |
| rs146560255                         | C/T               | rs139362540                | A/G               |
| rs146205151                         | -/A               | rs139300653                | -/A               |
| rs146159952                         | A/G               | rs139251223                | A/G               |
| rs145532449                         | C/G               | rs139145072                | A/G               |
| rs145446993                         | A/G               | rs138612783                | A/G               |
| rs145112012                         | G/T               | rs138582560                | A/G               |
| rs138414368                         | A/G               | rs111863295                | C/T               |
| rs138377835                         | A/G               | rs111519226                | C/G               |
| rs138300828                         | C/T               | rs111314092                | C/T               |
| rs138067637                         | C/T               | rs80074170                 | A/T               |
| rs138035024                         | A/G               | rs79408883                 | A/C               |
| rs137973748                         | C/G               | rs78741041                 | G/T               |
| rs137917558                         | A/G               | rs78677246                 | A/T               |
| rs117912126                         | A/T               | rs78299006                 | A/G               |
| rs117579809                         | G/T               | rs78085183                 | A/T               |
| rs116730458                         | C/T               | rs77844754                 | C/T               |
| rs116411189                         | A/C               | rs77585983                 | A/T               |
| rs116339664                         | C/T               | rs77576083                 | A/G               |
| rs116203404                         | A/T               | rs77154058                 | G/T               |
| rs115091892                         | G/T               | rs76999025                 | A/G               |
| rs114970855                         | A/G               | rs76496151                 | C/T               |
| rs114176478                         | A/G               | rs76471225                 | G/T               |
| rs113992989                         | C/T               | rs76085408                 | G/T               |
| rs113720830                         | C/T               | rs75409415                 | A/G               |
| rs113713558                         | A/C               | rs75397255                 | C/G               |
| rs113627177                         | G/T               | rs74336073                 | A/G               |
| rs113489608                         | A/C               | rs73791750                 | C/T               |
| rs113408729                         | G/T               | rs73791749                 | A/T               |
| rs113255561                         | A/G               | rs73791748                 | C/T               |



| Table D2                            |                   |                            |                   |
|-------------------------------------|-------------------|----------------------------|-------------------|
| Human Histidyl tRNA synthetase SNPs |                   |                            |                   |
| Gene Bank Accession Number          | Nucleotide Change | Gene Bank Accession Number | Nucleotide Change |
| rs113249111                         | C/T               | rs73791747                 | A/T               |
| rs113209109                         | A/G               | rs73273304                 | C/T               |
| rs113066628                         | G/T               | rs73271596                 | C/T               |
| rs112967222                         | C/T               | rs73271594                 | C/T               |
| rs112957918                         | A/T               | rs73271591                 | A/G               |
| rs112859141                         | A/G               | rs73271586                 | A/T               |
| rs112769834                         | C/G               | rs73271585                 | A/G               |
| rs112769758                         | A/C               | rs73271584                 | A/G               |
| rs112701444                         | A/C               | rs73271581                 | C/T               |
| rs112585944                         | A/G               | rs73271578                 | A/T               |
| rs112439761                         | A/G               | rs72800925                 | G/T               |
| rs112427345                         | A/C               | rs72800924                 | C/T               |
| rs112265354                         | C/T               | rs72800922                 | A/T               |
| rs112113896                         | C/G               | rs72432753                 | -/A               |
| rs112033118                         | C/T               | rs72427948                 | -/A               |
| rs112029988                         | A/G               | rs72388191                 | -/A               |
| rs72317985                          | -/A               | rs6873628                  | C/T               |
| rs71583608                          | G/T               | rs5871749                  | -/C               |
| rs67251579                          | -/A               | rs4334930                  | A/T               |
| rs67180750                          | -/A               | rs3887397                  | A/G               |
| rs63429961                          | A/T               | rs3776130                  | A/C               |
| rs61093427                          | C/T               | rs3776129                  | C/T               |
| rs61059042                          | -/A               | rs3776128                  | A/G               |
| rs60936249                          | -/AA              | rs3177856                  | A/C               |
| rs60916571                          | -/A               | rs2563307                  | A/G               |
| rs59925457                          | C/T               | rs2563306                  | A/G               |
| rs59702263                          | -/A               | rs2563305                  | C/T               |
| rs58302597                          | C/T               | rs2563304                  | A/G               |
| rs57408905                          | A/T               | rs2530242                  | C/G               |
| rs35790592                          | A/C               | rs2530241                  | A/G               |
| rs35609344                          | -/A               | rs2530240                  | A/G               |
| rs35559471                          | -/A               | rs2530239                  | A/G               |
| rs35217222                          | -/C               | rs2530235                  | A/C               |
| rs34903998                          | -/A               | rs2230361                  | C/T               |
| rs34790864                          | C/G               | rs2073512                  | C/T               |
| rs34732372                          | C/T               | rs1131046                  | C/T               |
| rs34291233                          | -/C               | rs1131045                  | C/G               |



| Table D2                            |                   |                            |                                   |
|-------------------------------------|-------------------|----------------------------|-----------------------------------|
| Human Histidyl tRNA synthetase SNPs |                   |                            |                                   |
| Gene Bank Accession Number          | Nucleotide Change | Gene Bank Accession Number | Nucleotide Change                 |
| rs34246519                          | -/T               | rs1131044                  | C/T                               |
| rs34176495                          | -/C               | rs1131043                  | C/G                               |
| rs13359823                          | A/G               | rs1131042                  | A/C                               |
| rs13180544                          | A/C               | rs1131041                  | C/G                               |
| rs12653992                          | A/C               | rs1131040                  | A/G                               |
| rs12652092                          | A/G               | rs1131039                  | C/T                               |
| rs11954514                          | A/C               | rs1131038                  | A/G                               |
| rs11745372                          | C/T               | rs1131037                  | A/G                               |
| rs11548125                          | A/G               | rs1131036                  | A/G                               |
| rs11548124                          | C/G               | rs1131035                  | C/T                               |
| rs11344157                          | -/C               | rs1131034                  | A/G                               |
| rs11336085                          | -/A               | rs1131033                  | A/G                               |
| rs11318345                          | -/A               | rs1131032                  | A/G                               |
| rs11309606                          | -/A               | rs1089305                  | A/G                               |
| rs10713463                          | -/A               | rs1089304                  | A/C                               |
| rs7706544                           | C/T               | rs1065342                  | A/C                               |
| rs7701545                           | A/T               | rs1050252                  | C/T                               |
| rs6880190                           | C/T               | rs1050251                  | A/T                               |
| rs1050250                           | A/G               | rs145769024                | -/AAACAAAACAAAACA (SEQ ID NO:164) |
| rs1050249                           | C/T               | rs10534452                 | -/AAAAC                           |
| rs1050248                           | A/C/T             | rs10534451                 | -/AAACAAAACA (SEQ ID NO:165)      |
| rs1050247                           | C/T               | rs59554063                 | -/CAAAACAAAA (SEQ ID NO:166)      |
| rs1050246                           | C/G               | rs58606188                 | -/CAAAACAAAACAAAA (SEQ ID NO:167) |
| rs1050245                           | C/T               | rs71835204                 | (LARGEDELETION)/-                 |
| rs1050222                           | C/T               | rs71766955                 | (LARGEDELETION)/-                 |
| rs813897                            | A/G               | rs144998196                | -/AAACAAAACA (SEQ ID NO:168)      |
| rs812381                            | C/G               | rs68038188                 | -/ACAAAACAAA (SEQ ID NO:169)      |
| rs811382                            | C/T               | rs71980275                 | -/AAAAC                           |
| rs801189                            | C/T               | rs71848069                 | -/AAAC                            |
| rs801188                            | A/C               | rs60987104                 | -/AAAC                            |
| rs801187                            | A/T               | rs801185                   | C/T                               |
| rs801186                            | A/G               | rs702396                   | C/G                               |



[0091] Additionally homologs and orthologs of the human gene exist in other species, as listed in Table D3, and it would thus be a routine matter to select a naturally occurring amino acid, or nucleotide variant present in a SNP, or other naturally occurring homolog in place of any of the human HRS polypeptide sequences listed in Tables D1, D4-D6, or D8.

| Table D3                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |            |
|--------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| Homologs of Human Histidyl tRNA synthetase |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |            |
| Type / species /Residues                   | Amino acid Sequences                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | SEQ ID NO: |
| Mus musculus                               | MADRAALEELVRLQGAHVRLKEQKASAEQIEEEVTKLLKLKAQL<br>GQDEGKQKFVLKTPKGTRDYSPRQMAVREKVFDVIIRCFKRHGAEV<br>IDTPVFELKETLTGKYGEDSKLIYDLKDQGGELLSLRYDLTVPFARY<br>LAMNKLNIKRYHIAKVYRRDNPAMTRGRYREFYQCDFDIAGQFDP<br>MIPDAECLKIMCEILSSLQIGNFLVKVNDRRILDGMFAVCGVPDSKFR<br>TICSSVDKLDKVSWEVKNEMVGEKGLAPEVADRIGDYVQQHGGV<br>SLVEQLLQDPKLSQNKQAVEGLGDLKLLFEYLTLFGIDDKISFDLSLA<br>RGLDYYTGVIYEAVLLQMPTQAGEEPLGVGSIAAGGRYDGLVGMF<br>DPKGRKVPCVGLSIGVERIFSIVEQRLEAEEKVRTTETQVLVASAQK<br>KLLLEERLKL VSELWDAGIKAELL YKKNPKLLNQLQYWEEAGIPLVAI<br>IGEQLRDLGVIKLRSVASREEVDVRREDLVEEIRRTNQPLSTC      | 19         |
| Canis lupus familiaris                     | MAERAALEELVRLQGERVRGLKQOKASAEQIEEEVAKLLKLKAQLG<br>PDEGKQKFVLKTPKGTRDYSPRQMAVREKVFDVIISCFKRHGAEVID<br>TPVFELKETLTGKYGEDSKLIYDLKDQGGELLSLRYDLTVPFARYLA<br>MNKLNIKRYHIAKVYRRDNPAMTRGRYREFYQCDFDIAGQFDPMI<br>PDAECLEIMCEILRSLQIGDFL VKVNDRRILDGMFAICGVPDSKFR TIC<br>SSVDKLDKVSWEVKNEMVGEKGLAPEVADHIGDYVQQHGGISLV<br>EQLLQDPELSQNKQALEGLGDLKLLFEYLT LFGIADKISFDLSLARGL<br>DYYTGVIYEAVLLQTPVQAGEEPLGVGSVAAGGRYDGLVGMFDPK<br>GRKVPCVGLSIGVERIFSIVEQRLEATEEKVRTTETQVLVASAQKLL<br>EERLKL VSELWNAGIKAELL YKKNPKLLNQLQYCEEAGIPLVAIIGE<br>QELKDGVIKLRSVASREEVDVPREDLVEEIKRRTSQPFCIC  | 20         |
| Bos taurus                                 | MADRAALEDLVRVQGERVRGLKQOKASAEQIEEEVAKLLKLKAQL<br>GPDEGKPKFVLKTPKGTRDYSPRQMAVREKVFDVIISCFKRHGAEVI<br>DTPVFELKETLTGKYGEDSKLIYDLKDQGGELLSLRYDLTVPFARYL<br>AMNKLNIKRYHIAKVYRRDNPAMTRGRYREFYQCDFDIAGQFDP<br>MLPDAECLKIMCEILSSLQIGDFL VKVNDRRILDGMFAICGVPDSKFR<br>TICSSVDKLDKVSWEVKNEMVGEKGLAPEVADRIGDYVQQHGGV<br>SLVEQLLQDPKLSQNKQALEGLGDLKLLFEYLT LFGIADKISFDLSLA<br>RGLDYYTGVIYEAVLLQPPARAGEEPLGVGSVAAGGRYDGLVGMF<br>DPKGRKVPCVGLSIGVERIFSIVEQRLEALEEKVRTTETQVLVASAQK<br>KLLLEERLKLISELWDAGIKAELL YKKNPKLLNQLQYCEETGIPLVAII<br>GEQELKDGVIKLRSVASREEVDVRREDLVEEIKR RTSQPLCIC | 21         |
| Rattus norvegicus                          | MADRAALEELVRLQGAHVRLKEQKASAEQIEEEVTKLLKLKAQL<br>GHDEGKQKFVLKTPKGTRDYSPRQMAVREKVFDVIIRCFKRHGAEV<br>IDTPVFELKETLTGKYGEDSKLIYDLKDQGGELLSLRYDLTVPFARY<br>LAMNKLNIKRYHIAKVYRRDNPAMTRGRYREFYQCDFDIAGQFDP<br>MIPDAECLKIMCEILSSLQIGNFQVKVNDRRILDGMFAVCGVPDSKF<br>RTICSSVDKLDKVSWEVKNEMVGEKGLAPEVADRIGDYVQQHGG<br>VSLVEQLLQDPKLSQNKQAVEGLGDLKLLFEYLT LFGIDDKISFDLSL<br>ARGLDYYTGVIYEAVLLQMPTQAGEEPLGVGSIAAGGRYDGLVGM<br>FDPKGRKVPCVGLSIGVERIFSIVEQKLEAEEKVRTTETQVLVASAQ<br>KKLLEERLKLISELWDAGIKAELL YKKNPKLLNQLQYCEEAGIPLVAI<br>IGEQLKDGVIKLRSVTSREEVDVRREDLVEEIRRTSQPLSM         | 22         |
| Gallus gallus                              | MADEAAVRQQAEEVVRRLKQDKAEPDEIAKEVAKLLEMKAHLGGD<br>EGKHKFVLKTPKGTRDYGPKQMAIRERVFSAIACFKRHGAEVIDTP<br>VFELKETLTGKYGEDSKLIYDLKDQGGELLSLRYDLTVPFARYLAM<br>NKITNIKRYHIAKVYRRDNPAMTRGRYREFYQCDFDIAGQFDPMPD<br>AECLKIVQEILSDLQLGDFLIKVNDRRILDGMFAVCGVPDSKFR TIC<br>SVDKLDKMPWEEVRNEMVGEKGLSPEAADRIGEYVQLHGGMDLIE<br>QLLQDPKLSQNKLVKEGLGDMKLLFEYLT LFGITGKISFDLSLARGL<br>DYYTGVIYEAVLLQQNDHGEESVSVGSVAGGGRYDGLVGMFDPKG<br>RKVPCVGISIGIERIFSILEQRVEAEEKIRTTETQVLVASAQKKLEER                                                                                                        | 23         |



| Table D3                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |            |
|--------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| Homologs of Human Histidyl tRNA synthetase |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |            |
| Type / species /Residues                   | Amino acid Sequences                                                                                                                                                                                                                                                                                                                                                                                                                                                              | SEQ ID NO: |
|                                            | LKLISELWDAGIKAEVLYKKNPKLLNQLQYCEDTGIPLVAIVGEQEL<br>KDGVVKLRVVATGEEVNIRRESLVEEIRRTNQL                                                                                                                                                                                                                                                                                                                                                                                              |            |
| Danio rerio                                | MAALGLVSMRLCAGLMGRRSAVRLHSLRVCSGMTISQIDEEVARLL<br>QLKAQLGGDEGKHVFLKTAKGTRDYNPKQMAIREKVFNIINC<br>RHGAETIDSPVFELKETLTGKYGEDSKLIYDLKDQGGELLSRYDLT<br>VPFARYLAMNKITNIKRYHIAKVYRRDNPAMTRGRYREFYQCDFDI<br>AGQYDAMIPDAECLKLVEILSELDLGDGDFRIKVNDRRLDGMFAICG<br>VPDEKFRITCSTVDKLDKLAWEVKKEMVNEKGLSEEVADRIRDYV<br>SMQGGKDLAERLLQDPKLSQSKQACAGITDMKLLFSYLELFQITDK<br>VFDLSLARGLDYTGVIYEAILTQANPAPASTPAEQNGAEDAGVSV<br>GSVAGGGRYDGLVGMFDPKAGKCPVWGSALALRGSSPSWSRRQSC<br>LQRRCAPLKLKCLWLQHRRTF | 24         |

[0092] Accordingly, in any of the methods therapeutic compositions and kits of the disclosure, the terms "HRS polypeptide" "HRS protein" or "HRS protein fragment" includes all naturally-occurring and synthetic forms of the histidyl-tRNA synthetase that possesses a non canonical activity, such as an anti-inflammatory activity and/or retains at least one epitope which specifically cross reacts with an auto-antibody or auto reactive T-cell from a subject with a disease associated with autoantibodies to histidyl-tRNA synthetase. Such HRS polypeptides include the full length human protein, as well as the HRS peptides derived from the full length protein listed in **Tables D1, D3-D6, or D8**. In some embodiments of the present disclosure, the term HRS polypeptide refers to a polypeptide sequence derived from human histidyl-tRNA synthetase (SEQ ID NO:1 in **Table D1**) of about 45 or 50 to about 250 amino acids in length. It will be appreciated that in any of HRS-Fc conjugates described herein the N-terminal acid of the HRS polypeptide (for example, the N-terminal Met) may be deleted from any of the sequences listed in **Tables D1, D3-D6, or D8** when creating the fusion protein or conjugate.

[0093] In some embodiments of the present disclosure, the HRS polypeptide is between about 20-509, 20-508, 20-507, 50-506, 20-505, 50-504, 20-503, 20-502, 20-501, 20-500, 20-400, 20-300, 20-250, 20-200, or 20-100 amino acids in length. For instance, in specific embodiments of the present disclosure the polypeptide is between about 20-25, 20-35, 20-40, 20-45, 20-55, 20-60, 20-65, 20-70, 20-75, 20-80, 20-85, 20-90, 20-95, or 20-100 amino acids in length, or about 30-35, 30-40, 30-45, 30-55, 30-60, 30-65, 30-70, 30-75, 30-80, 30-85, 30-90, 30-95, or 30-100 amino acids in length, or about 40-45, 40-55, 40-60, 40-65, 40-70, 40-75, 40-80, 40-85, 40-90, 40-95, or 40-100 amino acids in length, or about 45-50, 45-55, 50-55, 50-60, 50-65, 50-70, 50-75, 50-80, 50-85, 50-90, 50-95, or 50-100 amino acids in length, or about 60-65, 60-70, 60-75, 60-80, 60-85, 60-90, 60-95, or 60-100 amino acids in length, or about 70-75, 70-80, 70-85, 70-90, 70-95, or 70-100 amino acids in length, or about 80-85, 80-90, 80-95, or 80-100 amino acids in length. In certain embodiments of the present disclosure, the HRS polypeptide is about 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 150, 200, 250, 300, 350, 400, 450, 500, 501, 502, 503, 504, 505, 506, 507, 508, or 509 amino acids in length.

[0094] In some embodiments of the present disclosure, the HRS polypeptide does not significantly compete for disease associated auto-antibody binding (e.g., Jo-1 antibody) to wild type histidyl-tRNA synthetase in a competitive ELISA up to a concentration of about 1 to 5 x 10<sup>-7</sup>M, or higher. Accordingly, in some embodiments of the present disclosure, the HRS polypeptide has a lower affinity to disease associated auto-antibody than



wild type histidyl-tRNA synthetase (SEQ ID NO:1) as measured in a competitive ELISA. In some embodiments of the present disclosure, the HRS polypeptide has an apparent affinity for the disease associated auto-antibody (e.g., Jo-1 antibody) which is at least about 10 fold less, or at least about 20 fold less, or at least about 50 fold less, or at least about 100 fold less than the affinity of the disease associated auto-antibody to wild type human (SEQ ID NO:1).

**[0095]** Thus all such homologues, orthologs, and naturally-occurring, or synthetic isoforms of histidyl-tRNA synthetase (e.g., any of the proteins listed in **Tables D1, D3-D6, or D8**) are included in any of the methods, HRS-Fc conjugates, kits and compositions of the disclosure, as long as they retain at least one epitope which specifically cross reacts with an auto-antibody or auto reactive T-cell from a subject with a disease associated with autoantibodies to histidyl tRNA synthetase, or possess a non canonical activity. The HRS polypeptides may be in their native form, *i.e.*, as different variants as they appear in nature in different species which may be viewed as functionally equivalent variants of human histidyl -tRNA synthetase, or they may be functionally equivalent natural derivatives thereof, which may differ in their amino acid sequence, e.g., by truncation (e.g., from the N- or C-terminus or both) or other amino acid deletions, additions, insertions, substitutions, or post-translational modifications. Naturally-occurring chemical derivatives, including post-translational modifications and degradation products of any HRS polypeptide, are also specifically included in any of the methods and compositions of the disclosure including, e.g., pyroglutamyl, iso-aspartyl, proteolytic, phosphorylated, glycosylated, oxidatized, isomerized, and deaminated variants of a HRS polypeptide or HRS-Fc conjugate. HRS polypeptides and HRS-Fc conjugates can also be composed of naturally-occurring amino acids and/or non-naturally occurring amino acids, as described herein.

**[0096]** As noted above, embodiments of the present disclosure of the present disclosure include all homologues, orthologs, and naturally-occurring isoforms of histidyl-tRNA synthetase (e.g., any of the proteins listed in or derivable from, or their corresponding nucleic acids listed in, the Tables or the Sequence Listing) and "variants" of these HRS reference polypeptides. The recitation polypeptide "variant" refers to polypeptides that are distinguished from a reference HRS polypeptide by the addition, deletion, and/or substitution of at least one amino acid residue, and which typically retain (e.g., mimic) or modulate (e.g., antagonize) one or more non-canonical activities of a reference HRS polypeptide. Variants also include polypeptides that have been modified by the addition, deletion, and/or substitution of at least one amino acid residue to have improved stability or other pharmaceutical properties.

**[0097]** In certain embodiments of the present disclosure, a polypeptide variant is distinguished from a reference polypeptide by one or more substitutions, which may be conservative or non-conservative, as described herein and known in the art. In certain embodiments of the present disclosure, the polypeptide variant comprises conservative substitutions and, in this regard, it is well understood in the art that some amino acids may be changed to others with broadly similar properties without changing the nature of the activity of the polypeptide. In some embodiments of the present disclosure, the variant comprises one or more conserved residues, including one or more of Leu7, Gln14, Gly15, Val18, Arg19, Leu21, Lys22, Lys25, Ala26, Val35, Leu38, Leu39, Leu41, and Lys 42 (based on the numbering of SEQ ID NO:1).

**[0098]** Specific examples of HRS polypeptide variants useful in any of the methods and compositions of the disclosure include full-length HRS polypeptides, or truncations or splice variants thereof (e.g., any of the proteins listed in or derivable from the Tables or Sequence Listing) which i) retain detectable non canonical activity and/or retain at least one epitope which specifically cross reacts with an auto-antibody or auto reactive T-cell from a subject with a disease associated with autoantibodies to histidyl-tRNA synthetase, and ii) have one or more additional amino acid insertions, substitutions, deletions, and/or truncations. In certain embodiments of the present disclosure, a variant polypeptide includes an amino acid sequence having at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or more sequence identity or similarity to a corresponding sequence of a HRS reference polypeptide, as described herein, (e.g., any of the proteins listed in or derivable from the Tables or Sequence Listing) and substantially



retains the non-canonical activity of that reference polypeptide. Also included are sequences differing from the reference HRS sequences by the addition, deletion, or substitution of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150 or more amino acids but which retain the properties of the reference HRS polypeptide. In certain embodiments of the present disclosure, the amino acid additions or deletions occur at the C-terminal end and/or the N-terminal end of the HRS reference polypeptide. In certain embodiments of the present disclosure, the amino acid additions include 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50 or more wild-type residues (*i.e.*, from the corresponding full-length HRS polypeptide) that are proximal to the C-terminal end and/or the N-terminal end of the HRS reference polypeptide.

[0099] In some embodiments of the present disclosure, the HRS polypeptides comprise a polypeptide fragment of the full length histidyl tRNA synthetase of about 45 to 250 or about 50 to 250 amino acids, which comprises, consists, or consists essentially of the amino acids of the HRS polypeptide sequence set forth in one or more of SEQ ID NOS:1-106, 170-181, or 185-191. In some embodiments of the present disclosure, the HRS polypeptide comprises, consists, or consists essentially of residues 1-141, 1-408, 1-113, or 1-60 of SEQ ID NO:1. In some aspects of the present disclosure, the HRS polypeptide is a splice variant that comprises, consists, or consists essentially of residues 1-60+175-509, 1-60+211-509 or 1-60+101-509 of SEQ ID NO:1. In particular aspects of the present disclosure, the HRS polypeptide comprises, consists, or consists essentially of residues 1-48 or 1-506 of SEQ ID NO:1.

[0100] In certain embodiments of the present disclosure, a HRS polypeptide of the disclosure comprises, consists, or consists essentially of the minimal active fragment of a full-length HRS polypeptide capable of modulating an anti-inflammatory activity *in vivo* or having antibody or auto-reactive T-cell blocking activities. In one aspect of the present disclosure, such a minimal active fragment comprises, consists, or consists essentially of the WHEP domain, (*i.e.*, about amino acids 1-43 of SEQ ID NO:1). In some aspects of the present disclosure, the minimal active fragment comprises, consists, or consists essentially of the aminoacylation domain, (*i.e.*, about amino acids 54-398 of SEQ ID NO:1). In some aspects of the present disclosure, the minimal active fragment comprises, consists, or consists essentially of the anticodon binding domain (*i.e.*, about amino acids 406-501 of SEQ ID NO:1). Other exemplary active fragments are shown in Table D4 below.

| Table D4                            |                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |            |
|-------------------------------------|-----------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| Exemplary HRS polypeptide fragments |                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |            |
| Name                                | Amino Acid Residue Range of SEQ ID NO:1 | Amino acid sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | SEQ ID NO: |
| HRS(1-500)                          | Protein / Human / 1-500                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSRQMAVREKVFDVII<br>RCFKRHGAVIDTPVFELKETLMGKYGEDSKLIYDLKDQGG<br>ELLSLRYDLTVPFARYLAMNKLNIKRYHIAKVYRRDNPAM<br>TRGRYREFYQCDFDIAGNFDPMIPDAECLKIMCEILSSLQIGD<br>FLVKVNDRRILDGMFAICGVSDSKFRTICSSVDKLDKVSWE<br>EVKNEMVGEKGLAPEVADRIGDYVQQHGGVSLVEQLLQDP<br>KLSQNKQALEGLGDLKLLFEYLTDFGIDDKISFDLSLARGLD<br>YYTGVIYEAVLLQTPAQAGEEPLGVGSVAAGGRYDGLVGM<br>FDPKGRKVPCVGLSIGVERIFSIVEQRLEALEEKIRTTETQVL<br>VASAQKKLLEERLKLVSELWDAGIKAELLYKKNPKLLNQL<br>QYCEEAGIPLVAHGEQELKDGVIKLSVTSREEVDVRREDL<br>VEEIKR | 170        |
| HRS(1-501)                          | Protein / Human / 1-501                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSRQMAVREKVFDVII<br>RCFKRHGAVIDTPVFELKETLMGKYGEDSKLIYDLKDQGG<br>ELLSLRYDLTVPFARYLAMNKLNIKRYHIAKVYRRDNPAM<br>TRGRYREFYQCDFDIAGNFDPMIPDAECLKIMCEILSSLQIGD<br>FLVKVNDRRILDGMFAICGVSDSKFRTICSSVDKLDKVSWE<br>EVKNEMVGEKGLAPEVADRIGDYVQQHGGVSLVEQLLQDP<br>KLSQNKQALEGLGDLKLLFEYLTDFGIDDKISFDLSLARGLD<br>YYTGVIYEAVLLQTPAQAGEEPLGVGSVAAGGRYDGLVGM<br>FDPKGRKVPCVGLSIGVERIFSIVEQRLEALEEKIRTTETQVL<br>VASAQKKLLEERLKLVSELWDAGIKAELLYKKNPKLLNQL<br>QYCEEAGIPLVAHGEQELKDGVIKLSVTSREEVDVRREDL<br>VEEIKR | 171        |



| Table D4                            |                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |            |
|-------------------------------------|-----------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| Exemplary HRS polypeptide fragments |                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |            |
| Name                                | Amino Acid Residue Range of SEQ ID NO:1 | Amino acid sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | SEQ ID NO: |
|                                     |                                         | EVKNEMVGEKGLAPEVADRIGDYVQQHGGVSLVEQLLQDP<br>KLSQNKQALEGLGDLKLLFEYLTGIDDKISFDLSLARGLD<br>YYTGVIYEAVLLQTPAQAGEEPLGVGSVAAGGRYDGLVGM<br>FDPKGRKVPCVGLSIGVERIFSIVEQRLEALEEKIRTTETQVL                                                                                                                                                                                                                                                                                                                                                                                  |            |
|                                     |                                         | VASAQKKLLEERLKLVSELWDAGIKAELLYKKNPKLLNQL<br>QYCEEAGIPLVAIIGEQLKDGVILRSVTSREEVDVRREDL<br>VEEIKRR                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |            |
| HRS(1-502)                          | Protein / Human / 1-502                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVREKVFDVII<br>RCFKRHGAVIDTPVFELKETLMGKYGEDSKLIYDLKDQGG<br>ELLSLRYDLTPPFARYLAMNKLNIKRYHIAKVYRRDNPAM<br>TRGRYREFYQCDFDIAGNFDPMPDAECLKIMCEILSSLQIGD<br>FLVKVNDRIIDGMFAICGVSDSKFRTICSSVDKLDKVSWE<br>EVKNEMVGEKGLAPEVADRIGDYVQQHGGVSLVEQLLQDP<br>KLSQNKQALEGLGDLKLLFEYLTGIDDKISFDLSLARGLD<br>YYTGVIYEAVLLQTPAQAGEEPLGVGSVAAGGRYDGLVGM<br>FDPKGRKVPCVGLSIGVERIFSIVEQRLEALEEKIRTTETQVL<br>VASAQKKLLEERLKLVSELWDAGIKAELLYKKNPKLLNQL<br>QYCEEAGIPLVAIIGEQLKDGVILRSVTSREEVDVRREDL<br>VEEIKRRT   | 172        |
| HRS(1-503)                          | Protein / Human / 1-503                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVREKVFDVII<br>RCFKRHGAVIDTPVFELKETLMGKYGEDSKLIYDLKDQGG<br>ELLSLRYDLTPPFARYLAMNKLNIKRYHIAKVYRRDNPAM<br>TRGRYREFYQCDFDIAGNFDPMPDAECLKIMCEILSSLQIGD<br>FLVKVNDRIIDGMFAICGVSDSKFRTICSSVDKLDKVSWE<br>EVKNEMVGEKGLAPEVADRIGDYVQQHGGVSLVEQLLQDP<br>KLSQNKQALEGLGDLKLLFEYLTGIDDKISFDLSLARGLD<br>YYTGVIYEAVLLQTPAQAGEEPLGVGSVAAGGRYDGLVGM<br>FDPKGRKVPCVGLSIGVERIFSIVEQRLEALEEKIRTTETQVL<br>VASAQKKLLEERLKLVSELWDAGIKAELLYKKNPKLLNQL<br>QYCEEAGIPLVAIIGEQLKDGVILRSVTSREEVDVRREDL<br>VEEIKRRTG  | 173        |
| HRS(1-504)                          | Protein / Human / 1-504                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVREKVFDVII<br>RCFKRHGAVIDTPVFELKETLMGKYGEDSKLIYDLKDQGG<br>ELLSLRYDLTPPFARYLAMNKLNIKRYHIAKVYRRDNPAM<br>TRGRYREFYQCDFDIAGNFDPMPDAECLKIMCEILSSLQIGD<br>FLVKVNDRIIDGMFAICGVSDSKFRTICSSVDKLDKVSWE<br>EVKNEMVGEKGLAPEVADRIGDYVQQHGGVSLVEQLLQDP<br>KLSQNKQALEGLGDLKLLFEYLTGIDDKISFDLSLARGLD<br>YYTGVIYEAVLLQTPAQAGEEPLGVGSVAAGGRYDGLVGM<br>FDPKGRKVPCVGLSIGVERIFSIVEQRLEALEEKIRTTETQVL<br>VASAQKKLLEERLKLVSELWDAGIKAELLYKKNPKLLNQL<br>QYCEEAGIPLVAIIGEQLKDGVILRSVTSREEVDVRREDL<br>VEEIKRRTGQ | 174        |
| HRS(1-505)                          | Protein / Human / 1-505                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVREKVFDVII<br>RCFKRHGAVIDTPVFELKETLMGKYGEDSKLIYDLKDQGG<br>ELLSLRYDLTPPFARYLAMNKLNIKRYHIAKVYRRDNPAM<br>TRGRYREFYQCDFDIAGNFDPMPDAECLKIMCEILSSLQIGD<br>FLVKVNDRIIDGMFAICGVSDSKFRTICSSVDKLDKVSWE<br>EVKNEMVGEKGLAPEVADRIGDYVQQHGGVSLVEQLLQDP<br>KLSQNKQALEGLGDLKLLFEYLTGIDDKISFDLSLARGLD<br>YYTGVIYEAVLLQTPAQAGEEPLGVGSVAAGGRYDGLVGM<br>FDPKGRKVPCVGLSIGVERIFSIVEQRLEALEEKIRTTETQVL                                                                                                       | 175        |



| Table D4                            |                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |            |
|-------------------------------------|-----------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| Exemplary HRS polypeptide fragments |                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |            |
| Name                                | Amino Acid Residue Range of SEQ ID NO:1 | Amino acid sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | SEQ ID NO: |
|                                     |                                         | FDPKGRKVPCVGLSIGVERIFSIVEQRLEALEEKIRTTETQVL<br>VASAQKKLLEERLKLVSELWDAGIKAELLYKKNPKLLNQL<br>QYCEEAGIPLVAIIGEQLKDGVIKLSVTSREEVDVRREDL<br>VEEIKRRTGQP                                                                                                                                                                                                                                                                                                                                                                                                                         |            |
| HisRS 1 <sup>N8</sup><br>HRS(1-506) | Protein / Human / 1-506                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVREKVFDVII<br>RCFKRHGAVIDTPVFELKETLMGKYGEDSKLIYDLKDQGG<br>ELLSLRYDLTVPFARYLAMNKL TNIKRYHIAKVYRRDNPAM                                                                                                                                                                                                                                                                                                                                                                                            | 25         |
|                                     |                                         | TRGRYREFYQCDFDIAGNFDPMIPDAECLKIMCEILSSLQIGD<br>FLVKVNDRRILDGMFAICGVSDSKFRTICSSVDKLDKVSWE<br>EVKNEMVGEKGLAPEVADRIGDYVQQHGGVSLVEQLLQDP<br>KLSQNKQALEGLGDLKLLFEYLTFLGIDDKISFDLSLARGLD<br>YYTGVIYEAVLLQTPAQAGEEPLGVGSVAAGGRYDGLVGM<br>FDPKGRKVPCVGLSIGVERIFSIVEQRLEALEEKIRTTETQVL<br>VASAQKKLLEERLKLVSELWDAGIKAELLYKKNPKLLNQL<br>QYCEEAGIPLVAIIGEQLKDGVIKLSVTSREEVDVRREDL<br>VEEIKRRTGQPL                                                                                                                                                                                      |            |
| HRS(1-507)                          | Protein / Human / 1-507                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVREKVFDVII<br>RCFKRHGAVIDTPVFELKETLMGKYGEDSKLIYDLKDQGG<br>ELLSLRYDLTVPFARYLAMNKL TNIKRYHIAKVYRRDNPAM<br>TRGRYREFYQCDFDIAGNFDPMIPDAECLKIMCEILSSLQIGD<br>FLVKVNDRRILDGMFAICGVSDSKFRTICSSVDKLDKVSWE<br>EVKNEMVGEKGLAPEVADRIGDYVQQHGGVSLVEQLLQDP<br>KLSQNKQALEGLGDLKLLFEYLTFLGIDDKISFDLSLARGLD<br>YYTGVIYEAVLLQTPAQAGEEPLGVGSVAAGGRYDGLVGM<br>FDPKGRKVPCVGLSIGVERIFSIVEQRLEALEEKIRTTETQVL<br>VASAQKKLLEERLKLVSELWDAGIKAELLYKKNPKLLNQL<br>QYCEEAGIPLVAIIGEQLKDGVIKLSVTSREEVDVRREDL<br>VEEIKRRTGQPLC  | 176        |
| HRS(1-508)                          | Protein / Human / 1-508                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVREKVFDVII<br>RCFKRHGAVIDTPVFELKETLMGKYGEDSKLIYDLKDQGG<br>ELLSLRYDLTVPFARYLAMNKL TNIKRYHIAKVYRRDNPAM<br>TRGRYREFYQCDFDIAGNFDPMIPDAECLKIMCEILSSLQIGD<br>FLVKVNDRRILDGMFAICGVSDSKFRTICSSVDKLDKVSWE<br>EVKNEMVGEKGLAPEVADRIGDYVQQHGGVSLVEQLLQDP<br>KLSQNKQALEGLGDLKLLFEYLTFLGIDDKISFDLSLARGLD<br>YYTGVIYEAVLLQTPAQAGEEPLGVGSVAAGGRYDGLVGM<br>FDPKGRKVPCVGLSIGVERIFSIVEQRLEALEEKIRTTETQVL<br>VASAQKKLLEERLKLVSELWDAGIKAELLYKKNPKLLNQL<br>QYCEEAGIPLVAIIGEQLKDGVIKLSVTSREEVDVRREDL<br>VEEIKRRTGQPLCI | 177        |
| HRS(1-509)                          | Protein / Human / 1-509                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVREKVFDVII<br>RCFKRHGAVIDTPVFELKETLMGKYGEDSKLIYDLKDQGG<br>ELLSLRYDLTVPFARYLAMNKL TNIKRYHIAKVYRRDNPAM<br>TRGRYREFYQCDFDIAGNFDPMIPDAECLKIMCEILSSLQIGD<br>FLVKVNDRRILDGMFAICGVSDSKFRTICSSVDKLDKVSWE<br>EVKNEMVGEKGLAPEVADRIGDYVQQHGGVSLVEQLLQDP<br>KLSQNKQALEGLGDLKLLFEYLTFLGIDDKISFDLSLARGLD<br>YYTGVIYEAVLLQTPAQAGEEPLGVGSVAAGGRYDGLVGM<br>FDPKGRKVPCVGLSIGVERIFSIVEQRLEALEEKIRTTETQVL<br>VASAQKKLLEERLKLVSELWDAGIKAELLYKKNPKLLNQL<br>QYCEEAGIPLVAIIGEQLKDGVIKLSVTSREEVDVRREDL                   | 178        |



| Table D4                            |                                         |                                                           |            |
|-------------------------------------|-----------------------------------------|-----------------------------------------------------------|------------|
| Exemplary HRS polypeptide fragments |                                         |                                                           |            |
| Name                                | Amino Acid Residue Range of SEQ ID NO:1 | Amino acid sequence                                       | SEQ ID NO: |
|                                     |                                         | VEEIKRRRTGQPLECIC                                         |            |
| HisRS 1 <sup>N6</sup><br>HRS(1-48)  | Protein /<br>Human / 1-48               | MAERAAL EELVKLQGERV<br>RGLKQKASAE LIEEEVAKLLK<br>LKAQLGPD | 26         |

[0101] For some HRS polypeptides, about or at least about 20-40, 20-45, 20-50, 20-55, or 20-60, 20-65, or 20-67 contiguous or non-contiguous amino acids of the HRS polypeptide are from amino acids 1-67 of SEQ ID NO:1. In particular embodiments of the present disclosure, about 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, or 67 contiguous or non-contiguous amino acids of the HRS polypeptide are from amino acids 1-67 of SEQ ID NO:1. The HRS polypeptide may comprise one or more of a WHEP domain, an aminoacylation domain, an anticodon binding domain, or any combination thereof. In particular embodiments of the present disclosure, the HRS polypeptide lacks a functional aminoacylation domain. In some embodiments of the present disclosure, the polypeptide consists essentially of the WHEP domain from human HRS. Without wishing to be bound by any one theory, the unique orientation, or conformation, of the WHEP domain in certain HRS polypeptides may contribute to the enhanced non canonical, and/or antibody blocking activities observed in these proteins.

[0102] Hence, in certain embodiments of the present disclosure, the HRS polypeptide comprises, consists, or consists essentially of a human HRS WHEP domain sequence. In some embodiments of the present disclosure, the human HRS WHEP domain sequence is defined by certain conserved residues. For example, in some aspects of the present disclosure the HRS polypeptide comprises, consists, or consists essentially of the human HRS WHEP domain consensus sequence in **Table D5** below.

[0103] In certain embodiments of the present disclosure, the HRS polypeptide may comprise 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, or all 29 amino acids of a flexible linker connecting the minimum domain to a heterologous protein (e.g., Fc domain), or splice variant.

[0104] The recitations "sequence identity" or, for example, comprising a "sequence 50% identical to," as used herein, refer to the extent that sequences are identical on a nucleotide-by-nucleotide basis or an amino acid-by-amino acid basis over a window of comparison. Thus, a "percentage of sequence identity" may be calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g., A, T, C, G, I) or the identical amino acid residue (e.g., Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn, Gln, Cys and Met) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity.

[0105] Terms used to describe sequence relationships between two or more polypeptides include "reference sequence," "comparison window," "sequence identity," "percentage of sequence identity" and "substantial identity." A "reference sequence" is at least 12 but frequently 15 to 18 and often at least 25 monomer units, inclusive of nucleotides and amino acid residues, in length. Because two polypeptides may each comprise (1) a sequence (i.e., only a portion of the complete polypeptides sequence) that is similar between the two



polypeptides, and (2) a sequence that is divergent between the two polypeptides, sequence comparisons between two (or more) polypeptides are typically performed by comparing sequences of the two polypeptides over a "comparison window" to identify and compare local regions of sequence similarity. A "comparison window" refers to a conceptual segment of at least 6 contiguous positions, usually about 50 to about 100, more usually about 100 to about 150 in which a sequence is compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. The comparison window may comprise additions or deletions (*i.e.*, gaps) of about 20% or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by computerized implementations of algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Drive Madison, WI, USA) or by inspection and the best alignment (*i.e.*, resulting in the highest percentage homology over the comparison window) generated by any of the various methods selected. Reference also may be made to the BLAST family of programs as for example disclosed by Altschul et al., 1997, Nucl. Acids Res. 25:3389. A detailed discussion of sequence analysis can be found in Unit 19.3 of Ausubel et al., "Current Protocols in Molecular Biology," John Wiley & Sons Inc, 1994-1998, Chapter 15.

**[0106]** Calculations of sequence similarity or sequence identity between sequences (the terms are used interchangeably herein) can be performed as follows. To determine the percent identity of two amino acid sequences, or of two nucleic acid sequences, the sequences can be aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In certain embodiments of the present disclosure, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, 60%, and even more preferably at least 70%, 80%, 90%, 100% of the length of the reference sequence. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position.

**[0107]** The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

**[0108]** The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment of the present disclosure, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch, (1970, J. Mol. Biol. 48: 444-453) algorithm which has been incorporated into the GAP program in the GCG software package, using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment of the present disclosure, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package, using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A particularly preferred set of parameters (and the one that should be used unless otherwise specified) are a Blossum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5. The percent identity between two amino acid or nucleotide sequences can also be determined using the algorithm of E. Meyers and W. Miller (1989, Cabios, 4: 11-17) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

**[0109]** The nucleic acid and protein sequences described herein can be used as a "query sequence" to perform a search against public databases, for example, to identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of



Altschul, et al., (1990, J. Mol. Biol, 215: 403-10). BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to nucleic acid molecules of the disclosure. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to protein molecules of the disclosure. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al. (Nucleic Acids Res. 25:3389-3402, 1997). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used.

[0110] In certain embodiments of the present disclosure, variant polypeptides differ from the corresponding HRS reference sequences by at least 1% but less than 20%, 15%, 10% or 5% of the residues. If this comparison requires alignment, the sequences should be aligned for maximum similarity. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences. The differences are, suitably, differences or changes at a non-essential residue or a conservative substitution. In certain embodiments of the present disclosure, the molecular weight of a variant HRS polypeptide differs from that of the HRS reference polypeptide by about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, or more.

[0111] Also included are biologically active "fragments" of the HRS reference polypeptides, *i.e.*, biologically active fragments of the HRS protein fragments. Representative biologically active fragments generally participate in an interaction, e.g., an intramolecular or an inter-molecular interaction. An inter-molecular interaction can be a specific binding interaction or an enzymatic interaction. An inter-molecular interaction can be between a HRS polypeptide and a cellular binding partner, such as a cellular receptor or other host molecule that participates in the non-canonical activity of the HRS polypeptide.

[0112] A biologically active fragment of an HRS reference polypeptide can be a polypeptide fragment which is, for example, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 220, 240, 260, 280, 300, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 38, 359, 360, 361, 362, 363, 364, 365, 380, 400, 450, 500, 505, or more contiguous or non-contiguous amino acids, including all integers (e.g., 101, 102, 103) and ranges (e.g., 50-100, 50-150, 50-200) in between, of the amino acid sequences set forth in any one of the HRS reference polypeptides described herein. In certain embodiments of the present disclosure, a biologically active fragment comprises a non-canonical activity-related sequence, domain, or motif. In certain embodiments of the present disclosure, the C-terminal or N-terminal region of any HRS reference polypeptide may be truncated by about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 350, 400, 450, 500 or more amino acids, or by about 10-50, 20-50, 50-100, 100-150, 150-200, 200-250, 250-300, 300-350, 350-400, 400-450, 450-500 or more amino acids, including all integers and ranges in between (e.g., 101, 102, 103, 104, 105), so long as the truncated HRS polypeptide retains the non-canonical activity of the reference polypeptide. Certain exemplary truncated HRS polypeptides and a human HRS WHEP domain consensus sequence are shown in Table D5 below.

| Table D5                             |                                                                                         |            |
|--------------------------------------|-----------------------------------------------------------------------------------------|------------|
| Exemplary truncated HRS polypeptides |                                                                                         |            |
| C-terminal truncations               |                                                                                         |            |
| HRS range                            | Sequence                                                                                | SEQ ID NO: |
| 1-80                                 | MAERAAL EELVKLQGERVRGLKQQKASAE LIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVREKVF DVI | 27         |
| 1-79                                 |                                                                                         | 28         |



| Table D5                             |                                                                                      |            |
|--------------------------------------|--------------------------------------------------------------------------------------|------------|
| Exemplary truncated HRS polypeptides |                                                                                      |            |
| C-terminal truncations               |                                                                                      |            |
| HRS range                            | Sequence                                                                             | SEQ ID NO: |
|                                      | MAERAACLEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVREKVFDV |            |
| 1-78                                 | MAERAACLEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVREK VFD | 29         |
| 1-77                                 | MAERAACLEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVREK VF  | 30         |
| 1-76                                 | MAERAACLEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVREKV    | 31         |
| 1-75                                 | MAERAACLEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVREK     | 32         |
| 1-74                                 | MAERAACLEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVRE      | 33         |
| 1-73                                 | MAERAACLEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVR       | 34         |
| 1-72                                 | MAERAACLEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAV        | 35         |
| 1-71                                 | MAERAACLEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMA         | 36         |
| 1-70                                 | MAERAACLEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQM          | 37         |
| 1-69                                 | MAERAACLEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQ           | 38         |
| 1-68                                 | MAERAACLEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPR            | 39         |
| 1-67                                 | MAERAACLEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSP             | 40         |
| 1-66                                 | MAERAACLEELVKLOGERVRLKOOKASAELIEEEVAKLLK                                             | 41         |



| Table D5                             |                                                                       |            |
|--------------------------------------|-----------------------------------------------------------------------|------------|
| Exemplary truncated HRS polypeptides |                                                                       |            |
| C-terminal truncations               |                                                                       |            |
| HRS range                            | Sequence                                                              | SEQ ID NO: |
|                                      | LKAQLGPDESKQKFVLKTPKGTRDYS                                            |            |
| 1-65                                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDY | 42         |
| 1-64                                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRD  | 43         |
| 1-63                                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTR   | 44         |
| 1-62                                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGT    | 45         |
| 1-61                                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKG     | 46         |
| 1-60                                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPK      | 47         |
| 1-59                                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTP       | 48         |
| 1-58                                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKT        | 49         |
| 1-57                                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLK         | 50         |
| 1-56                                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVL          | 51         |
| 1-55                                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFV           | 52         |
| 1-54                                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKF            | 53         |
| 1-53                                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQK             | 54         |



| Table D5                             |                                                                                     |            |
|--------------------------------------|-------------------------------------------------------------------------------------|------------|
| Exemplary truncated HRS polypeptides |                                                                                     |            |
| C-terminal truncations               |                                                                                     |            |
| HRS range                            | Sequence                                                                            | SEQ ID NO: |
|                                      | LKAQLGPDESKQK                                                                       |            |
| 1-52                                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQ                            | 55         |
| 1-51                                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESK                             | 56         |
| 1-50                                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDES                              | 57         |
| 1-49                                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDE                               | 58         |
| 1-48                                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLKLKAQLGPD                                    | 59         |
| 1-47                                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLKLKAQLGP                                     | 60         |
| 1-46                                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLKLKAQLG                                      | 61         |
| 1-45                                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLKLKAQL                                       | 62         |
| 1-44                                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLKLKAQ                                        | 63         |
| 1-43                                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKA                                     | 64         |
| 1-42                                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LK                                      | 65         |
| 1-41                                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>L                                       | 66         |
| 1-40                                 | MAERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK                                            | 67         |
| N-terminal truncations               |                                                                                     |            |
| 2-80                                 | AERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVREKVFDVI | 68         |
| 3-80                                 | ERAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVREKVFDVI  | 69         |
| 4-80                                 | RAALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVREKVFDVI   | 70         |
| 5-80                                 | MAERAALVKLQGERVRGLKQQKASAELIEEEVAKLLK                                               | 71         |



| N-terminal truncations |                                                                                  |    |
|------------------------|----------------------------------------------------------------------------------|----|
|                        | AALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVREKVFDVI |    |
| 6-80                   | ALEELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVREKVFDVI  | 72 |
| 7-80                   | LEELVKLQGERVRGLKQQKASAELIEEEVAKLLK                                               | 73 |
|                        | LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVREKVFDVI                                         |    |
| 8-80                   | EELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVREKVFDVI    | 74 |
| 9-80                   | ELVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVREKVFDVI     | 75 |
| 10-80                  | LVKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVREKVFDVI      | 76 |
| 11-80                  | VKLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVREKVFDVI       | 77 |
| 12-80                  | KLQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVREKVFDVI        | 78 |
| 13-80                  | LQGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVREKVFDVI         | 79 |
| 14-80                  | QGERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVREKVFDVI          | 80 |
| 15-80                  | GERVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVREKVFDVI           | 81 |
| 16-80                  | RVRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVREKVFDVI             | 82 |
| 17-80                  | VRGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVREKVFDVI              | 83 |
| 18-80                  | RGLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSPRQMAVREKVFDVI               | 84 |



| N-terminal truncations |                                                                  |     |
|------------------------|------------------------------------------------------------------|-----|
| 19-80                  | GLKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSRQMAVREKVFDVI | 85  |
| 20-80                  | LKQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSRQMAVREKVFDVI  | 86  |
| 21-80                  | KQQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSRQMAVREKVFDVI   | 87  |
| 22-80                  | QQKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSRQMAVREKVFDVI    | 88  |
| 23-80                  | QKASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSRQMAVREKVFDVI     | 89  |
| 24-80                  | KASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSRQMAVREKVFDVI      | 90  |
| 25-80                  | ASAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSRQMAVREKVFDVI       | 91  |
| 26-80                  | SAELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSRQMAVREKVFDVI        | 92  |
| 27-80                  | AELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSRQMAVREKVFDVI         | 93  |
| 28-80                  | ELIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSRQMAVREKVFDVI          | 94  |
| 29-80                  | LIEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSRQMAVREKVFDVI           | 95  |
| 30-80                  | IEEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSRQMAVREKVFDVI            | 96  |
| 31-80                  | EEEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSRQMAVREKVFDVI             | 97  |
| 32-80                  | EEVAKLLK<br>LKAQLGPDESKQKFVLKTPKGTRDYSRQMAVREKVFDVI              | 98  |
| 33-80                  | EVAKLLKLKAQLGPDESKQKFVLKTPKGTRDYSRQMAVREKVFDVI                   | 99  |
| 34-80                  | VAKLLKLKAQLGPDESKQKFVLKTPKGTRDYSRQMAVREKVFDVI                    | 100 |



| N-terminal truncations |                                                                                                            |     |
|------------------------|------------------------------------------------------------------------------------------------------------|-----|
| 35-80                  | AKLLK LKAQLGPDESKQKFVLKTPKGTRDYSRQMAVREKVFDVI                                                              | 101 |
| 36-80                  | KLLK LKAQLGPDESKQKFVLKTPKGTRDYSRQMAVREKVFDVI                                                               | 102 |
| 37-80                  | LLK LKAQLGPDESKQKFVLKTPKGTRDYSRQMAVREKVFDVI                                                                | 103 |
| 38-80                  | LK LKAQLGPDESKQKFVLKTPKGTRDYSRQMAVREKVFDVI                                                                 | 104 |
| 39-80                  | K LKAQLGPDESKQKFVLKTPKGTRDYSRQMAVREKVFDVI                                                                  | 105 |
| 40-80                  | LKAQLGPDESKQKFVLKTPKGTRDYSRQMAVREKVFDVI                                                                    | 106 |
| HRS                    | X <sub>A</sub> -L-X <sub>B</sub> -Q-G-X-X-V-R-X-L-K-X-X-K-A-X <sub>C</sub> -V-X-X-L-L-X-L-K-X <sub>D</sub> | 322 |
| WHEP<br>consensus      | Where                                                                                                      |     |
|                        | X is any amino acid                                                                                        |     |
|                        | X <sub>A</sub> is 0-50 amino acids                                                                         |     |
|                        | X <sub>B</sub> is about 5-7 amino acids, preferably 6 amino acids                                          |     |
|                        | X <sub>C</sub> is about 7-9 amino acids, preferably 8 amino acids                                          |     |
|                        | X <sub>D</sub> is 0-50 amino acids                                                                         |     |

[0113] It will be appreciated that in any of the HRS-Fc conjugates of the disclosure, the N-terminal acid of the HRS polypeptide (for example, the N-terminal Met) may additionally be deleted from any of the exemplary truncated HRS polypeptides or other HRS sequences described herein.

[0114] Typically, the biologically-active fragment has no less than about 1%, 10%, 25%, or 50% of an activity of the biologically-active (*i.e.*, non-canonical activity) HRS reference polypeptide from which it is derived. Exemplary methods for measuring such non-canonical activities are described in the Examples.

[0115] In some embodiments of the present disclosure, HRS proteins, variants, and biologically active fragments thereof, bind to one or more cellular binding partners with an affinity of at least about 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 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, 40, 50, 100, or 150 nM. In some embodiments of the present disclosure, the binding affinity of a HRS protein fragment for a selected cellular binding partner, particularly a binding partner that participates in a non-canonical activity, can be stronger than that of the corresponding full length HRS polypeptide or a specific alternatively spliced HRS polypeptide variant, by at least about 1.5x, 2x, 2.5x, 3x, 3.5x, 4x, 4.5x, 5x, 6x, 7x, 8x, 9x, 10x, 15x, 20x, 25x, 30x, 40x, 50x, 60x, 70x, 80x, 90x, 100x, 200x, 300x, 400x, 500x, 600x, 700x, 800x, 900x, 1000x or more (including all integers in between).

[0116] As noted above, a HRS polypeptide may be altered in various ways including amino acid substitutions, deletions, truncations, and insertions. Methods for such manipulations are generally known in the art. For example, amino acid sequence variants of a HRS reference polypeptide can be prepared by mutations in the DNA. Methods for mutagenesis and nucleotide sequence alterations are well known in the art. See, for example, Kunkel (1985, Proc. Natl. Acad. Sci. USA. 82: 488-492), Kunkel et al., (1987, Methods in Enzymol, 154: 367-382), U.S. Pat. No. 4,873,192, Watson, J. D. et al., ("Molecular Biology of the Gene", Fourth Edition, Benjamin/Cummings, Menlo Park, Calif., 1987) and the references cited therein. Guidance as to appropriate amino acid substitutions that do not affect biological activity of the protein of interest may be found in the model of Dayhoff et al., (1978) Atlas of Protein Sequence and Structure (Natl. Biomed. Res. Found., Washington, D.C.).

[0117] Biologically active truncated and/or variant HRS polypeptides may contain conservative amino acid substitutions at various locations along their sequence, as compared to a reference HRS amino acid residue,



and such additional substitutions may further enhance the activity or stability of the HRS polypeptides with altered cysteine content. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, which can be generally sub-classified as follows:

**Acidic:** The residue has a negative charge due to loss of H ion at physiological pH and the residue is attracted by aqueous solution so as to seek the surface positions in the conformation of a peptide in which it is contained when the peptide is in aqueous medium at physiological pH. Amino acids having an acidic side chain include glutamic acid and aspartic acid.

**Basic:** The residue has a positive charge due to association with H ion at physiological pH or within one or two pH units thereof (e.g., histidine) and the residue is attracted by aqueous solution so as to seek the surface positions in the conformation of a peptide in which it is contained when the peptide is in aqueous medium at physiological pH. Amino acids having a basic side chain include arginine, lysine and histidine.

**Charged:** The residues are charged at physiological pH and, therefore, include amino acids having acidic or basic side chains (i.e., glutamic acid, aspartic acid, arginine, lysine and histidine).

**Hydrophobic:** The residues are not charged at physiological pH and the residue is repelled by aqueous solution so as to seek the inner positions in the conformation of a peptide in which it is contained when the peptide is in aqueous medium. Amino acids having a hydrophobic side chain include tyrosine, valine, isoleucine, leucine, methionine, phenylalanine and tryptophan.

**Neutral/polar:** The residues are not charged at physiological pH, but the residue is not sufficiently repelled by aqueous solutions so that it would seek inner positions in the conformation of a peptide in which it is contained when the peptide is in aqueous medium. Amino acids having a neutral/polar side chain include asparagine, glutamine, cysteine, histidine, serine and threonine.

**[0118]** This description also characterizes certain amino acids as "small" since their side chains are not sufficiently large, even if polar groups are lacking, to confer hydrophobicity. With the exception of proline, "small" amino acids are those with four carbons or less when at least one polar group is on the side chain and three carbons or less when not. Amino acids having a small side chain include glycine, serine, alanine and threonine. The gene-encoded secondary amino acid proline is a special case due to its known effects on the secondary conformation of peptide chains. The structure of proline differs from all the other naturally-occurring amino acids in that its side chain is bonded to the nitrogen of the  $\alpha$ -amino group, as well as the  $\alpha$ -carbon. Several amino acid similarity matrices are known in the art (see e.g., PAM120 matrix and PAM250 matrix as disclosed for example by Dayhoff et al., 1978, A model of evolutionary change in proteins). Matrices for determining distance relationships In M. O. Dayhoff, (ed.), Atlas of protein sequence and structure, Vol. 5, pp. 345-358, National Biomedical Research Foundation, Washington DC; and by Gonnet et al., (Science, 256: 14430-1445, 1992), however, include proline in the same group as glycine, serine, alanine and threonine. Accordingly, for the purposes of the present disclosure, proline is classified as a "small" amino acid.

**[0119]** The degree of attraction or repulsion required for classification as polar or nonpolar is arbitrary and, therefore, amino acids specifically contemplated by the disclosure have been classified as one or the other. Most amino acids not specifically named can be classified on the basis of known behavior.

**[0120]** Amino acid residues can be further sub-classified as cyclic or non-cyclic, and aromatic or non-aromatic, self-explanatory classifications with respect to the side-chain substituent groups of the residues, and as small or large. The residue is considered small if it contains a total of four carbon atoms or less, inclusive of the carboxyl carbon, provided an additional polar substituent is present; three or less if not. Small residues are, of course, always non-aromatic. Dependent on their structural properties, amino acid residues may fall in two or



more classes. For the naturally-occurring protein amino acids, sub-classification according to this scheme is presented in Table A.

Table A: Amino acid sub-classification.

| Sub-classes                               | Amino acids                                                                  |
|-------------------------------------------|------------------------------------------------------------------------------|
| Acidic                                    | Aspartic acid, Glutamic acid                                                 |
| Basic                                     | Noncyclic: Arginine, Lysine; Cyclic: Histidine                               |
| Charged                                   | Aspartic acid, Glutamic acid, Arginine, Lysine, Histidine                    |
| Small                                     | Glycine, Serine, Alanine, Threonine, Proline                                 |
| Polar/neutral                             | Asparagine, Histidine, Glutamine, Cysteine, Serine, Threonine                |
| Polar/large                               | Asparagine, Glutamine                                                        |
| Hydrophobic                               | Tyrosine, Valine, Isoleucine, Leucine, Methionine, Phenylalanine, Tryptophan |
| Aromatic                                  | Tryptophan, Tyrosine, Phenylalanine                                          |
| Residues that influence chain orientation | Glycine and Proline                                                          |

[0121] Conservative amino acid substitution also includes groupings based on side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulphur-containing side chains is cysteine and methionine. For example, it is reasonable to expect that replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid will not have a major effect on the properties of the resulting variant polypeptide. Whether an amino acid change results in a functional truncated and/or variant HRS polypeptide can readily be determined by assaying its non-canonical activity, as described herein. Conservative substitutions are shown in Table B under the heading of exemplary substitutions. Amino acid substitutions falling within the scope of the disclosure, are, in general, accomplished by selecting substitutions that do not differ significantly in their effect on maintaining (a) the structure of the peptide backbone in the area of the substitution, (b) the charge or hydrophobicity of the molecule at the target site, (c) the bulk of the side chain, or (d) the biological function. After the substitutions are introduced, the variants are screened for biological activity.

Table B: Exemplary Amino Acid Substitutions.

| Original Residue | Exemplary Substitutions         | Preferred Substitutions |
|------------------|---------------------------------|-------------------------|
| Ala              | Val, Leu, Ile                   | Val                     |
| Arg              | Lys, Gln, Asn                   | Lys                     |
| Asn              | Gln, His, Lys, Arg              | Gln                     |
| Asp              | Glu                             | Glu                     |
| Cys              | Ser, Ala, Leu, Val              | Ser, Ala                |
| Gln              | Asn, His, Lys,                  | Asn                     |
| Glu              | Asp, Lys                        | Asp                     |
| Gly              | Pro                             | Pro                     |
| His              | Asn, Gln, Lys, Arg              | Arg                     |
| Ile              | Leu, Val, Met, Ala, Phe, Norleu | Leu                     |



| Original Residue | Exemplary Substitutions         | Preferred Substitutions |
|------------------|---------------------------------|-------------------------|
| Leu              | Norleu, Ile, Val, Met, Ala, Phe | Ile                     |
| Lys              | Arg, Gln, Asn                   | Arg                     |
| Met              | Leu, Ile, Phe                   | Leu                     |
| Phe              | Leu, Val, Ile, Ala              | Leu                     |
| Pro              | Gly                             | Gly                     |
| Ser              | Thr                             | Thr                     |
| Thr              | Ser                             | Ser                     |
| Trp              | Tyr                             | Tyr                     |
| Tyr              | Trp, Phe, Thr, Ser              | Phe                     |
| Val              | Ile, Leu, Met, Phe, Ala, Norleu | Leu                     |

**[0122]** Alternatively, similar amino acids for making conservative substitutions can be grouped into three categories based on the identity of the side chains. The first group includes glutamic acid, aspartic acid, arginine, lysine, histidine, which all have charged side chains; the second group includes glycine, serine, threonine, cysteine, tyrosine, glutamine, asparagine; and the third group includes leucine, isoleucine, valine, alanine, proline, phenylalanine, tryptophan, methionine, as described in Zubay, G., *Biochemistry*, third edition, Wm.C. Brown Publishers (1993).

**[0123]** The NMR structure of the human HRS WHEP domain has been determined (see Nameki *et al.*, Accession 1X59\_A). Further, the crystal structures of full-length human HRS and an internal catalytic domain deletion mutant of HRS (HRS $\Delta$ CD) have also been determined (see Xu *et al.*, *Structure*. 20:1470-7, 2012; and U.S. Application No. 61/674,639). In conjunction with the primary amino acid sequence of HRS, these detailed physical descriptions of the protein provide precise insights into the roles played by specific amino acids within the protein. Persons skilled in the art can thus use this information to identify structurally-conserved domains, linking regions, secondary structures such as alpha-helices, surface or solvent-exposed amino acids, non-exposed or internal regions, catalytic sites, and ligand-interacting surfaces, among other structural features. Such persons can then use that and other information to readily engineer HRS variants that retain or improve the non-canonical activity of interest, for instance, by conserving or altering the characteristics of the amino acid residues within or adjacent to these and other structural features, such as by conserving or altering the polarity, hydropathy index, charge, size, and/or positioning (*i.e.*, inward, outward) of selected amino acid side chain(s) relative to wild-type residues (see, *e.g.*, Zaiwara *et al.*, *Mol Biotechnol.* 51:67-102, 2012; Perona and Hadd, *Biochemistry*. 51:8705-29, 2012; Morin *et al.*, *Trends Biotechnol.* 29:159-66, 2011; Collins *et al.*, *Annu. Rev. Biophys.* 40:81-98, 2011; and U.S. Application No. 61/674,639).

**[0124]** Thus, a predicted non-essential amino acid residue in a truncated and/or variant HRS polypeptide is typically replaced with another amino acid residue from the same side chain family. Alternatively, mutations can be introduced randomly along all or part of a HRS coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for an activity of the parent polypeptide to identify mutants which retain that activity. Following mutagenesis of the coding sequences, the encoded peptide can be expressed recombinantly and the activity of the peptide can be determined. A "non-essential" amino acid residue is a residue that can be altered from the reference sequence of an embodiment of the present disclosure polypeptide without abolishing or substantially altering one or more of its non canonical activities. Suitably, the alteration does not substantially abolish one of these activities, for example, the activity is at least 20%, 40%, 60%, 70% or 80% 100%, 500%, 1000% or more of the reference HRS sequence. An "essential" amino acid residue is a residue that, when altered from the reference sequence of a HRS polypeptide, results in abolition of an activity of the parent molecule such that less than 20% of the reference activity is present. For example, such essential amino acid residues include those that are conserved in HRS polypeptides across different



species, including those sequences that are conserved in the active binding site(s) or motif(s) of HRS polypeptides from various sources.

[0125] Assays to determine anti-inflammatory activity, including routine measurements of cytokine release from in vitro cell based, and animal studies are well established in the art (see, for example, Wittmann et al., J Vis Exp. (65):e4203. doi: 10.3791/4203, 2012; Feldman et al., Mol Cell. 47:585-95, 2012; Clutterbuck et al., J Proteomics. 74:704-15, 2011, Giddings and Maitra, J Biomol Screen. 15:1204-10, 2010; Wijnhoven et al., Glycoconj J. 25:177-85, 2008; and Frow et al., Med Res Rev. 24:276-98, 2004) and can be readily used to profile and optimize anti-inflammatory activity. An exemplary *in vivo* experimental system is also described in the accompanying Examples.

[0126] In some embodiments of the present disclosure, HRS polypeptides may have one or more cysteine substitutions, where one or more naturally-occurring (non-cysteine) residues are substituted with cysteine (e.g., to alter stability, to facilitate thiol-based conjugation of an Fc fragment, to facilitate thiol-based attachment of PEG or other molecules). In some embodiments of the present disclosure, cysteine substitutions are near the N-terminus and/or C-terminus of the HRS polypeptide (e.g., SEQ ID NOS:1-106, 170-181, or 185-191), or other surface exposed regions of a HRS polypeptide. Particular embodiments of the present disclosure include where one or more of residues within 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 amino acids relative to the N-terminus and/or C-terminus of any one of SEQ ID NOS: 1-106, 170-181, or 185-191 are substituted with a cysteine residue. In some embodiments of the present disclosure, cysteine residues may be added to the HRS polypeptide through the creation of N, or C-terminal fusion proteins. Such fusion proteins may be of any length, but will typically be about 1-5, or about 5-10, about 10 to 20, or about 20 to 30 amino acids in length. In some embodiments of the present disclosure, fusion to the C-terminus is preferred.

[0127] Specific exemplary embodiments of the present disclosure of such cysteine modified proteins are shown in **Table D6**, based on the HRS polypeptide HRS(1-60). This approach is directly applicable to the HRS polypeptides of Table D5, and other HRS polypeptides described herein.

| Table D6        |                                                                                                                                                                                                     |            |
|-----------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| Name            | Protein Sequences                                                                                                                                                                                   | SEQ ID NO: |
| HRS(1-60)-M1MC- | <u>M</u> CAERAALEE LVKLQGERVR GLKQQKASAE LIEEEVAKLL<br>KLKAQLGPDE SKQKFVLKTP K                                                                                                                      | 179        |
| HRS(1-60)-A26C- | MAERAALEEL VKLQGERVRG LKQQK <u>C</u> SAEL IIEEVAKLLK<br>LKAQLGPDES KQKFVLKTPK                                                                                                                       | 180        |
| HRS(1-60)-C61   | MAERAALEEL VKLQGERVRG LKQQKASAEL IIEEVAKLLK<br>LKAQLGPDES KQKFVLKTPK <u>C</u>                                                                                                                       | 181        |
| DNA sequences   |                                                                                                                                                                                                     |            |
| HRS(1-60)-M1MC- | ATGTGTGCAGAAAGAGCCGCCCTGGAAGAGTTAGTTAAGTTGCAAGGT<br>GAACGTGTCCGTGGTCTGAAGCAGCAGAAGGCTAGCGCGGAGCTGAT<br>CGAAGAAGAGGTGGCCAAACTGCTGAAGCTGAAGGCGCAGCTGGGCC<br>CGGACGAGAGCAAACAAAAGTTCGTCCTGAAAACCCCGAAA | 182        |
| HRS(1-60)-A26C- | ATGGCAGAACGTGCGGCATTGGAAGAATTGGTTAAACTGCAAGGTGA<br>ACGTGTTTCGTGGTCTGAAGCAGCAGAAGTGCAGCGCGGAGCTGATCGA<br>AGAAGAGGTGGCCAAACTGCTGAAGCTGAAGGCGCAGCTGGGCCCGG                                             | 183        |



| DNA sequences |                                                                                                                                                                                                     |     |
|---------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
|               | ACGAGAGCAAACAAAAGTTCGTCCTGAAAACCCCGAAA                                                                                                                                                              |     |
| HRS(1-60)-C61 | ATGGCAGAACGTGCGGCATTGGAAGAATTGGTTAACTGCAAGGTGA<br>ACGTGTTCGTGGTCTGAAGCAGCAGAAGGCTAGCGCGGAGCTGATCGA<br>AGAAGAGGTGGCCAAACTGCTGAAGCTGAAGGCGCAGCTGGGCCCCGG<br>ACGAGAGCAAACAAAAGTTCGTCCTGAAAACCCCGAAATGC | 184 |

[0128] In some embodiments of the present disclosure, the HRS polypeptide can include mutants in which the endogenous or naturally-occurring cysteine residues have been mutated to alternative amino acids, or deleted. In some embodiments of the present disclosure, the insertion or substitution of cysteine residue(s) into the HRS polypeptide may be combined with the elimination of other surface exposed reactive cysteine residues. Accordingly, in some embodiments of the present disclosure, the HRS polypeptide may comprise one or more substitutions and/or deletions at Cys83, Cys174, Cys191, Cys196, Cys224, Cys235, Cys379, Cys455, Cys507, and/or Cys509 (as defined by SEQ ID NO:1), for instance, to remove naturally-occurring cysteine residues.

[0129] Specific embodiments of the present disclosure include any one of SEQ ID NOS: 1-106, 170-181, or 185-191, or variants or fragments thereof, having at mutation or deletion of any one or more of Cys83, Cys174, Cys191, Cys196, Cys224, Cys235, Cys379, Cys455, or the deletion of Cys507 and Cys509, for instance, by the deletion of the C-terminal 3 amino acids ( $\Delta$ 507-509). Exemplary mutations at these positions include for example the mutation of cysteine to serine, alanine, leucine, valine or glycine. In certain embodiments of the present disclosure, amino acid residues for specific cysteine substitutions can be selected from naturally-occurring substitutions that are found in HRS orthologs from other species and organisms. Exemplary substitutions of this type are presented in Table D7.

| Table D7<br>Naturally-occurring sequence variation at positions occupied by cysteine residues in human HRS |              |           |          |            |              |          |          |        |                |           |              |        |
|------------------------------------------------------------------------------------------------------------|--------------|-----------|----------|------------|--------------|----------|----------|--------|----------------|-----------|--------------|--------|
| H.sapiens<br>cysteine<br>residue #                                                                         | P.troglodyte | M.mulatta | B.taurus | M.musculus | R.norvegicus | G.gallus | X.laevis | D.reio | D.melanogaster | C.elegans | S.cerevisiae | E.coli |
| 83                                                                                                         | C            | C         | C        | C          | C            | C        | C        | C      | V              | T         | L            | V      |
| 174                                                                                                        | C            | C         | C        | C          | C            | C        | C        | C      | C              | C         | C            | L      |
| 191                                                                                                        | C            | C         | C        | C          | C            | C        | C        | C      | C              | V         | C            | A/L    |
| 196                                                                                                        | C            | C         | C        | C          | C            | Q        | H        | Y      | S              | M         | V            | L/A    |
| 224                                                                                                        | C            | C         | C        | C          | C            | C        | C        | C      | C              | S         | A            | A      |
| 235                                                                                                        | C            | C         | C        | C          | C            | C        | C        | C      | C              | C         | S            | E      |
| 379                                                                                                        | C            | C         | C        | C          | C            | C        | C        | V      | C              | C         | C            | A      |
| 455                                                                                                        | C            | C         | C        | C          | C            | C        | C        | -      | C              | C         | A            | A      |
| 507                                                                                                        | C            | R         | C        | S          | S            | -        | -        | -      | -              | S/Q       | S/E          | -      |
| 509                                                                                                        | C            | C         | C        | C          | -            | -        | -        | -      | -              | I         | I/G          | -      |

[0130] In some embodiments of the present disclosure, the naturally-occurring cysteines selected for mutagenesis are selected based on their surface exposure. Accordingly, in one aspect of the present disclosure the cysteine residues selected for substitution are selected from Cys224, Cys235, Cys507 and Cys509. In some embodiments of the present disclosure, the last three (C-terminal) residues of SEQ ID NO:1 are deleted so as to delete residues 507 to 509. In some embodiments of the present disclosure, the cysteines are selected for mutation or deletion so as to eliminate an intramolecular cysteine pair, for example Cys174 and Cys191.

[0131] Specific additional examples of desired cysteine mutations/substitutions (indicated in bold underline) to



reduce surface exposed cysteine residues include those listed below in Table D8.

| Table D8                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |            |
|-------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| HRS polypeptides with Substitutions to Remove Surface Exposed Cysteines |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |            |
| Name                                                                    | Protein Sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | SEQ ID NO: |
| HRS(1-506) C174A                                                        | MAERAAL <del>EEL</del> VKLQGERVRGLKQQKASAELIEEEVAKLLKLKAQLGPDESKQKFVLKTPKGTRDYS <del>PRQ</del> MAVREKVFDVIIRCFKRHGAEVIDTPVFELKETL MGKYGEDSKLIYDLKDQGGELL <del>SLRYDL</del> TVPFARYLAMNKLTNIKRYHIAK VYRRDNPAMTRGRYREFYQ <del>AD</del> FDIAGNFDPMPDAECLKIMCEILSSLQIGD FLVKVNDRRILDGMFAICGVSDSKFRTICSSVDKLDKVSWE <del>EV</del> VKNEMVGEK GLAPEVADRIGDYVQQHGGVSLVEQLLQDPKLSQNKQALEGLGDLKLLFEY LTLFGIDDKISFDLSLARGLDY <del>YT</del> GV <del>VI</del> YEA <del>VLL</del> QTPAQAGEEPLGVGSVAAG GR <del>Y</del> DGLVGMFDPKGRK <del>VPC</del> VGLSIGVERIFSIVEQRLEALEEKIRTTETQVLV ASAQKLL <del>EER</del> LKL <del>VSEL</del> WDAGIKAELLYKKNPKLLNQLQYCEEAGIPLVAI IGEQELKDGVIK <del>LSV</del> TSREEVDVRREDLVEEIKRRTGQPL                                                                           | 185        |
| HRS(1-506) C174V                                                        | MAERAAL <del>EEL</del> VKLQGERVRGLKQQKASAELIEEEVAKLLKLKAQLGPDESKQKFVLKTPKGTRDYS <del>PRQ</del> MAVREKVFDVIIRCFKRHGAEVIDTPVFELKETL MGKYGEDSKLIYDLKDQGGELL <del>SLRYDL</del> TVPFARYLAMNKLTNIKRYHIAK VYRRDNPAMTRGRYREFYQ <del>VD</del> FDIAGNFDPMPDAECLKIMCEILSSLQIGD FLVKVNDRRILDGMFAICGVSDSKFRTICSSVDKLDKVSWE <del>EV</del> VKNEMVGEK GLAPEVADRIGDYVQQHGGVSLVEQLLQDPKLSQNKQALEGLGDLKLLFEY LTLFGIDDKISFDLSLARGLDY <del>YT</del> GV <del>VI</del> YEA <del>VLL</del> QTPAQAGEEPLGVGSVAAG GR <del>Y</del> DGLVGMFDPKGRK <del>VPC</del> VGLSIGVERIFSIVEQRLEALEEKIRTTETQVLV ASAQKLL <del>EER</del> LKL <del>VSEL</del> WDAGIKAELLYKKNPKLLNQLQYCEEAGIPLVAI IGEQELKDGVIK <del>LSV</del> TSREEVDVRREDLVEEIKRRTGQPL                                                                           | 186        |
| HRS(1-506) C191A                                                        | MAERAAL <del>EEL</del> VKLQGERVRGLKQQKASAELIEEEVAKLLKLKAQLGPDESKQKFVLKTPKGTRDYS <del>PRQ</del> MAVREKVFDVIIRCFKRHGAEVIDTPVFELKETL MGKYGEDSKLIYDLKDQGGELL <del>SLRYDL</del> TVPFARYLAMNKLTNIKRYHIAK VYRRDNPAMTRGRYREFYQ <del>CD</del> FDIAGNFDPMPDAE <del>ALK</del> IMCEILSSLQIGD FLVKVNDRRILDGMFAICGVSDSKFRTICSSVDKLDKVSWE <del>EV</del> VKNEMVGEK GLAPEVADRIGDYVQQHGGVSLVEQLLQDPKLSQNKQALEGLGDLKLLFEY LTLFGIDDKISFDLSLARGLDY <del>YT</del> GV <del>VI</del> YEA <del>VLL</del> QTPAQAGEEPLGVGSVAAG GR <del>Y</del> DGLVGMFDPKGRK <del>VPC</del> VGLSIGVERIFSIVEQRLEALEEKIRTTETQVLV ASAQKLL <del>EER</del> LKL <del>VSEL</del> WDAGIKAELLYKKNPKLLNQLQYCEEAGIPLVAI IGEQELKDGVIK <del>LSV</del> TSREEVDVRREDLVEEIKRRTGQPL                                                              | 187        |
| HRS(1-506) C191S                                                        | MAERAAL <del>EEL</del> VKLQGERVRGLKQQKASAELIEEEVAKLLKLKAQLGPDESKQKFVLKTPKGTRDYS <del>PRQ</del> MAVREKVFDVIIRCFKRHGAEVIDTPVFELKETL MGKYGEDSKLIYDLKDQGGELL <del>SLRYDL</del> TVPFARYLAMNKLTNIKRYHIAK VYRRDNPAMTRGRYREFYQ <del>CD</del> FDIAGNFDPMPDAE <del>SL</del> KIMCEILSSLQIGD <del>F</del> LVKVNDRRILDGMFAICGVSDSKFRTICSSVDKLDKVSWE <del>EV</del> VKNEMVGEK <del>G</del> LAPEVADRIGDYVQQHGGVSLVEQLLQDPKLSQNKQALEGLGDLKLLFEY <del>L</del> TLFGIDDKISFDLSLARGLDY <del>YT</del> GV <del>VI</del> YEA <del>VLL</del> QTPAQAGEEPLGVGSVAAG <del>G</del> RYDGLVGMFDPKGRK <del>VPC</del> VGLSIGVERIFSIVEQRLEALEEKIRTTETQVL <del>V</del> A SAQKLL <del>EER</del> LKL <del>VSEL</del> WDAGIKAELLYKKNPKLLNQLQYCEEAGIPL <del>V</del> AI IGEQELKDGVIK <del>LSV</del> TSREEVDVRREDLVEEIKRRTGQPL | 188        |
| HRS(1-506) C191V                                                        | MAERAAL <del>EEL</del> VKLQGERVRGLKQQKASAELIEEEVAKLLKLKAQLGPDESKQKFVLKTPKGTRDYS <del>PRQ</del> MAVREKVFDVIIRCFKRHGAEVIDTPVFELKETL MGKYGEDSKLIYDLKDQGGELL <del>SLRYDL</del> TVPFARYLAMNKLTNIKRYHIAK VYRRDNPAMTRGRYREFYQ <del>CD</del> FDIAGNFDPMPDAE <del>V</del> LKIMCEILSSLQIGD FLVKVNDRRILDGMFAICGVSDSKFRTICSSVDKLDKVSWE <del>EV</del> VKNEMVGEK GLAPEVADRIGDYVQQHGGVSLVEQLLQDPKLSQNKQALEGLGDLKLLFEY LTLFGIDDKISFDLSLARGLDY <del>YT</del> GV <del>VI</del> YEA <del>VLL</del> QTPAQAGEEPLGVGSVAAG GR <del>Y</del> DGLVGMFDPKGRK <del>VPC</del> VGLSIGVERIFSIVEQRLEALEEKIRTTETQVLV ASAQKLL <del>EER</del> LKL <del>VSEL</del> WDAGIKAELLYKKNPKLLNQLQYCEEAGIPLVAI IGEQELKDGVIK <del>LSV</del> TSREEVDVRREDLVEEIKRRTGQPL                                                              | 189        |
| HRS(1-506) C224S                                                        | MAERAAL <del>EEL</del> VKLQGERVRGLKQQKASAELIEEEVAKLLKLKAQLGPDESKQKFVLKTPKGTRDYS <del>PRQ</del> MAVREKVFDVIIRCFKRHGAEVIDTPVFELKETL                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |            |



| Table D8                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |            |
|-------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| HRS polypeptides with Substitutions to Remove Surface Exposed Cysteines |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |            |
| Name                                                                    | Protein Sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | SEQ ID NO: |
|                                                                         | MGKYGEDSKLIYDLKDQGGELLSLRYDLTVPFARYLAMNKLtnikryhiak<br>VYRRDNPAMTRGRYREFYQCDFDIAGNFDPMPDAECLKIMCEILSSLQIGD<br>FLVKVNDRRILDGMFAISGVSDSKFRTICSSVDKLDKVSWEDEVKNEMVGEK                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | 190        |
|                                                                         | GLAPEVADRIGDYVQQHGGVSLVEQLLQDPKLSQNKQALEGLGDLKLLFEY<br>LTLFGIDDKISFDLSLARGLDYYTGVIYEAVLLQTPAQAGEEPLGVGSVAAG<br>GRYDGLVGMFDPKGRKVPCVGLSIGVERIFSIVEQRLEALEEKIRTTETQVLV<br>ASAQKKLLEERLKLVSSELWDAGIKAELLYKKNPKLLNQLQYCEEAGIPLVAI<br>IGEQELKDGVIKLSVTSREEVDVRREDLVEEIKRRTGQPL                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |            |
| HRS(1-506)<br>C235S                                                     | MAERAAL EELVKLQGERVRLKQKQKASAELEEEVAKLLKLKAQLGPDESK<br>QKFVLKTPKGTRDYSRQMAVREKVFDVHIRCFKRHGAEVIDTPVFELKETL<br>MGKYGEDSKLIYDLKDQGGELLSLRYDLTVPFARYLAMNKLtnikryhiak<br>VYRRDNPAMTRGRYREFYQCDFDIAGNFDPMPDAECLKIMCEILSSLQIGD<br>FLVKVNDRRILDGMFAICGVSDSKFRTISSSSVDKLDKVSWEDEVKNEMVGEK<br>GLAPEVADRIGDYVQQHGGVSLVEQLLQDPKLSQNKQALEGLGDLKLLFEY<br>LTLFGIDDKISFDLSLARGLDYYTGVIYEAVLLQTPAQAGEEPLGVGSVAAG<br>GRYDGLVGMFDPKGRKVPCVGLSIGVERIFSIVEQRLEALEEKIRTTETQVLV<br>ASAQKKLLEERLKLVSSELWDAGIKAELLYKKNPKLLNQLQYCEEAGIPLVAI<br>IGEQELKDGVIKLSVTSREEVDVRREDLVEEIKRRTGQPL                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 191        |
| HRS(1-506)<br>C174A                                                     | ATGGCGGAACGTGCCGCACTGGAAGAATTGGTTAAATTACAGGGAGAAC<br>GCGTACGTGGTCTTAAACAACAAAAAGCCTCTGCGGAATTGATTGAAGA<br>AGAAGTTGCCAAATTACTGAAACTGAAAGCTCAACTTGGACCCGATGAA<br>AGTAAACAACAAATTTGTGTTGAAAACGCCCAAAGGAACCCGTGATTATA<br>GTCCACGTCAAATGGCCGTTTCGTGAAAAAGTGTTGACGTTATTATTTCGC<br>TGTTTTAAACGTACGGTGCTGAAGTAATCGATACCCCGTATTTGAATT<br>GAAAGAGACTCTGATGGGCAAATATGGTGAAGATTCTAAACTGATTTAT<br>GATTTGAAAGACCAAGGAGGTGAAGTCTGAGCCTGCGCTACGACTTAA<br>CTGTGCCTTTTGCCCGTTACTTAGCCATGAATAAaTTaACCAACATCAAAC<br>GTTACCATATTGCAAAAGTATATCGCCGCGACAACCCTGCAATGACTCGT<br>GGACGCTATCGCGAATTCTATCAGGCTGATTTTGATATTGCCGGAATTT<br>CGACCCGATGATCCCGGATGCCGAGTGTTTGAAAATTATGTGTGAAATTC<br>TGAGTTCGTTGCAGATCGGAGACTTTCTTGTAAGTAATGACCGCCGT<br>ATTCTGGATGGTATGTTTGCTATTTGCGGTGTTTCTGATTCCAAATTCGCT<br>ACAATCTGCTCAAGCGTGGACAAATTGGATAAAGTGTCTTGGGAAGAAG<br>TAAAAAATGAAATGGTGGGAGAAAAAGGCCTGGCTCCAGAAGTAGCAG<br>ACCGTATTGGTGACTATGTTCAACAACATGGCGGTGTGTCCTTAGTCGAA<br>CAGTTATTACAGGATCCTAAACTGAGCCAAAATAAACAAGCACTTGAAG<br>GACTGGGAGATCTGAAATTACTCTTTGAATATCTGACCTTATTTGGGATT<br>GATGATAAAATTAGCTTTGATCTGAGCTTGGCCCGCGGTCTTGATTATTA<br>TACCGGCGTGATTACGAAGCTGTTCTCTTGCAAACCCAGCCAGGCGG<br>GCGAAGAGCCTTTGGGAGTCGGCAGTGTTGGCAGCCGCTGGTTCGTTATGA<br>TGGTTTGGTAGGAATGTTTGACCCTAAAGGCCGTAAAGTACCATGTGTG<br>GGGCTTTCTATCGGTGTGCAACGTATCTTTTCTATTGTTGAACAACGTCTT<br>GAAGCTTTGGAGGAAAAAGATCCGTACCACGGAAacCCAAGTCTTAGTTGC<br>aAGTGCCCAAAAAAACTGTTAGAAGAACGCCTGAAACTCGTATCAGAA<br>CTTTGGGACGCCGGCATCAAGGCCGAAGTCTGTATAAAAAAGAACCCGA<br>AATTGTTAAACCAACTCCAGTATTGTGAAGAAGCTGGGATCCCACTCGT<br>AGCTATTATTGGTGAGCAAGAATTAAGATGGCGTGATTAAACTGCGT<br>TCAGTAACAAGCCGTGAAGAGGTAGATGTACGTCGGAAGACTTAGTGG<br>AAGAAATTAAACGCCGCACCGGTCAACCGTTA | 192        |
| HRS(1-506)<br>C174V                                                     | ATGGCGGAACGTGCCGCACTGGAAGAATTGGTTAAATTACAGGGAGAAC<br>GCGTACGTGGTCTTAAACAACAAAAAGCCTCTGCGGAATTGATTGAAGA<br>AGAAGTTGCCAAATTACTGAAACTGAAAGCTCAACTTGGACCCGATGAA<br>AGTAAACAACAAATTTGTGTTGAAAACGCCCAAAGGAACCCGTGATTATA<br>GTCCACGTCAAATGGCCGTTTCGTGAAAAAGTGTTGACGTTATTATTTCGC<br>TGTTTTAAACGTACGGTGCTGAAGTAATCGATACCCCGTATTTGAATT<br>GAAAGAGACTCTGATGGGCAAATATGGTGAAGATTCTAAACTGATTTAT<br>GATTTGAAAGACCAAGGAGGTGAAGTCTGAGCCTGCGCTACGACTTAA<br>CTGTGCCTTTTGCCCGTTACTTAGCCATGAATAAaTTaACCAACATCAAAC<br>GTTACCATATTGCAAAAGTATATCGCCGCGACAACCCTGCAATGACTCGT                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | 193        |



| Table D8                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |            |
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| HRS polypeptides with Substitutions to Remove Surface Exposed Cysteines |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |            |
| Name                                                                    | Protein Sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | SEQ ID NO: |
|                                                                         | GGACGCTATCGCGAATTCTATCAGGTTGATTTTGATATTGCCGGAATTT                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |            |
|                                                                         | CGACCCGATGATCCCGGATGCCGAGTGTTTGAAAATTATGTGTGAAATTC<br>TGAGTTCGTTGCAGATCGGAGACTTTCTTGTAAGTTAATGACCGCCGT<br>ATTCTGGATGGTATGTTTGCTATTTGCGGTGTTTCTGATTCCAAATCCGT<br>ACAATCTGCTCAAGCGTGGACAAATTGGATAAAGTGTCTTGGGAAGAAG<br>TAAAAAATGAAATGGTGGGAGAAAAAGGCCTGGCTCCAGAAGTAGCAG<br>ACCGTATTGGTGACTATGTTCAACAACATGGCGGTGTGTCCTTAGTCGAA<br>CAGTTATTACAGGATCCTAAACTGAGCCAAAATAACAAGCACTTGAAG<br>GACTGGGAGATCTGAAATTACTCTTTGAATATCTGACCTTATTTGGGATT<br>GATGATAAAATTAGCTTTGATCTGAGCTTGGCCCCGCGTCTTGATTATTA<br>TACCGGCGTGATTTACGAAGCTGTTCTCTTGCAAACCCAGCCCAGGCGG<br>GCGAAGAGCCTTTGGGAGTCGGCAGTGTGGCAGCCGGTGGTCGTTATGA<br>TGGTTTGGTAGGAATGTTTGACCCTAAAGGCCGTAAAGTACCATGTGTG<br>GGGCTTTCTATCGGTGTCTGAACGTATCTTTTCTATTGTTGAACAACGTCTT<br>GAAGCTTTGGAGGAAAAAGATCCGTACCACGGAA <sub>ac</sub> CCAAGTCTTAGTTGC<br><sub>a</sub> AGTGCCCAAAAAAACTGTTAGAAGAACGCCTGAAACTCGTATCAGAA<br>CTTTGGGACGCCGGCATCAAGGCCGAAGTCTGTATAAAAAAGAACCCGA<br>AATTGTAAACCAACTCCAGTATTGTGAAGAAGCTGGGATCCCACTCGT<br>AGCTATTATTGGTGAGCAAGAATTAAGATGGCGTGATTAAACTGCGT<br>TCAGTAACAAGCCGTGAAGAGGTAGATGTACGTCGCGAAGACTTAGTGG<br>AAGAAATTAAACGCCGCACCGGTCAACCGTTA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |            |
| HRS(1-506)<br>C191A                                                     | ATGGCGGAACGTGCCGCACTGGAAGAATTGGTTAAATTACAGGGAGAAC<br>GCGTACGTGGTCTTAAACAACAAAAAGCCTCTGCGGAATTGATTGAAGA<br>AGAAGTTGCCAAATTACTGAAACTGAAAGCTCAACTTGGACCCGATGAA<br>AGTAAACAAAAATTTGTGTTGAAAACGCCCAAAGGAACCCGTGATTATA<br>GTCCACGTCAAATGGCCGTTCTGTAAAAAGTGTTCGACGTTATTATTCGC<br>TGTTTTAAACGTACGGTGCTGAAGTAATCGATACCCCGTATTTGAATT<br>GAAAGAGACTCTGATGGGCAAATATGGTGAAGATTCTAAACTGATTTAT<br>GATTTGAAAGACCAAGGAGGTGAACTGCTGAGCCTGCGCTACGACTTAA<br>CTGTGCCTTTTGCCCGTTACTTAGCCATGAATAA <sub>a</sub> TT <sub>a</sub> ACCAACATCAAAC<br>GTTACCATATTGCAAAAGTATATCGCCGCGACAACCCTGCAATGACTCGT<br>GGACGCTATCGCGAATTCTATCAGTGTGATTTTGATATTGCCGGAATTT<br>CGACCCGATGATCCCGGATGCCGAGGCTTTGAAAATTATGTGTGAAATT<br>CTGAGTTCGTTGCAGATCGGAGACTTTCTTGTAAGTTAATGACCGCCG<br>TATTCTGGATGGTATGTTTGCTATTTGCGGTGTTTCTGATTCCAAATCCG<br>TACAATCTGCTCAAGCGTGGACAAATTGGATAAAGTGTCTTGGGAAGAA<br>GTAAAAAATGAAATGGTGGGAGAAAAAGGCCTGGCTCCAGAAGTAGCA<br>GACCGTATTGGTGACTATGTTCAACAACATGGCGGTGTGTCCTTAGTCGA<br>ACAGTTATTACAGGATCCTAAACTGAGCCAAAATAACAAGCACTTGAA<br>GGACTGGGAGATCTGAAATTACTCTTTGAATATCTGACCTTATTTGGGAT<br>TGATGATAAAATTAGCTTTGATCTGAGCTTGGCCCCGCGTCTTGATTATT<br>ATACCGGCGTGATTTACGAAGCTGTTCTCTTGCAAACCCAGCCCAGGCG<br>GGCGAAGAGCCTTTGGGAGTCGGCAGTGTGGCAGCCGGTGGTCGTTATG<br>ATGGTTTGGTAGGAATGTTTGACCCTAAAGGCCGTAAAGTACCATGTGT<br>GGGGCTTTCTATCGGTGTCTGAACGTATCTTTTCTATTGTTGAACAACGTC<br>TTGAAGCTTTGGAGGAAAAAGATCCGTACCACGGAA <sub>ac</sub> CCAAGTCTTAGTT<br>GC <sub>a</sub> AGTGCCCAAAAAAACTGTTAGAAGAACGCCTGAAACTCGTATCAG<br>AACTTTGGGACGCCGGCATCAAGGCCGAAGTCTGTATAAAAAAGAACCC<br>GAAATTGTAAACCAACTCCAGTATTGTGAAGAAGCTGGGATCCCACTC<br>GTAGCTATTATTGGTGAGCAAGAATTAAGATGGCGTGATTAAACTGC<br>GTTTCAGTAACAAGCCGTGAAGAGGTAGATGTACGTCGCGAAGACTTAGT<br>GGAAGAAATTAAACGCCGCACCGGTCAACCGTTA | 194        |
| HRS(1-506)<br>C191S                                                     | ATGGCGGAACGTGCCGCACTGGAAGAATTGGTTAAATTACAGGGAGAAC<br>GCGTACGTGGTCTTAAACAACAAAAAGCCTCTGCGGAATTGATTGAAGA<br>AGAAGTTGCCAAATTACTGAAACTGAAAGCTCAACTTGGACCCGATGAA<br>AGTAAACAAAAATTTGTGTTGAAAACGCCCAAAGGAACCCGTGATTATA<br>GTCCACGTCAAATGGCCGTTCTGTAAAAAGTGTTCGACGTTATTATTCGC<br>TGTTTTAAACGTACGGTGCTGAAGTAATCGATACCCCGTATTTGAATT<br>GAAAGAGACTCTGATGGGCAAATATGGTGAAGATTCTAAACTGATTTAT<br>GATTTGAAAGACCAAGGAGGTGAACTGCTGAGCCTGCGCTACGACTTAA<br>CTGTGCCTTTTGCCCGTTACTTAGCCATGAATAA <sub>a</sub> TT <sub>a</sub> ACCAACATCAAAC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | 195        |



| Table D8                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |            |
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| HRS polypeptides with Substitutions to Remove Surface Exposed Cysteines |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |            |
| Name                                                                    | Protein Sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | SEQ ID NO: |
|                                                                         | GTTACCATATTGCAAAAAGTATATCGCCGCGACAACCCTGCAATGACTCGT<br>GGACGCTATCGCGAATTCTATCAGTGTGATTTTGATATTGCCGGAAATTT<br>CGACCCGATGATCCCGGATGCCGAGAGTTTGAAAATTATGTGTGAAATT<br>CTGAGTTCGTTGCAGATCGGAGACTTTCTTGTAAGTTAATGACCGCCG<br>TATTCTGGATGGTATGTTTGCTATTTGCGGTGTTTCTGATTCCAAATCCG<br>TACAATCTGCTCAAGCGTGGACAAATTGGATAAAGTGTCTTGGAAGAA<br>GTAAAAAATGAAATGGTGGGAGAAAAAGGCCTGGCTCCAGAAAGTAGCA<br>GACCGTATTGGTGACTATGTTCAACAACATGGCGGTGTGTCCTTAGTCGA<br>ACAGTTATTACAGGATCCTAAACTGAGCCAAAATAAACAAGCACTTGAA<br>GGACTGGGAGATCTGAAATTACTCTTTGAATATCTGACCTTATTTGGGAT<br>TGATGATAAAATTAGCTTTGATCTGAGCTTGGCCCGCGGTCTTGATTATT<br>ATACCGGCGTGATTTACGAAGCTGTTCTCTTGCAAACCCCAGCCCAGGCG<br>GGCGAAGAGCCTTTGGGAGTCGGCAGTGTGGCAGCCGGTGGTCGTTATG<br>ATGGTTTGGTAGGAATGTTTGACCCTAAAGGCCGTAAAGTACCATGTGT<br>GGGGCTTTCTATCGGTGTCGAACGTATCTTTCTATTGTTGAACAACGTC<br>TTGAAGCTTTGGAGGAAAAGATCCGTACCACGGAAacCCAAGTCTTAGTT<br>GCaAGTGCCCAAAAAAACTGTTAGAAGAACGCCTGAAACTCGTATCAG<br>AACTTTGGGACGCGCGCATCAAGGCCGAACTGCTGTATAAAAAGAACCC<br>GAAATTGTTAAACCAACTCCAGTATTGTGAAGAAGCTGGGATCCCACTC<br>GTAGCTATTATTGGTGAGCAAGAATTAAGATGGCGTGATTAAACTGC<br>GTTTCAGTAACAAGCCGTGAAGAGGTAGATGTACGTCGCGAAGACTTAGT<br>GGAAGAAATTAAACGCCGCACCGGTCAACCGTTA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |            |
| HRS(1-506)<br>C191V                                                     | ATGGCGGAACGTGCCGCACTGGAAGAATTGGTTAAATTACAGGGAGAAC<br>GCGTACGTGGTCTTAAACAACAAAAAGCCTCTGCGGAATTGATTGAAGA<br>AGAAGTTGCCAAATTACTGAAACTGAAAGCTCAACTTGGACCCGATGAA<br>AGTAAACAAAAATTTGTGTTGAAAACGCCCAAAGGAACCCGTGATTATA<br>GTCCACGTCAAATGGCCGTTTCGTGAAAAAGTGTTCGACGTTATTATTCGC<br>TGTTTTAAACGTACCGGTGCTGAAGTAATCGATACCCCCGTATTTGAATT<br>GAAAGAGACTCTGATGGGCAAATATGGTGAAGATTCTAAACTGATTTAT<br>GATTTGAAAGACCAAGGAGGTGAACTGCTGAGCCTGCGCTACGACTTAA<br>CTGTGCCCTTTTGCCCGTTACTTAGCCATGAATAAaTTaACCAACATCAAAC<br>GTTACCATATTGCAAAAAGTATATCGCCGCGACAACCCTGCAATGACTCGT<br>GGACGCTATCGCGAATTCTATCAGTGTGATTTTGATATTGCCGGAAATTT<br>CGACCCGATGATCCCGGATGCCGAGGTTTGTAAAATTATGTGTGAAATTC<br>TGAGTTCGTTGCAGATCGGAGACTTTCTTGTAAGTTAATGACCGCCGT<br>ATTCTGGATGGTATGTTTGCTATTTGCGGTGTTTCTGATTCCAAATCCGT<br>ACAATCTGCTCAAGCGTGGACAAATTGGATAAAGTGTCTTGGAAGAAG<br>TAAAAAATGAAATGGTGGGAGAAAAAGGCCTGGCTCCAGAAAGTAGCAG<br>ACCGTATTGGTGACTATGTTCAACAACATGGCGGTGTGTCCTTAGTCGAA<br>CAGTTATTACAGGATCCTAAACTGAGCCAAAATAAACAAGCACTTGAAG<br>GACTGGGAGATCTGAAATTACTCTTTGAATATCTGACCTTATTTGGGATT<br>GATGATAAAATTAGCTTTGATCTGAGCTTGGCCCGCGGTCTTGATTATTA<br>TACCGGCGTGATTTACGAAGCTGTTCTCTTGCAAACCCCAGCCCAGGCGG<br>GCGAAGAGCCTTTGGGAGTCGGCAGTGTGGCAGCCGGTGGTCGTTATGA<br>TGGTTTGGTAGGAATGTTTGACCCTAAAGGCCGTAAAGTACCATGTGTG<br>GGGCTTTCTATCGGTGTGCAACGTATCTTTTCTATTGTTGAACAACGTCTT<br>GAAGCTTTGGAGGAAAAGATCCGTACCACGGAAacCCAAGTCTTAGTTGC<br>aAGTGCCCAAAAAAACTGTTAGAAGAACGCCTGAAACTCGTATCAGAA<br>CTTTGGGACGCGCGCATCAAGGCCGAACTGCTGTATAAAAAGAACCCGA<br>AATTGTAAACCAACTCCAGTATTGTGAAGAAGCTGGGATCCCACTCGT<br>AGCTATTATTGGTGAGCAAGAATTAAGATGGCGTGATTAAACTGCGT<br>TCAGTAACAAGCCGTGAAGAGGTAGATGTACGTCGCGAAGACTTAGTGG<br>AAGAAATTAAACGCCGCACCGGTCAACCGTTA | 196        |
| HRS(1-506)<br>C224S                                                     | ATGGCGGAACGTGCCGCACTGGAAGAATTGGTTAAATTACAGGGAGAAC<br>GCGTACGTGGTCTTAAACAACAAAAAGCCTCTGCGGAATTGATTGAAGA<br>AGAAGTTGCCAAATTACTGAAACTGAAAGCTCAACTTGGACCCGATGAA<br>AGTAAACAAAAATTTGTGTTGAAAACGCCCAAAGGAACCCGTGATTATA<br>GTCCACGTCAAATGGCCGTTTCGTGAAAAAGTGTTCGACGTTATTATTCGC<br>TGTTTTAAACGTACCGGTGCTGAAGTAATCGATACCCCCGTATTTGAATT<br>GAAAGAGACTCTGATGGGCAAATATGGTGAAGATTCTAAACTGATTTAT                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | 197        |
|                                                                         | GATTTGAAAGACCAAGGAGGTGAACTGCTGAGCCTGCGCTACGACTTAA<br>CTGTGCCCTTTTGCCCGTTACTTAGCCATGAATAAaTTaACCAACATCAAAC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |            |



| Table D8                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |            |
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| HRS polypeptides with Substitutions to Remove Surface Exposed Cysteines |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |            |
| Name                                                                    | Protein Sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | SEQ ID NO: |
|                                                                         | GTTACCATATTGCAAAAAGTATATCGCCGCGACAACCCTGCAATGACTCGT<br>GGACGCTATCGCGAATTCTATCAGTGTGATTTTGATATTGCCGGAATTT<br>CGACCCGATGATCCCGGATGCCGAGTGTTTGAAAATTATGTGTGAAATTC<br>TGAGTTCGTTGCAGATCGGAGACTTTCTTGTAAGTAAATGACCGCCGT<br>ATTCTGGATGGTATGTTTGCTATTTCCGGTGTTTCTGATTCCAAATCCGT<br>ACAATCTGCTCAAGCGTGGACAAATTGGATAAAGTGTCTTGGGAAGAAG<br>TAAAAAATGAAATGGTGGGAGAAAAAGGCCTGGCTCCAGAAGTAGCAG<br>ACCGTATTGGTGACTATGTTCAACAACATGGCGGTGTGTCCTTAGTCGAA<br>CAGTTATTACAGGATCCTAAACTGAGCCAAAATAAACAAGCACTTGAAG<br>GACTGGGAGATCTGAAATTACTCTTTGAATATCTGACCTTATTTGGGATT<br>GATGATAAAATTAGCTTTGATCTGAGCTTGGCCCGCGGTCTTGATTATTA<br>TACCGGCGTGATTTACGAAGCTGTTCTCTTGCAAACCCAGCCCAGGCGG<br>GCGAAGAGCCTTTGGGAGTCGGCAGTGTGGCAGCCGGTGGTTCGTTATGA<br>TGGTTTGGTAGGAATGTTTGACCCCTAAAGGCCGTAAAGTACCATGTGTG<br>GGGCTTTCTATCGGTGTGCAACGTATCTTTTCTATTGTTGAACAACGTCTT<br>GAAGCTTTGGAGGAAAAGATCCGTACCACGGAAacCCAAGTCTTAGTTGC<br>aAGTGCCCAAAAAAACTGTTAGAAGAACGCCTGAAACTCGTATCAGAA<br>CTTTGGGACGCCGGCATCAAGGCCGAAGTGTGTATAAAAAAGAACCCGA<br>AATTGTAAACCAACTCCAGTATTGTGAAGAAGCTGGGATCCCACTCGT<br>AGCTATTATTGGTGAGCAAGAATTAAGATGGCGTGATTAAACTGCGT<br>TCAGTAACAAGCCGTGAAGAGGTAGATGTACGTCGCGAAGACTTAGTGG<br>AAGAAATTAACGCCGCACCGGTCAACCGTTA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |            |
| HRS(1-506)<br>C235S                                                     | ATGGCGGAACGTGCCGCACTGGAAGAATTGGTTAAATTACAGGGAGAAC<br>GCGTACGTGGTCTTAAACAACAAAAAGCCTCTGCGGAATTGATTGAAGA<br>AGAAGTTGCCAAATTACTGAAACTGAAAGCTCAACTTGGACCCGATGAA<br>AGTAAACAAAAATTTGTGTTGAAAACGCCCAAAGGAACCCGTGATTATA<br>GTCCACGTCAAATGGCCGTTCTGTGAAAAAGTGTTGACGTTATTATTCGC<br>TGTTTTAAACGTACGGTGCTGAAGTAATCGATACCCCGTATTTGAATT<br>GAAAGAGACTCTGATGGGCAAATATGGTGAAGATTCTAAACTGATTTAT<br>GATTTGAAAGACCAAGGAGGTGAAGTGTGAGCCTGCGCTACGACTTAA<br>CTGTGCCTTTTGCCCGTTACTTAGCCATGAATAAaTTaACCAACATCAAAC<br>GTTACCATATTGCAAAAAGTATATCGCCGCGACAACCCTGCAATGACTCGT<br>GGACGCTATCGCGAATTCTATCAGTGTGATTTTGATATTGCCGGAATTT<br>CGACCCGATGATCCCGGATGCCGAGTGTTTGAAAATTATGTGTGAAATTC<br>TGAGTTCGTTGCAGATCGGAGACTTTCTTGTAAGTAAATGACCGCCGT<br>ATTCTGGATGGTATGTTTGCTATTTGCGGTGTTTCTGATTCCAAATCCGT<br>ACAATCTCTCAAGCGTGGACAAATTGGATAAAGTGTCTTGGGAAGAAG<br>TAAAAAATGAAATGGTGGGAGAAAAAGGCCTGGCTCCAGAAGTAGCAG<br>ACCGTATTGGTGACTATGTTCAACAACATGGCGGTGTGTCCTTAGTCGAA<br>CAGTTATTACAGGATCCTAAACTGAGCCAAAATAAACAAGCACTTGAAG<br>GACTGGGAGATCTGAAATTACTCTTTGAATATCTGACCTTATTTGGGATT<br>GATGATAAAATTAGCTTTGATCTGAGCTTGGCCCGCGGTCTTGATTATTA<br>TACCGGCGTGATTTACGAAGCTGTTCTCTTGCAAACCCAGCCCAGGCGG<br>GCGAAGAGCCTTTGGGAGTCGGCAGTGTGGCAGCCGGTGGTTCGTTATGA<br>TGGTTTGGTAGGAATGTTTGACCCCTAAAGGCCGTAAAGTACCATGTGTG<br>GGGCTTTCTATCGGTGTGCAACGTATCTTTTCTATTGTTGAACAACGTCTT<br>GAAGCTTTGGAGGAAAAGATCCGTACCACGGAAacCCAAGTCTTAGTTGC<br>aAGTGCCCAAAAAAACTGTTAGAAGAACGCCTGAAACTCGTATCAGAA<br>CTTTGGGACGCCGGCATCAAGGCCGAAGTGTGTATAAAAAAGAACCCGA<br>AATTGTAAACCAACTCCAGTATTGTGAAGAAGCTGGGATCCCACTCGT<br>AGCTATTATTGGTGAGCAAGAATTAAGATGGCGTGATTAAACTGCGT<br>TCAGTAACAAGCCGTGAAGAGGTAGATGTACGTCGCGAAGACTTAGTGG<br>AAGAAATTAACGCCGCACCGGTCAACCGTTA | 198        |

[0132] In some embodiments of the present disclosure, such cysteine substituted mutants are modified to engineer-in, insert, or otherwise introduce a new surface exposed cysteine residue at a defined surface exposed position, where the introduced residue does not substantially interfere with the non-canonical activity of the HRS polypeptide. Specific examples include for example the insertion (or re-insertion back) of additional cysteine residues at the N- or C-terminus of any of the reduced cysteine HRS polypeptides described above. In some embodiments of the present disclosure, the insertion of such N- or C-terminal surface exposed cysteines involves the re-insertion of the last 1, last 2, or last 3 naturally occurring C-terminal amino acids of



the full length human HRS to a reduced cysteine variant of a HRS polypeptide *e.g.*, the re-insertion of all or part of the sequence CIC (Cys Ile Cys). Exemplary reduced cysteine mutants include for example any combination of mutations (or the deletion of) at residues Cys174, Cys191, Cys224, and Cys235, and or the deletion or substitution of Cys507 and Cys509 (based on the numbering of full length human HRS (SEQ ID NO:1) in any of the HRS polypeptides of SEQ ID NOS: 1-106, 170-181, or 185-191 or **Tables D1, D3-D6 or D8**.

**[0133]** For some types of site-specific conjugation or attachment to heterologous molecules such as Fc regions or PEG or other heterologous molecules, HRS polypeptides may have one or more glutamine substitutions, where one or more naturally-occurring (non-glutamine) residues are substituted with glutamine, for example, to facilitate transglutaminase-catalyzed attachment of the molecule(s) to the glutamine's amide group. In some embodiments of the present disclosure, glutamine substitutions are introduced near the N-terminus and/or C-terminus of the HRS polypeptide (*e.g.*, SEQ ID NOS:1-106, 170-181, or 185-191 or the HRS polypeptides of **Tables D1, D3-D6 or D8**). Particular embodiments of the present disclosure include where one or more of residues within 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 amino acids relative to the N-terminus and/or C-terminus of any one of SEQ ID NOS:1-106, 170-181, or 185-191 are substituted with a glutamine residue. These and related HRS polypeptides can also include substitutions (*e.g.*, conservative substitutions) to remove any naturally-occurring glutamine residues, if desired, and thereby regulate the degree of site-specific conjugation or attachment.

**[0134]** For certain types of site-specific conjugation or attachment to heterologous molecules such as Fc regions or PEG or other heterologous molecules, HRS polypeptides may have one or more lysine substitutions, where one or more naturally-occurring (non-lysine) residues are substituted with lysine, for example, to facilitate acylation or alkylation-based attachment of molecule(s) to the lysine's amino group. These methods also typically result in attachment of molecule(s) to the N-terminal residue. In some embodiments of the present disclosure, lysine substitutions are near the N-terminus and/or C-terminus of the HRS polypeptide (*e.g.*, SEQ ID NOS:1-106, 170-181, or 185-191 or the HRS polypeptides of **Tables D1, D3-D6 or D8**). Particular embodiments of the present disclosure include where one or more of residues within 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 amino acids to the N-terminus and/or C-terminus of any one of SEQ ID NOS:1-106, 170-181, or 185-191 (or the HRS polypeptides of **Tables D1, D3-D6 or D8**) are substituted with a lysine residue. These and related HRS polypeptides can also include substitutions (*e.g.*, conservative substitutions) to remove any naturally-occurring lysine residues, if desired, and thereby regulate the degree of site-specific conjugation or attachment.

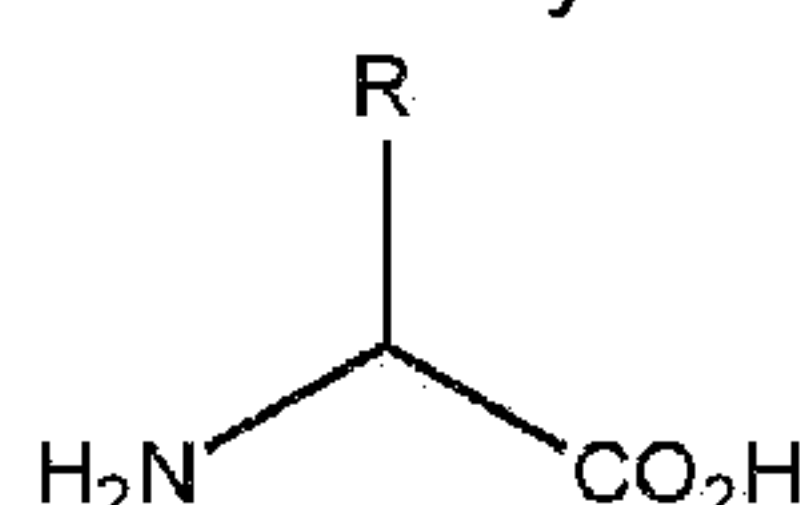
**[0135]** Site-specific conjugation to HRS polypeptides may also be performed by substituting one or more solvent accessible surface amino acids of a HRS polypeptide. For example, suitable solvent accessible amino acids may be determined based on the predicted solvent accessibility using the SPIDDER server (<http://sppider.cchmc.org/>) using the published crystal structure of an exemplary HRS polypeptide (see Xu et al., Structure. 20:1470-7, 2012; and U.S. Application No. 61/674,639). Based on this analysis several amino acids on the surface may potentially be used as mutation sites to introduce functional groups suitable for conjugation or attachment. The surface accessibility score of amino acids based on the crystal structure can be calculated, where the higher scores represent better accessibility. In particular embodiments of the present disclosure, higher scores (for example, >40) are preferred. Accordingly in some embodiments of the present disclosure an amino acid position have a surface accessibility score of greater than 40 may used to introduce a cysteine, lysine, glutamine, or other non-naturally-occurring amino acid.

**[0136]** In particular embodiments of the present disclosure, a solvent accessible surface amino acid is selected from the group consisting of: alanine, glycine, and serine, and can be substituted with naturally occurring amino acids including, but not limited to, cysteine, glutamine, or lysine, or a non-naturally occurring amino acid that is optimized for site specific conjugation or attachment.



**[0137]** In various embodiments of the present disclosure, the present disclosure contemplates site-specific conjugation or attachment at any amino acid position in a HRS polypeptide by virtue of substituting a non-naturally-occurring amino acid comprising a functional group that will form a covalent bond with the functional group attached to a heterologous molecules such as an Fc region or PEG or other heterologous molecule. Non-natural amino acids can be inserted or substituted at, for example, one or more of residues within 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 amino acids relative to the N-terminus and/or C-terminus of any one of SEQ ID NOS:1-106, 170-181, or 185-191 (or the HRS polypeptides of **Tables D1, D3-D6 or D8**); at the N-terminus and/or C-terminus of any one of SEQ ID NOS:1-106, 170-181, or 185-191 (or the HRS polypeptides of **Tables D1, D3-D6 or D8**); or a solvent accessible surface amino acid residue as described herein.

**[0138]** In particular embodiments of the present disclosure, non-naturally occurring amino acids include, without limitation, any amino acid, modified amino acid, or amino acid analogue other than selenocysteine and the following twenty genetically encoded alpha-amino acids: alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine. The generic structure of an alpha-amino acid is illustrated by the following formula:



**[0139]** A non-natural amino acid is typically any structure having the foregoing formula wherein the R group is any substituent other than one used in the twenty natural amino acids. See, e.g., biochemistry texts such as Biochemistry by L. Stryer, 3rd ed. 1988, Freeman and Company, New York, for structures of the twenty natural amino acids. Note that the non-natural amino acids disclosed herein may be naturally occurring compounds other than the twenty alpha-amino acids above. Because the non-natural amino acids disclosed herein typically differ from the natural amino acids in side chain only, the non-natural amino acids form amide bonds with other amino acids, e.g., natural or non-natural, in the same manner in which they are formed in naturally occurring proteins. However, the non-natural amino acids have side chain groups that distinguish them from the natural amino acids. For example, R in foregoing formula optionally comprises an alkyl-, aryl-, aryl halide, vinyl halide, alkyl halide, acetyl, ketone, aziridine, nitrile, nitro, halide, acyl-, keto-, azido-, hydroxyl-, hydrazine, cyano-, halo-, hydrazide, alkenyl, alkynyl, ether, thio ether, epoxide, sulfone, boronic acid, boronate ester, borane, phenylboronic acid, thiol, seleno-, sulfonyl-, borate, boronate, phospho, phosphono, phosphine, heterocyclic-, pyridyl, naphthyl, benzophenone, a constrained ring such as a cyclooctyne, thio ester, enone, imine, aldehyde, ester, thioacid, hydroxylamine, amino, carboxylic acid, alpha-keto carboxylic acid, alpha or beta unsaturated acids and amides, glyoxyl amide, or organosilane group, or the like or any combination thereof.

**[0140]** Specific examples of unnatural amino acids include, but are not limited to, p-acetyl-L-phenylalanine, O-methyl-L-tyrosine, an L-3-(2-naphthyl)alanine, a 3-methyl-phenylalanine, an O-4-allyl-L-tyrosine, a 4-propyl-L-tyrosine, a tri-O-acetyl-GlcNAc $\beta$ -serine,  $\beta$ -O-GlcNAc-L-serine, a tri-O-acetyl-GalNAc- $\alpha$ -threonine, an  $\alpha$ -GalNAc-L-threonine, an L-Dopa, a fluorinated phenylalanine, an isopropyl-L-phenylalanine, a p-azido-L-phenylalanine, a p-acyl-L-phenylalanine, a p-benzoyl-L-phenylalanine, an L-phosphoserine, a phosphonoserine, a phosphonotyrosine, a p-iodo-phenylalanine, a p-bromophenylalanine, a p-amino-L-phenylalanine, an isopropyl-L-phenylalanine, those listed below, or elsewhere herein, and the like.

**[0141]** Accordingly, one may select a non-naturally occurring amino acid comprising a functional group that forms a covalent bond with any preferred functional group of a desired molecule (e.g., Fc region, PEG). Non-natural amino acids, once selected, can either be purchased from vendors, or chemically synthesized. Any number of non-natural amino acids may be incorporated into the target molecule and may vary according to



the number of desired molecules that are to be attached. The molecules may be attached to all or only some of the non-natural amino acids. Further, the same or different non-natural amino acids may be incorporated into a HRS polypeptide, depending on the desired outcome. In certain embodiments of the present disclosure, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more non-natural amino acids are incorporated into a HRS polypeptide any or all of which may be conjugated to a molecule comprising a desired functional group.

**[0142]** In certain aspects of the present disclosure, the use of non-natural amino acids can be utilized to modify (e.g., increase) a selected non-canonical activity of a HRS polypeptide, or to alter the *in vivo* or *in vitro* half-life of the protein. Non-natural amino acids can also be used to facilitate (selective) chemical modifications (e.g., pegylation) of a HRS protein, as described elsewhere herein. For instance, certain non-natural amino acids allow selective attachment of polymers such as an Fc region or PEG to a given protein, and thereby improve their pharmacokinetic properties.

**[0143]** Specific examples of amino acid analogs and mimetics can be found described in, for example, Roberts and Vellaccio, *The Peptides: Analysis, Synthesis, Biology*, Eds. Gross and Meinhofer, Vol. 5, p. 341, Academic Press, Inc., New York, N.Y. (1983). Other examples include peralkylated amino acids, particularly permethylated amino acids. See, for example, *Combinatorial Chemistry*, Eds. Wilson and Czarnik, Ch. 11, p. 235, John Wiley & Sons Inc., New York, N.Y. (1997). Yet other examples include amino acids whose amide portion (and, therefore, the amide backbone of the resulting peptide) has been replaced, for example, by a sugar ring, steroid, benzodiazepine or carbo cycle. See, for instance, *Burger's Medicinal Chemistry and Drug Discovery*, Ed. Manfred E. Wolff, Ch. 15, pp. 619-620, John Wiley & Sons Inc., New York, N.Y. (1995). Methods for synthesizing peptides, polypeptides, peptidomimetics and proteins are well known in the art (see, for example, U.S. Pat. No. 5,420,109; M. Bodanzsky, *Principles of Peptide Synthesis* (1st ed. & 2d rev. ed.), Springer-Verlag, New York, N.Y. (1984 & 1993), see Chapter 7; Stewart and Young, *Solid Phase Peptide Synthesis*, (2d ed.), Pierce Chemical Co., Rockford, Ill. (1984)). Accordingly, the HRS polypeptides of the present disclosure may be composed of naturally occurring and non-naturally occurring amino acids as well as amino acid analogs and mimetics.

### ***Polynucleotides***

**[0144]** Certain embodiments of the present disclosure relate to polynucleotides that encode a HRS polypeptide or a HRS-Fc fusion protein. Also included are polynucleotides that encode any one or more of the Fc regions described herein, alone or in combination with a HRS coding sequence. Among other uses, these embodiments of the present disclosure may be utilized to recombinantly produce a desired HRS, Fc region, or HRS-Fc polypeptide or variant thereof, or to express the HRS, Fc region, or HRS-Fc polypeptide in a selected cell or subject. It will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that encode a HRS polypeptide HRS-Fc fusion protein as described herein. Some of these polynucleotides may bear minimal homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated by the present disclosure, for example polynucleotides that are optimized for human, yeast or bacterial codon selection.

**[0145]** As will be recognized by the skilled artisan, polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide of the present disclosure, and a polynucleotide may, but need not, be linked to other molecules and/or support materials.

**[0146]** Polynucleotides may comprise a native sequence (i.e., an endogenous sequence that encodes an HRS-Fc fusion polypeptide or a portion thereof) or may comprise a variant, or a biological functional equivalent of such a sequence. Polynucleotide variants may contain one or more substitutions, additions,



deletions and/or insertions, as further described below, preferably such that the activity of the encoded polypeptide is not substantially diminished relative to the unmodified polypeptide.

**[0147]** In additional embodiments of the present disclosure, the present disclosure provides isolated polynucleotides comprising various lengths of contiguous stretches of sequence identical to or complementary to a HRS polypeptide or HRS-Fc fusion protein, wherein the isolated polynucleotides encode a truncated HRS polypeptide as described herein

**[0148]** Therefore, multiple polynucleotides can encode the HRS polypeptides, Fc regions, and fusion proteins of the disclosure. Moreover, the polynucleotide sequence can be manipulated for various reasons. Examples include but are not limited to the incorporation of preferred codons to enhance the expression of the polynucleotide in various organisms (see generally Nakamura et al., Nuc. Acid. Res. 28:292, 2000). In addition, silent mutations can be incorporated in order to introduce, or eliminate restriction sites, decrease the density of CpG dinucleotide motifs (see for example, Kameda et al., Biochem. Biophys. Res. Commun. 349:1269-1277, 2006) or reduce the ability of single stranded sequences to form stem-loop structures: (see, e.g., Zuker M., Nucl. Acid Res. 31:3406-3415, 2003). In addition, mammalian expression can be further optimized by including a Kozak consensus sequence (*i.e.*, (a/g)cc(a/g)ccATGg) (SEQ ID NO:199) at the start codon. Kozak consensus sequences useful for this purpose are known in the art (Mantyh et al., PNAS 92: 2662-2666, 1995; Mantyh et al., Prot. Exp. & Purif. 6:124, 1995). Exemplary wild type and codon optimized versions of various HRS polypeptides are provided in **Table D9** below.

| Table D9                      |                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |            |
|-------------------------------|-----------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| Codon Optimized DNA Sequences |                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |            |
| Name                          | Amino Acid Residue Range of SEQ ID NO:1 | Nucleic acid sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | SEQ ID NO: |
| Wild type (Full length)       | 1-509                                   | ATGGCAGAGCGTGCGGCGCTGGAGGAGCTGGTGAAACTTCA<br>GGGAGAGCGCGTGCGAGGCCTCAAGCAGCAGAAGGCCAGCG                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | 111        |
| HisRS)                        |                                         | CCGAGCTGATCGAGGAGGAGGTGGCGAAACTCCTGAAACTG<br>AAGGCACAGCTGGGTCCTGATGAAAGCAAACAGAAATTTGTG<br>CTCAAAACCCCCAAGGGCACAAAGAGACTATAGTCCCCGGCAG<br>ATGGCAGTTCGCGAGAAGGTGTTTGACGTAATCATCCGTTGC<br>TTCAAGCGCCACGGTGCAGAAGTCATTGATACACCTGTATTT<br>GAACTAAAGGAAACACTGATGGGAAAGTATGGGGAAGACTC<br>CAAGCTTATCTATGACCTGAAGGACCAGGGCGGGGAGCTCCT<br>GTCCCTTCGCTATGACCTCACTGTTCCCTTTTGCTCGGTATTGG<br>CAATGAATAAACTGACCAACATTAACGCTACCACATAGCAA<br>AGGTATATCGGCGGGGATAACCCAGCCATGACCCGTGGCCGAT<br>ACCGGGAATTCTACCAGTGTGATTTTGACATTGCTGGGAACTT<br>TGATCCCATGATCCCTGATGCAGAGTGCCTGAAGATCATGTG<br>CGAGATCCTGAGTTCACCTCAGATAGGCGACTTCCTGCTCAA<br>GGTAAACGATCGACGCATTCTAGATGGGATGTTTGCTATCTG<br>TGGTGTCTTGACAGCAAGTCCGTACCATCTGCTCCTCAGTA<br>GACAAGCTGGACAAGGTGTCCTGGGAAGAGGTGAAGAATGA<br>GATGGTGGGAGAGAAGGGCCTTGCACCTGAGGTGGCTGACC<br>GCATTGGGGACTATGTCCAGCAACATGGTGGGGTATCCCTGG<br>TGGAACAGCTGCTCCAGGATCCTAAACTATCCAAAACAAGC<br>AGGCCTTGAGGGCCTGGGAGACCTGAAGTTGCTCTTTGAGT<br>ACCTGACCCTATTTGGCATTGATGACAAAATCTCCTTTGACCT<br>GAGCCTTGCTCGAGGGCTGGATTACTACACTGGGGTGATCTA<br>TGAGGCAGTGCTGCTACAGACCCAGCCCAGGCAGGGGAAG<br>AGCCCCTGGGTGTGGGCAGTGTGGCTGCTGGAGGACGCTATG<br>ATGGGCTAGTGGGCATGTTTCGACCCCAAAGGGCGCAAGGTGC<br>CATGTGTGGGCTCAGCATTGGGGTGGAGCGGATTTTCTCCA<br>TCGTGGAACAGAGACTAGAGGCTTTGGAGGGAAGATACGG<br>ACCACGGAGACACAGGTGCTTGTGGCATCTGCACAGAAGAA<br>GCTGCTAGAGGAAAGACTAAAGCTTGTCTCAGAACTGTGGGA<br>TGCTGGGATCAAGGCTGAGCTGCTGTACAAGAAGAACCCAAA |            |



| Table D9                      |                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |            |
|-------------------------------|-----------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| Codon Optimized DNA Sequences |                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |            |
| Name                          | Amino Acid Residue Range of SEQ ID NO:1 | Nucleic acid sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | SEQ ID NO: |
|                               |                                         | GCTACTGAACCAAGTTACAGTACTGTGAGGAGGCAGGCATCCC<br>ACTGGTGGCTATCATCGGCGAGCAGGAAGTCAAGGATGGGGT<br>CATCAAGCTCCGTTCAAGTGACGAGCAGGGAAGAGGTGGATGT<br>CCGAAGAGAAGACCTTGTGGAGGAAATCAAAAGGAGAACAG<br>GCCAGCCCCCTCTGCATCTGC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |            |
| HisRS 1 <sup>N1</sup>         | 1-141                                   | ATGGCAGAACGTGCCGCCCTGGAAGAGCTGGTAAAACTGCA<br>AGGCGAGCGTGTTCTGGTCTGAAACAGCAGAAAGCAAGCG<br>CTGAACTGATCGAAGAAGAAGTGGCGAAACTGCTGAAACTG<br>AAAGCACAGCTGGGTCCTGATGAATCAAAACAAAAATTCGTC<br>CTGAAAACTCCGAAAGGAACCCGTGACTATTCTCCTCGTCAA<br>ATGGCCGTCCGTGAAAAAGTGTTTCGACGTGATCATTGCTGC<br>TTTAAACGCCATGGTGCCGAAGTGATTGATACCCCGGTGTTT<br>GAGCTGAAAGAGACACTGATGGGCAAATATGGTGAGGACAG<br>CAAAGTGAATTTATGACCTGAAAGATCAGGGTGGTGAAGTCT<br>GAGTCTGCGCTATGATCTGACAGTTCCGTTTGCCCGTTATCTG<br>GCAATG                                                                                                                                                                                                                                                                                           | 112        |
| HisRS 1 <sup>N2</sup>         | 1-408                                   | ATGGCAGAACGTGCCGCCCTGGAAGAGCTGGTAAAACTGCA<br>AGGCGAGCGTGTTCTGGTCTGAAACAGCAGAAAGCAAGCG<br>CTGAACTGATCGAAGAAGAAGTGGCGAAACTGCTGAAACTG<br>AAAGCACAGCTGGGTCCTGATGAATCAAAACAAAAATTCGTC<br>CTGAAAACTCCGAAAGGAACCCGTGACTATTCTCCTCGTCAA<br>ATGGCCGTCCGTGAAAAAGTGTTTCGACGTGATCATTGCTGC<br>TTTAAACGCCATGGTGCCGAAGTGATTGATACCCCGGTGTTT<br>GAGCTGAAAGAGACACTGATGGGCAAATATGGTGAGGACAG<br>CAAAGTGAATTTATGACCTGAAAGATCAGGGTGGTGAAGTCT<br>GAGTCTGCGCTATGATCTGACAGTTCCGTTTGCCCGTTATCTG<br>GCAATGAATAAACTGACCAACATTAAACGCTATCACATTGCT<br>AAAGTCTATCGCCGTGACAATCCTGCTATGACCCGTGGTTCGT<br>ATCGTGAGTTCTATCAGTGTGACTTCGATATTGCCGGCAACTT                                                                                                                                                         | 113        |
|                               |                                         | TGATCCGATGATCCCGGATGCTGAATGCCTGAAAATCATGTG<br>TGAGATCCTGAGCAGTCTGCAGATTGGCGATTTCTGGTGAA<br>AGTCAACGATCGCCGTATTCTGGATGGCATGTTCCGCATCTGT<br>GGTGTTAGCGACTCCAAATTCCGTACCATCTGTAGTAGTGTG<br>GACAACTGGATAAAGTGAGCTGGGAGGAGGTGAAAAACGA<br>AATGGTGGGCGAGAAAGGTCTGGCTCCTGAAGTGGCTGACCG<br>TATTGGTGATTATGTCCAGCAGCACGGTGGAGTATCACTGGT<br>TGAGCAACTGCTGCAAGACCCTAAACTGAGTCAGAATAAACA<br>GGCCCTGGAGGGACTGGGAGATCTGAAACTGCTGTTTCGAGTA<br>TCTGACCCTGTTCCGTATCGATGACAAAATCTCCTTTGACCTG<br>TCACTGGCTCGTGGACTGGACTATTATACCGGCGTGATCTATG<br>AAGCTGTACTGCTGCAAACTCCAGCACAAAGCAGGTGAAGAGC<br>CTCTGGGTGTGGGTAGTGTAGCCGCTGGGGGACGTTATGATG<br>GACTGGTGGGGATGTTTCGACCCTAAAGGCCGTAAAGTTCCTG<br>GTGTGGGTCTGAGTATCGGTGTTGAGCGTATCTTTTCCATCGT<br>CGAGCAACGTCTGGAAGCACTGGAGGAAAAAATCCGTACGA<br>CCGAA |            |
| HisRS 1 <sup>N3</sup>         | 1-113                                   | ATGGCAGAACGTGCCGCCCTGGAAGAGCTGGTAAAACTGCA<br>AGGCGAGCGTGTTCTGGTCTGAAACAGCAGAAAGCAAGCG<br>CTGAACTGATCGAAGAAGAAGTGGCGAAACTGCTGAAACTG<br>AAAGCACAGCTGGGTCCTGATGAATCAAAACAAAAATTCGTC<br>CTGAAAACTCCGAAAGGAACCCGTGACTATTCTCCTCGTCAA<br>ATGGCCGTCCGTGAAAAAGTGTTTCGACGTGATCATTGCTGC<br>TTTAAACGCCATGGTGCCGAAGTGATTGATACCCCGGTGTTT<br>GAGCTGAAAGAGACACTGATGGGCAAATATGGTGAGGACAG<br>CAAAGTGAATTTATGACCTGAAAGATCAGGGTGGTGAAGTCT                                                                                                                                                                                                                                                                                                                                                    | 114        |



| Table D9                      |                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |            |
|-------------------------------|-----------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| Codon Optimized DNA Sequences |                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |            |
| Name                          | Amino Acid Residue Range of SEQ ID NO:1 | Nucleic acid sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | SEQ ID NO: |
| HisRS 1 <sup>N4</sup>         | 1-60                                    | ATGGCAGAACGTGCCGCCCTGGAAGAGCTGGTAAAACTGCA<br>AGGCGAGCGTGTTTCGTGGTCTGAAACAGCAGAAAGCAAGCG<br>CTGAACTGATCGAAGAAGAAGTGGCGAAACTGCTGAAACTG<br>AAAGCACAGCTGGGTCTGATGAATCAAAACAAAAATTCTGTC<br>CTGAAAACTCCGAAG                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | 115        |
| HisRS 1 <sup>N8</sup>         | 1-506                                   | ATGGCAGAGCGTGCGGGCGCTGGAGGAGCTGGTGAAACTTCA<br>GGGAGAGCGCGTGCGAGGCCTCAAGCAGCAGAAGGCCAGCG<br>CCGAGCTGATCGAGGAGGAGGTGGCGAAACTCCTGAAACTG<br>AAGGCACAGCTGGGTCTGATGAAAGCAAACAGAAATTTGTG<br>CTCAAAACCCCCAAGGGCACAAGAGACTATAGTCCCCGGCAG<br>ATGGCAGTTTCGCGAGAAGGTGTTTGACGTAATCATCCGTTGC<br>TTCAAGCGCCACGGTGCAGAAGTCATTGATACACCTGTATTT<br>GAACTAAAGGAAACACTGATGGGAAAGTATGGGGAAGACTC<br>CAAGCTTATCTATGACCTGAAGGACCAGGGCGGGGAGCTCCT<br>GTCCCTTCGCTATGACCTCACTGTTCTTTTGCTCGGTATTTGG<br>CAATGAATAAACTGACCAACATTAAACGCTACCACATAGCAA<br>AGGTATATCGGCGGGATAAACCAGCCATGACCCGTGGCCGAT<br>ACCGGGAATTCTACCAGTGTGATTTTGACATTGCTGGGAACTT<br>TGATCCCATGATCCCTGATGCAGAGTGCCTGAAGATCATGTG<br>CGAGATCCTGAGTTCACCTTCAGATAGGCGACTTCCTGGTCAA<br>GGTAAACGATCGACGCATTCTAGATGGGATGTTTGCTATCTG<br>TGGTGTTCCTGACAGCAAGTTCCTGACCATCTGCTCCTCAGTA<br>GACAAGCTGGACAAGGTGTCCTGGGAAGAGGTGAAGAATGA<br>GATGGTGGGAGAGAAGGGCCTTGACCTGAGGTGGCTGACC<br>GCATTGGGGACTATGTCCAGCAACATGGTGGGGTATCCCTGG<br>TGGAACAGCTGCTCCAGGATCCTAAACTATCCCAAAACAAGC<br>AGCCCTTGGAAGGGCCTGGGAGACCTGAAGTTGCTCTTTGAGT<br>ACCTGACCCTATTTGGCATTGATGACAAAAATCTCCTTTGACCT<br>GAGCCTTGCTCGAGGGCTGGATTACTACACTGGGGTGATCTA<br>TGAGGCAGTGCTGCTACAGACCCCAGCCCAGGCAGGGGAAG<br>AGCCCCTGGGTGTGGGCAGTGTGGCTGCTGGAGGACGCTATG<br>ATGGGCTAGTGGGCATGTTGACCCCAAAGGGCGCAAGGTGC<br>CATGTGTGGGGCTCAGCATTGGGGTGGAGCGGATTTTCTCCA<br>TCGTGGAACAGAGACTAGAGGCTTTGGAGGAGAAGATACGG | 116        |
|                               |                                         | ACCACGGAGACACAGGTGCTTGTGGCATCTGCACAGAAGAA<br>GCTGCTAGAGGAAAGACTAAAGCTTGTCTCAGAACTGTGGGA<br>TGCTGGGATCAAGGCTGAGCTGCTGTACAAGAAGAACCCAAA<br>GCTACTGAACCAGTTACAGTACTGTGAGGAGGCAGGCATCCC<br>ACTGGTGGCTATCATCGGCGAGCAGGAACTCAAGGATGGGGT<br>CATCAAGCTCCGTTCAGTGACGAGCAGGGAAGAGGTGGATGT<br>CCGAAGAGAAGACCTTGTGGAGGAAATCAAAAGGAGAACAG<br>GCCAGCCCCCTC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |            |
| HisRS 1 <sup>N6</sup>         | 1-48                                    | ATGGCAGAACGTGCCGCCCTGGAAGAGCTGGTAAAACTGCA<br>AGGCGAGCGTGTTTCGTGGTCTGAAACAGCAGAAAGCAAGCG<br>CTGAACTGATCGAAGAAGAAGTGGCGAAACTGCTGAAACTG<br>AAAGCACAGCTGGGTCTGAT                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 117        |
| HisRS1 <sup>I1</sup>          | 191-333                                 | TGCCTGAAAATCATGTGTGAGATCCTGAGTAGTCTGCAAATT<br>GGCGACTTTCTGGTCAAAGTGAACGATCGCCGTATTCTGGAT<br>GGCATGTTTCGCCATCTGTGGTGTAGCGACTCCAAATTCCGTA<br>CAATCTGTAGCAGCGTGGACAAACTGGATAAAGTGTCTCTGGG<br>AAGAGGTGAAAAACGAAATGGTGGGTGAAAAAGGTCTGGCT<br>CCGGAGGTTGCTGACCGTATCGGTGATTATGTTTCAGCAGCAC<br>CCCCCTCTTACTCTGCTTCAACAAGTCTCTCAACAGCCCAAA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | 118        |



| Table D9                      |                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |            |
|-------------------------------|-----------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| Codon Optimized DNA Sequences |                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |            |
| Name                          | Amino Acid Residue Range of SEQ ID NO:1 | Nucleic acid sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | SEQ ID NO: |
|                               |                                         | GGCGGTGTTAGTCTGGTTGAAACACCTGCTGCAAGACCCGAAA<br>CTGTCTCAGAACAAACAGGCCCTGGAAGGACTGGGAGATCTG<br>AAACTGCTGTTTCGAGTATCTGACGCTGTTCCGGCATTGATGAC<br>AAAATTTCTTTTCGACCTGTCACTGGCACGTGGACTGGACTATT<br>ATACCGGT                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |            |
| HisRS1 <sup>C1</sup>          | 405-509                                 | CGTACCACCGAAACCCAAAGTTCTGGTTGCCTCAGCTCAGAAA<br>AAACTGCTGGAAGAACGCCTGAAACTGGTTAGCGAACTGTGG<br>GATGCTGGCATTAAAGCCGAACTGCTGTATAAAAAAACC<br>AAACTGCTGAATCAGCTGCAGTATTGTGAGGAAGCGGGTATT<br>CCTCTGGTGGCCATTATCGGAGAACAGGAACTGAAAGACGGC<br>GTTATTAACTGCGTAGCGTGACCTCTCGTGAAGAAGTTGAC<br>GTTCCGCGTGAAGATCTGGTCGAGGAAATCAAACGTCGTACC<br>GGTCAACCTCTGTGTATTGTC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | 119        |
| HisRS 1 <sup>N5</sup>         | 1-243 + 27aa                            | ATGGCAGAACGTGCCGCCCTGGAAGAGCTGGTAAACTGCA<br>AGCCGAGCGTGTTTCGTGGTCTGAAACAGCAGAAAGCAAGCG<br>CTGAACTGATCGAAGAAGAAGTGGCGAAACTGCTGAAACTG<br>AAAGCACAGCTGGGTCTGATGAATCAAAACAAAAATTCGTC<br>CTGAAAACTCCGAAAGGAACCCGTGACTATTCTCCTCGTCAA<br>ATGGCCGTCCGTGAAAAAGTGTTTCGACGTGATCATTGCTGC<br>TTTAAACGCCATGGTGCCGAAGTGATTGATACCCCGGTGTTT<br>GAGCTGAAAGAGACACTGATGGGCAAATATGGTGAGGACAG<br>CAAACGTATCTATGACCTGAAAGACCAAGGCGGTGAACTGCT<br>GTCCCTGCGTTATGATCTGACTGTGCCGTTTGCCCGTTATCTG<br>GCCATGAATAAACTGACGAACATTAAACGCTATCACATTGCC<br>AAAGTGATCGCCGTGACAATCCTGCTATGACTCGTGGACGT<br>TATCGTGAATTCTATCAGTGTGACTTCGATATTGCCGGCAACT<br>TCGACCTATGATTCCGGATGCTGAATGCCTGAAAATCATGT<br>GTGAGATCCTGAGCAGCCTGCAAATTGGTGACTTCCTGGTGA<br>AAGTGAATGACCGTCGTATCCTGGATGGCATGTTTGCCATTTG<br>TGGTGTGAGCGATTCCAAATCCGTACCATCTGTAGTAGTGTG<br>GACAACTGGATAAAGTGGGCTATCCGTGGTGAACTCTTGT<br>AGCCGTATTCTGAACTATCCTAAAACCAGCCGCCCGTGGCGT<br>GCTTGGGAAACT | 120        |
| HisRS1 <sup>C2</sup>          | 1-60 + 175-509                          | ATGGCAGAACGTGCCGCCCTGGAAGAGCTGGTAAACTGCA<br>AGCCGAGCGTGTTTCGTGGTCTGAAACAGCAGAAAGCAAGCG<br>CTGAACTGATCGAAGAAGAAGTGGCGAAACTGCTGAAACTG<br>AAAGCACAGCTGGGTCTGATGAATCAAAACAAAAATTCGTC<br>CTGAAAACTCCGAAAGACTTCGATATTGCCGGGAATTTTGAC<br>CCTATGATCCCTGATGCCGAATGTCTGAAAATCATGTGTGAG<br>ATCCTGAGCAGTCTGCAGATTGGTGACTTCCTGGTGAAAGTG<br>AACGATCGCCGTATTCTGGATGGAATGTTTGCCATTTGTGGCG                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 121        |
|                               |                                         | TGTCTGACAGCAAATTTCTGACGATCTGTAGCAGCGTGGATA<br>AACTGGATAAAGTGAGCTGGGAGGAGGTGAAAAATGAGATG<br>GTGGGCGAAAAAGGTCTGGCACCTGAAGTGGCTGACCGTATC<br>GGTGATTATGTTACAGCAACATGGCGGTGTTTCTCTGGTCAAC<br>AGCTGCTGCAAGACCCAAAACCTGAGCCAGAACAACAGGCA<br>CTGGAAGGACTGGGTGATCTGAAACTGCTGTTTGAGTATCTG<br>ACGCTGTTTGGCATCGATGACAAAATCTCGTTTGACCTGAGC<br>CTGGCACGTGGTCTGGATTATTATACCGGCGTGATCTATGAA<br>GCCGTCTGCTGCAAACTCCAGCACAAGCAGGTGAAGAACCT<br>CTGGGTGTTGGTAGTGTAGCGGCAGGCGGACGTTATGATGGA<br>CTGGTGGGGATGTTTGATCCGAAAGGCCGTAAAGTTCCGTGT<br>GTCCGTCTGAGTATCGGGGTTGAGCGTATCTTTAGCATTGTGG<br>AGCAACGTCTGGAAGCTCTGGAGGAAAAAATCCGTACCACCG<br>AAACCCAAGTTCTGGTTGCCTCAGCTCAGAAAAAACTGCTGG<br>AAGAACGCCTGAAACTGGTTAGCGAACTGTGGGATGCTGGCA<br>TTAAACGCCCAACTGCTCTATAAAAAAAGCCCAAACTGCTCA                                                                                                                                                      |            |



| Table D9                      |                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |            |
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| Codon Optimized DNA Sequences |                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |            |
| Name                          | Amino Acid Residue Range of SEQ ID NO:1 | Nucleic acid sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | SEQ ID NO: |
|                               |                                         | TTAAAGCCGAACTGCTGTATATAAAXXAAACCCGAAACTGCTGA<br>ATCAGCTGCAGTATTGTGAGGAAGCGGGTATTCTCTGGTGG<br>CCATTATCGGAGAACAGGAAGTAAAGACGGCGTTATTAAC<br>TGCGTAGCGTGACCTCTCGTGAAGAAGTTGACGTTCCGCCGTG<br>AAGATCTGGTTCGAGGAAATCAAACGTCGTACCGGTCAACCTC<br>TGTGTATTTC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |            |
| HisRS 1 <sup>03</sup>         | 1-60 + 211-509                          | ATGGCAGAACGTGCCGCCCTGGAAGAGCTGGTAAAACTGCA<br>AGGCGAGCGTGTTCTGTGGTCTGAAACAGCAGAAAGCAAGCG<br>CTGAACTGATCGAAGAAGAAGTGCGGAAACTGCTGAAACTG<br>AAAGCACAGCTGGGTCTCTGATGAATCAAAACAAAAATTCGTC<br>CTGAAAACTCCGAAAGTGAATGATCGCCGTATCCTGGATGGC<br>ATGTTTGCCATTTGTGGTGTGAGCGACTCGAAATTCGGTACGA<br>TTTGCTCTAGCGTCGATAAACTGGACAAAGTGTCTGGGAAG<br>AGGTGAAAAACGAGATGGTGGGTGAGAAAGGTCTGGCTCCT<br>GAAGTTGCCGACCGTATTGGTGATTATGTTGAGCAGCATGGC<br>GGTGTTCCTACTGGTTGAACAACTGCTGCAAGACCCGAAACTG<br>TCTCAGAATAAACAGGCGCTGGAAGGACTGGGAGATCTGAA<br>ACTGCTGTTTGAGTATCTGACCCTGTTCCGCATTGATGACAAA<br>ATCAGCTTCGACCTGAGCCTGGCACGTGGTCTGGATTATTATA<br>CCGGCGTGATCTATGAAGCCGTTCTGCTGCAGACACCAGCAC<br>AAGCAGGCGAAGAACCTCTGGGTGTTGGTTCTGTGGCAGCCG<br>GTGGTCGTTATGATGGACTGGTAGGCATGTTGATCCGAAAG<br>GCCGTAAAGTTCGGTGTGTGGGACTGAGTATCGGTGTTGAGC<br>GTATCTTTAGCATCGTGGAACAACGTCTGGAAGCGCTGGAGG<br>AGAAAATTCGTACCACCGAAACCCAAAGTTCTGGTTGCCTCAG<br>CTCAGAAAAAACTGCTGGAAGAAGCCTGAAACTGGTTAGCG<br>AACTGTGGGATGCTGGCATTAAAGCCGAACTGCTGTATAAAA<br>AAAACCCGAAACTGCTGAATCAGCTGCAGTATTGTGAGGAAG<br>CGGGTATTCTCTGGTGGCCATTATCGGAGAACAGGAACTGA<br>AAGACGGCGTTATTAAGCTGCGTAGCGTGACCTCTCGTGAAG<br>AAGTTGACGTTCCCGGTGAAGATCTGGTCGAGGAAATCAAAC<br>GTCGTACCGGTCAACCTCTGTGTATTTC | 122        |
| HisRS 1 <sup>C4</sup>         | 1-100 + 211-509                         | ATGGCAGAACGTGCCGCCCTGGAAGAGCTGGTAAAACTGCA<br>AGGCGAGCGTGTTCTGTGGTCTGAAACAGCAGAAAGCAAGCG<br>CTGAACTGATCGAAGAAGAAGTGCGGAAACTGCTGAAACTG<br>AAAGCACAGCTGGGTCTCTGATGAATCAAAACAAAAATTCGTC<br>CTGAAAACTCCGAAAGGAACTCGTGATTATAGCCCTCGCCAG<br>ATGGCTGTCCGTGAAAAAGTGTTGATGTGATCATTGCTGCT<br>TCAAACGTCATGGTGCCGAAGTCATTGATACCCCGGTGTTTCG<br>AGCTGAAAGTGAACGATCGCCGTATTCTGGATGGCATGTTTCG<br>CCATTTGTGGTGTTAGCGATAGCAAATTCGTAACAATCTGCTC<br>TAGCGTGGACAAACTGGACAAAGTGAGCTGGGAAGAGGTGA<br>AAAACGAGATGGTGGGTGAGAAAGGCCTGGCTCCTGAAGTT<br>GCCGACCGTATCGGAGATTATGTTGAGCAGCATGGCGGAGTT<br>TCACTGGTTGAACAACTGCTGCAAGACCCGAAACTGTCTCAG                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 123        |
|                               |                                         | AACAAACAGGCACTGGAAGGTCTGGGAGATCTGAAACTGCT<br>GTTTCGAGTATCTGACGCTGTTCCGTATTGACGACAAAATTTCC<br>TTCGACCTGTGCTGGCACGTGGTCTGGATTATTATACAGGCG<br>TGATCTATGAGGCTGTACTGCTGCAGACACCAGCACAAAGCAG<br>GTGAAGAGCCTCTGGGTGTTGGTTCAGTTGCTGCCGGTGGAC<br>GTTATGACGGAAGTGGTAGGGATGTTTGACCCAAAAGGCCGTA<br>AAGTCCCGTGTGTAGGACTGTCTATTGGCGTTGAGCGTATCTT<br>TAGCATCGTGGAGCAACGTCTGGAAGCTCTGGAGGAGAAAAAT<br>CCGTACCACCGAAACCCAAAGTTCTGGTTGCCTCAGCTCAGAA<br>AAAAGTGTGGAAGAAGCCTGAAACTGGTTAGCGAACTGTG<br>GGATGCTGGCATTAAAGCCGAACTGCTGTATAAAAAAAACCC<br>GAAAGTGTGATCAGCTGCAGTATTGTGAGGAAGCGGGTAT<br>TCCTCTGGTGGCCATTATCGGAGAACAGGAACTGAAAGACGG<br>CGTTATTAAGCTGCGTAGCGTGACCTCTCGTGAAGAAGTTGA<br>CGTTCCCGGTGAAGATCTGGTCGAGGAAATCAAACGTCGTAC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |            |



| Table D9                      |                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |            |
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| Codon Optimized DNA Sequences |                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |            |
| Name                          | Amino Acid Residue Range of SEQ ID NO:1 | Nucleic acid sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | SEQ ID NO: |
|                               |                                         | CGGTCAACCTCTGTGTATTGTC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |            |
| HisRS1 <sup>C5</sup>          | 1-174 + 211-509                         | ATGGCAGAACGTGCCGCCCTGGAAGAGCTGGTAAAACTGCAAGGCGAGCGTGTTTCGTGGTCTGAAACAGCAGAAAGCAAGCGCTGAACTGATCGAAGAAGAAGTGGCGAAACTGCTGAAACTGAAAGCACAGCTGGGTCTCTGATGAATCAAAACAAAAATTCGTCCTGAAAACCTCCGAAAGGAACTCGTGATTATAGCCCTCGCCAGATGGCTGTCCGTGAAAAAGTGTTTCGATGTGATCATTGCTGCTTCAAACGTCATGGTGCCGAAGTCATTGATACCCCGGTGTTTCGAGCTGAAAGAAACCCTGATGGGCAAATATGGGGAAGATTCCAAACTGATCTATGACCTGAAAGACCAGGGAGGTGAACTGCTGTCTCTGCGCTATGACCTGACTGTTCCCTTTTGCTCGCTATCTGGCCATGAATAAACTGACCAACATCAAACGCTATCATATCGCCAAGTGTATCGCCGTGACAATCCAGCAATGACCCGTGGTTCGTTATCGTGAAATTTTATCAGTGTGTGAACGATCGCCGTATTCTGGACGGCATGTTCCGCATTGTGGTGTGTCTGACTCCAAATTTTCGTACGATCTGCTCAAGCGTGGACAAACTGGACAAAGTGAGCTGGGAAGAGGTTGAAAAACGAGATGGTGGGTGAGAAAGGCCTGGCTCCTGAAGTTGCCGACCGTATCGGAGATTATGTTTCAGCAGCATGGCGGAGTTTCACTGGTTGAACAACCTGCTGCAAGACCCGAACTGTCACAGAACAAACAGGCACTGGAAGGTCTGGGGGATCTGAAACTGCTGTTTCGAGTATCTGACGCTGTTTCGGTATTGACGACAAAATCAGCTTCGATCTGAGCCTGGCACGTGGTCTGGACTATTATACCGGCGTGATTTATGAAGCCGTTCTGCTGCAGACTCCAGCACAGCAGGTGAAGAGCCTCTGGGTGTTGGAAGTGTGGCAGCCGGTGGCCGTTATGATGGTCTGGTTGGCATGTTTGACCCGAAAGGCCGTAAAGTCCCGTGTGTAGGACTGTCTATCGGCCGTGAGCGTATTTTTAGCATCGTGGAACAACGCCTGGAAGCTCTGGAAGAGAAAATCCGTACCACCGAAACCCAAGTTCTGGTTGCCTCAGCTCAGAAAAAACTGCTGGAAGAACGCCTGAAACTGGTTAGCGAACTGTGGGATGCTGGCATTAAAGCCGAACTGCTGTATAAAAAAACCCGAAACTGCTGAATCAGCTGCAGTATTGTGAGGAAGCGGGTATTCCTCTGGTGGCCATTATCGGAGAACAGGAACTGAAAGACGGCGTTATTAACCTGCGTAGCGTGACCTCTCGTGAAGAAGTTGACGTTCCCGTGAAGATCTGGTCGAGGAAATCAAACGTCGTACCGGTCAACCTCTGTGTATTGTC | 124        |
| HisRS1 <sup>C6</sup>          | 1-60 + 101-509                          | ATGGCAGAACGTGCCGCCCTGGAAGAGCTGGTAAAACTGCAAGGCGAGCGTGTTTCGTGGTCTGAAACAGCAGAAAGCAAGCGCTGAACTGATCGAAGAAGAAGTGGCGAAACTGCTGAAACTGAAAGCACAGCTGGGTCTCTGATGAATCAAAACAAAAATTCGTCCTGAAAACCTCCGAAAGAAACCCTGATGGGCAAAATATGGCGAAGATTCCAAACTGATCTATGACCTGAAAGACCAAGGCGGTGACTGCTGTCCCTGCGTTATGACCTGACTGTTCCGTTTGCTCGTTATCTGGCCATGAATAAACTGACCAACATTAACGCTATCACATTGCCAAAGTGTATCGCCGTGACAATCCTGCTATGACTCGTGGACGTTATCGTGAATTCTATCAGTGTGACTTCGATATTGCCG                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 125        |
|                               |                                         | GCAACTTCGACCCTATGATTCCGGATGCTGAATGCCTGAAAAATCATGTGTGAGATCCTGAGCAGCCTGCAAATTGGTGACTTCCTGGTGAAGTGAATGACCGTCGTATCCTGGATGCCATGTTTCGCATTTTGTGGTGTTAGCGATTCCAAATTCCTGACCATCTGTAGTAGTGTGGACAAACTGGATAAAGTGAGCTGGGAAGAGGTGAATAACGAAATGGTGGGCGAAAAAGGTCTGGCACCTGAGGTTGCTGATCGTATCGGTGACTATGTCCAGCAGCATGGAGGTGTTCACTGGTTGAGCAACTGCTGCAAGATCCGAAACTGTCTCAGACAAAACAGGCCCTGGAAGGACTGGCTGATCTGAAACTGCTGTTCGAGTATCTGACGCTGTTTCGGTATTGATGACAAAATCTCGTTCGACCTGTCTCTGGCTCGTGGACTGGATTATTATACGGGCGTATCTATGAAGCTGTCCTGCTGCAGACACCAGCACAAAGCAGGTGAAGAGCCTCTGGGTGTTGGAAGTGTGCTGCCGGTGGTTCGTATGACGGACTGGTTGGCATGTTTCGATCCGAAAGGCCGTAAA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |            |



| Table D9                      |                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |            |
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| Codon Optimized DNA Sequences |                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |            |
| Name                          | Amino Acid Residue Range of SEQ ID NO:1 | Nucleic acid sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | SEQ ID NO: |
|                               |                                         | GTTCCGTGTGTAGGACTGAGCATTGGCGTTGAGCGTATCTTTT<br>CCATCGTTGAGCAACGTCTGGAAGCACTGGAAGAGAAAAATCC<br>GTACCACCGAAACCCAAGTTCTGGTTGCCTCAGCTCAGAAAA<br>AACTGCTGGAAGAACGCCTGAAACTGGTTAGCGAACTGTGGG<br>ATGCTGGCATTAAAGCCGAACTGCTGTATAAAAAAACC<br>AACTGCTGAATCAGCTGCAGTATTGTGAGGAAGCGGGTATTC<br>CTCTGGTGGCCATTATCGGAGAACAGGAACTGAAAGACGGCG<br>TTATTAAACTGCGTAGCGTGACCTCTCGTGAAGAAGTTGACG<br>TTCGCCGTGAAGATCTGGTTCGAGGAAATCAAACGTCGTACCG<br>GTCAACCTCTGTGTATTG                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |            |
| HisRS 1 <sup>C7</sup>         | 1-100 + 175-509                         | ATGGCAGAACGTGCCGCCCTGGAAGAGCTGGTAAAACTGCA<br>AGGCGAGCGTGTTCTGTGGTCTGAAACAGCAGAAAGCAAGCG<br>CTGAACTGATCGAAGAAGAAGTGGCGAAACTGCTGAAACTG<br>AAAGCACAGCTGGGTCTGATGAATCAAAACAAAAATTCGTC<br>CTGAAAACTCCGAAAGGAACTCGTGATTATAGCCCTCGCCAG<br>ATGGCTGTCCGTGAAAAAGTGTTTCGATGTGATCATTTCGCTGCT<br>TCAAACGTCATGGTGCCGAAGTCATTGATACCCCGGTGTTTCG<br>AGCTGAAAGATTTCGATATTGCCGGCAACTTTGATCCGATGA<br>TTCCGGATGCTGAGTGTCTGAAAATCATGTGTGAGATCCTGA<br>GTAGTCTGCAGATTGGGGATTTCCTGGTGAAAGTGAACGATC<br>GCCGTATTCTGGACGGCATGTTTGCCATTTGTGGCGTTAGCGA<br>TAGCAAATTCGTACGATCTGTAGCAGTGTGGACAAACTGGA<br>TAAAGTCTCTTGGGAAGAGGTCAAAAACGAGATGGTTGGTGA<br>GAAAGGCCTGGCTCCTGAAGTGGCTGACCGTATTGGTGATTA<br>TGTCAGCAGCATGGTGGTGTTCCTGCTGGTTGAACAACTGCT<br>GCAAGACCCGAAACTGTCTCAGAACAAACAGGCACTGGAAG<br>GTCTGGGTGATCTGAAACTGCTGTTTCGAGTATCTGACGCTGTT<br>CGGTATTGACGACAAAATTTCTTCGACCTGTCACTGGCACGT<br>GGTCTGGATTATTATACAGGCGTAATCTATGAGGCTGTACTG<br>CTGCAAACTCCAGCACAAGCAGGTGAAGAACCTCTGGGAGTT<br>GGTAGTGTAGCGGCAGGGGGTCTTATGATGGGCTGGTCCGG<br>ATGTTTCGATCCAAAAGGCCGTAAAGTCCCGTGTGTTGGTCTG<br>TCTATTGGCGTTGAGCGTATCTTCTCCATCGTGGAGCAACGTC<br>TGGAAGCTCTGGAAGAAAAATCCGTACCACCGAAACCCAA<br>GTTCTGGTTGCCTCAGCTCAGAAAAAACTGCTGGAAGAACGC<br>CTGAAACTGGTTAGCGAACTGTGGGATGCTGGCATTAAAGCC<br>GAACTGCTGTATAAAAAAACCAGAACTGCTGAATCAGCTG<br>CAGTATTGTGAGGAAGCGGGTATTCTCTGGTGGCCATTATC<br>GGAGAACAGGAACTGAAAGACGGCGTTATTAACTGCGTAG<br>CGTGACCTCTCGTGAAGAAGTTGACGTTCCCGGTGAAGATCT<br>GGTCGAGGAAATCAAACGTCGTACCGGTCAACCTCTGTGTAT<br>TTGC | 126        |
| HisRS1 <sup>C10</sup>         | 369-509                                 | ATGTTTCGACCCAAAAGGCCGTAAAGTTCCGTGTGTAGGGCTG<br>TCTATCGGTGTTGAGCGTATCTTCTCCATCGTTGAGCAGCGTC<br>TGGAAGCACTGGAGGAAAAATCCGTACGACCGAGACTCAA<br>GTCCTGGTTGCTAGTGCCAGAAAAAACTGCTGGAAGAGCGC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 127        |
|                               |                                         | CTGAAACTGGTTAGTGAGCTGTGGGATGCCGGTATTAAAGCC<br>GAACTGCTGTATAAAAAAACCAGAACTGCTGAATCAGCTG<br>CAGTATTGTGAAGAAGCGGGCATTCCGCTGGTAGCGATTATC<br>GGGGAACAAGAACTGAAAGATGGCGTGATCAAACGCTGCGTAG<br>CGTTACAAGCCGTGAGGAAGTGGACGTCCGCCGTGAGGATCT<br>GGTTGAAGAGATTAAACGCCGTACAGGTCAGCCTCTGTGTAT<br>TTGC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |            |



**[0149]** Additional coding or non-coding sequences may, but need not, be present within a polynucleotide of the present disclosure, and a polynucleotide may, but need not, be linked to other molecules and/or support materials. Hence, the polynucleotides of the present disclosure, regardless of the length of the coding sequence itself, may be combined with other DNA or RNA sequences, such as promoters, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like, such that their overall length may vary considerably.

**[0150]** It is therefore contemplated that a polynucleotide fragment of almost any length may be employed; with the total length preferably being limited by the ease of preparation and use in the intended recombinant DNA protocol. Included are polynucleotides of about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 41, 43, 44, 45, 46, 47, 48, 49, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 220, 240, 260, 270, 280, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000 or more (including all integers in between) bases in length, including any portion or fragment (e.g., greater than about 6, 7, 8, 9, or 10 nucleotides in length) of a HRS reference polynucleotide (e.g., base number X-Y, in which X is about 1-3000 or more and Y is about 10-3000 or more), or its complement.

**[0151]** Embodiments of the present disclosure of the present disclosure also include "variants" of the HRS reference polynucleotide sequences. Polynucleotide "variants" may contain one or more substitutions, additions, deletions and/or insertions in relation to a reference polynucleotide. Generally, variants of a HRS reference polynucleotide sequence may have at least about 30%, 40% 50%, 55%, 60%, 65%, 70%, generally at least about 75%, 80%, 85%, desirably about 90% to 95% or more, and more suitably about 98% or more sequence identity to that particular nucleotide sequence (Such as for example, SEQ ID NOS:111-127, 182-184, 192-198; see also the Examples) as determined by sequence alignment programs described elsewhere herein using default parameters. In certain embodiments of the present disclosure, variants may differ from a reference sequence by about 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, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 41, 43, 44, 45, 46, 47, 48, 49, 50, 60, 70, 80, 90, 100 (including all integers in between) or more bases. In certain embodiments of the present disclosure, such as when the polynucleotide variant encodes a HRS polypeptide having a non-canonical activity, the desired activity of the encoded HRS polypeptide is not substantially diminished relative to the unmodified polypeptide. The effect on the activity of the encoded polypeptide may generally be assessed as described herein. In some embodiments of the present disclosure, the variants can alter the aggregation state of the HRS polypeptides, for example to provide for HRS polypeptides that exist in different embodiments of the present disclosure primarily as a monomer, dimer or multimer.

**[0152]** Certain embodiments of the present disclosure include polynucleotides that hybridize to a reference HRS polynucleotide sequence, (such as for example, SEQ ID NOS:111-127, 182-184, 192-198; see also the Examples) or to their complements, under stringency conditions described below. As used herein, the term "hybridizes under low stringency, medium stringency, high stringency, or very high stringency conditions" describes conditions for hybridization and washing. Guidance for performing hybridization reactions can be found in Ausubel *et al.*, (1998, *supra*), Sections 6.3.1-6.3.6. Aqueous and non-aqueous methods are described in that reference and either can be used.

**[0153]** Reference herein to low stringency conditions include and encompass from at least about 1% v/v to at least about 15% v/v formamide and from at least about 1 M to at least about 2 M salt for hybridization at 42°C, and at least about 1 M to at least about 2 M salt for washing at 42°C. Low stringency conditions also may include 1% Bovine Serum Albumin (BSA), 1 mM EDTA, 0.5 M NaHPO<sub>4</sub> (pH 7.2), 7% SDS for hybridization at 65°C, and (i) 2 × SSC, 0.1% SDS; or (ii) 0.5% BSA, 1 mM EDTA, 40 mM NaHPO<sub>4</sub> (pH 7.2), 5% SDS for washing at room temperature. One embodiment of the present disclosure of low stringency conditions includes hybridization in 6 × sodium chloride/sodium citrate (SSC) at about 45°C, followed by two washes in



0.2 × SSC, 0.1% SDS at least at 50°C (the temperature of the washes can be increased to 55°C for low stringency conditions).

**[0154]** Medium stringency conditions include and encompass from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5 M to at least about 0.9 M salt for hybridization at 42°C, and at least about 0.1 M to at least about 0.2 M salt for washing at 55°C. Medium stringency conditions also may include 1% Bovine Serum Albumin (BSA), 1 mM EDTA, 0.5 M NaHPO<sub>4</sub> (pH 7.2), 7% SDS for hybridization at 65° C, and (i) 2 × SSC, 0.1% SDS; or (ii) 0.5% BSA, 1 mM EDTA, 40 mM NaHPO<sub>4</sub> (pH 7.2), 5% SDS for washing at 60-65°C. One embodiment of the present disclosure of medium stringency conditions includes hybridizing in 6 × SSC at about 45°C, followed by one or more washes in 0.2 × SSC, 0.1% SDS at 60°C. High stringency conditions include and encompass from at least about 31% v/v to at least about 50% v/v formamide and from about 0.01 M to about 0.15 M salt for hybridization at 42° C, and about 0.01 M to about 0.02 M salt for washing at 55°C.

**[0155]** High stringency conditions also may include 1% BSA, 1 mM EDTA, 0.5 M NaHPO<sub>4</sub> (pH 7.2), 7% SDS for hybridization at 65°C, and (i) 0.2 × SSC, 0.1% SDS; or (ii) 0.5% BSA, 1 mM EDTA, 40 mM NaHPO<sub>4</sub> (pH 7.2), 1% SDS for washing at a temperature in excess of 65°C. One embodiment of the present disclosure of high stringency conditions includes hybridizing in 6 × SSC at about 45°C, followed by one or more washes in 0.2 × SSC, 0.1% SDS at 65° C. One embodiment of the present disclosure of very high stringency conditions includes hybridizing in 0.5 M sodium phosphate, 7% SDS at 65°C, followed by one or more washes in 0.2 × SSC, 1% SDS at 65°C.

**[0156]** Other stringency conditions are well known in the art and a skilled artisan will recognize that various factors can be manipulated to optimize the specificity of the hybridization. Optimization of the stringency of the final washes can serve to ensure a high degree of hybridization. For detailed examples, see Ausubel *et al.*, *supra* at pages 2.10.1 to 2.10.16 and Sambrook *et al.* (1989, *supra*) at sections 1.101 to 1.104. While stringent washes are typically carried out at temperatures from about 42°C to 68°C, one skilled in the art will appreciate that other temperatures may be suitable for stringent conditions. Maximum hybridization rate typically occurs at about 20°C to 25°C below the T<sub>m</sub> for formation of a DNA-DNA hybrid. It is well known in the art that the T<sub>m</sub> is the melting temperature, or temperature at which two complementary polynucleotide sequences dissociate. Methods for estimating T<sub>m</sub> are well known in the art (see Ausubel *et al.*, *supra* at page 2.10.8).

**[0157]** In general, the T<sub>m</sub> of a perfectly matched duplex of DNA may be predicted as an approximation by the formula:  $T_m = 81.5 + 16.6 (\log_{10} M) + 0.41 (\%G+C) - 0.63 (\% \text{ formamide}) - (600/\text{length})$  wherein: M is the concentration of Na<sup>+</sup>, preferably in the range of 0.01 molar to 0.4 molar; %G+C is the sum of guanosine and cytosine bases as a percentage of the total number of bases, within the range between 30% and 75% G+C; % formamide is the percent formamide concentration by volume; length is the number of base pairs in the DNA duplex. The T<sub>m</sub> of a duplex DNA decreases by approximately 1° C with every increase of 1% in the number of randomly mismatched base pairs. Washing is generally carried out at T<sub>m</sub> - 15° C for high stringency, or T<sub>m</sub> - 30°C for moderate stringency.

**[0158]** In one example of a hybridization procedure, a membrane (e.g., a nitrocellulose membrane or a nylon membrane) containing immobilized DNA is hybridized overnight at 42° C in a hybridization buffer (50% deionized formamide, 5 × SSC, 5 × Denhardt's solution (0.1% ficoll, 0.1% polyvinylpyrrolidone and 0.1% bovine serum albumin), 0.1% SDS and 200 mg/mL denatured salmon sperm DNA) containing a labeled probe. The membrane is then subjected to two sequential medium stringency washes (*i.e.*, 2 × SSC, 0.1% SDS for 15 min at 45° C, followed by 2 × SSC, 0.1% SDS for 15 min at 50° C), followed by two sequential higher stringency washes (*i.e.*, 0.2 × SSC, 0.1% SDS for 12 min at 55° C followed by 0.2 × SSC and 0.1% SDS solution for 12



min at 65-68°C.

***Production of HRS polypeptides and HRS-Fc Conjugates***

**[0159]** HRS-Fc conjugate polypeptides may be prepared by any suitable procedure known to those of skill in the art for example, by using standard solid-phase peptide synthesis (Merrifield, J. Am. Chem. Soc. 85:2149-2154 (1963)), or by recombinant technology using a genetically modified host. Protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Alternatively, various fragments may be chemically synthesized separately and combined using chemical methods to produce the desired molecule.

**[0160]** HRS-Fc conjugates can also be produced by expressing a DNA or RNA sequence encoding the HRS polypeptide or HRS-Fc conjugates in question in a suitable host cell by well-known techniques. The polynucleotide sequence coding for the HRS-Fc conjugate or HRS polypeptide may be prepared synthetically by established standard methods, e.g., the phosphoramidite method described by Beaucage et al., Tetrahedron Letters 22:1859-1869, 1981; or the method described by Matthes et al., EMBO Journal 3:801-805, 1984. According to the phosphoramidite method, oligonucleotides are synthesized, e.g., in an automatic DNA synthesizer, purified, duplexed and ligated to form the synthetic DNA construct. Alternatively the DNA or RNA construct can be constructed using standard recombinant molecular biological techniques including restriction enzyme mediated cloning and PCR based gene amplification. In some embodiments of the present disclosure for direct mRNA mediated expression the polynucleotide may be encapsulated in a nanoparticle or liposome to enable efficient delivery and uptake into the cell, and optionally include a modified cap or tail structure to enhance stability and translation.

**[0161]** The polynucleotide sequences may also be of mixed genomic, cDNA, RNA, and that of synthetic origin. For example, a genomic or cDNA sequence encoding a leader peptide may be joined to a genomic or cDNA sequence encoding the HRS polypeptide or HRS-Fc conjugate, after which the DNA or RNA sequence may be modified at a site by inserting synthetic oligonucleotides encoding the desired amino acid sequence for homologous recombination in accordance with well-known procedures or preferably generating the desired sequence by PCR using suitable oligonucleotides. In some embodiments of the present disclosure a signal sequence can be included before the coding sequence. This sequence encodes a signal peptide N-terminal to the coding sequence which communicates to the host cell to direct the polypeptide to the cell surface or secrete the polypeptide into the media. Typically the signal peptide is clipped off by the host cell before the protein leaves the cell. Signal peptides can be found in variety of proteins in prokaryotes and eukaryotes.

**[0162]** A variety of expression vector/host systems are known and may be utilized to contain and express polynucleotide sequences. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (e.g., baculovirus); plant cell systems transformed with virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems, including mammalian cell and more specifically human cell systems transformed with viral, plasmid, episomal or integrating expression vectors.

**[0163]** The "control elements" or "regulatory sequences" present in an expression vector are non-translated regions of the vector--enhancers, promoters, 5' and 3' untranslated regions--which interact with host cellular proteins to carry out transcription and translation. Such elements may vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the PBLUESCRIPT phagemid (Stratagene,



La Jolla, Calif.) or PSFORT1 plasmid (Gibco BRL, Gaithersburg, Md.) and the like may be used. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are generally preferred. If it is necessary to generate a cell line that contains multiple copies of the sequence encoding a polypeptide, vectors based on SV40 or EBV may be advantageously used with an appropriate selectable marker.

**[0164]** Certain embodiments of the present disclosure may employ *E. coli*-based expression systems (see, e.g., Structural Genomics Consortium et al., *Nature Methods*. 5:135-146, 2008). These and related embodiments of the present disclosure may rely partially or totally on ligation-independent cloning (LIC) to produce a suitable expression vector. In specific embodiments of the present disclosure, protein expression may be controlled by a T7 RNA polymerase (e.g., pET vector series), or modified pET vectors with alternate promoters, including for example the TAC promoter. These and related embodiments of the present disclosure may utilize the expression host strain BL21(DE3), a  $\lambda$ DE3 lysogen of BL21 that supports T7-mediated expression and is deficient in lon and ompT proteases for improved target protein stability. Also included are expression host strains carrying plasmids encoding tRNAs rarely used in *E. coli*, such as ROSETTA™ (DE3) and Rosetta 2 (DE3) strains. In some embodiments of the present disclosure other *E. coli* strains may be utilized, including other *E. coli* K-12 strains such as W3110 (F<sup>-</sup> lambda<sup>-</sup> IN(rrnD-rrnE)1 rph-1), and UT5600 (F, araC14, leuB6(Am), secA206(aziR), lacY1, proC14, tsx67,  $\Delta$ (ompTfepC)266, entA403, glnX44(AS),  $\lambda^-$ , trpE38, rfbC1, rpsL109(strR), xylA5, mtl-1, thiE1), which can result in reduced levels of post-translational modifications during fermentation. Cell lysis and sample handling may also be improved using reagents sold under the trademarks BENZONASE® nuclease and BUGBUSTER® Protein Extraction Reagent. For cell culture, auto-inducing media can improve the efficiency of many expression systems, including high-throughput expression systems. Media of this type (e.g., OVERNIGHT EXPRESS™ Autoinduction System) gradually elicit protein expression through metabolic shift without the addition of artificial inducing agents such as IPTG.

**[0165]** Particular embodiments of the present disclosure employ hexahistidine tags (such as those sold under the trademark HIS•TAG® fusions), followed by immobilized metal affinity chromatography (IMAC) purification, or related techniques. In certain aspects of the present disclosure, however, clinical grade proteins can be isolated from *E. coli* inclusion bodies, without or without the use of affinity tags (see, e.g., Shimp et al., *Protein Expr Purif.* 50:58-67, 2006). As a further example, certain embodiments of the present disclosure may employ a cold-shock induced *E. coli* high-yield production system, because over-expression of proteins in *Escherichia coli* at low temperature improves their solubility and stability (see, e.g., Qing et al., *Nature Biotechnology*. 22:877-882, 2004).

**[0166]** Also included are high-density bacterial fermentation systems. For example, high cell density cultivation of *Ralstonia eutropha* allows protein production at cell densities of over 150 g/L, and the expression of recombinant proteins at titers exceeding 10 g/L. In the yeast *Saccharomyces cerevisiae*, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH may be used. For reviews, see Ausubel et al. (supra) and Grant et al., *Methods Enzymol.* 153:516-544, 1987. Also included are *Pichia pandoris* expression systems (see, e.g., Li et al., *Nature Biotechnology*. 24, 210-215, 2006; and Hamilton et al., *Science*, 301:1244, 2003). Certain embodiments of the present disclosure include yeast systems that are engineered to selectively glycosylate proteins, including yeast that have humanized N-glycosylation pathways, among others (see, e.g., Hamilton et al., *Science*. 313:1441-1443, 2006; Wildt et al., *Nature Reviews Microbiol.* 3:119-28, 2005; and Gerngross et al., *Nature-Biotechnology*. 22:1409 -1414, 2004; U.S. Patent Nos. 7,629,163; 7,326,681; and 7,029,872). Merely by way of example, recombinant yeast cultures can be grown in Fernbach Flasks or 15L, 50L, 100L, and 200L fermentors, among others.

**[0167]** In cases where plant expression vectors are used, the expression of sequences encoding polypeptides may be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV may be used alone or in combination with the omega leader sequence from TMV



(Takamatsu, EMBO J. 6:307-311, 1987). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used (Coruzzi et al., EMBO J. 3:1671-1680, 1984; Broglie et al., Science. 224:838-843, 1984; and Winter et al., Results Probl. Cell Differ. 17:85-105, 1991). These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. Such techniques are described in a number of generally available reviews (see, e.g., Hobbs in McGraw Hill, Yearbook of Science and Technology, pp. 191-196, 1992).

**[0168]** An insect system may also be used to express a polypeptide of interest. For example, in one such system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* cells. The sequences encoding the polypeptide may be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of the polypeptide-encoding sequence will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses may then be used to infect, for example, *S. frugiperda* cells or *Trichoplusia* cells in which the polypeptide of interest may be expressed (Engelhard et al., PNAS USA. 91:3224-3227, 1994). Also included are baculovirus expression systems, including those that utilize SF9, SF21, and *T. ni* cells (see, e.g., Murphy and Piwnicka-Worms, Curr Protoc Protein Sci. Chapter 5:Unit5.4, 2001). Insect systems can provide post-translation modifications that are similar to mammalian systems.

**[0169]** In mammalian host cells, a number of expression systems are well known in the art and commercially available. Exemplary mammalian vector systems include for example, pCEP4, pREP4, and pREP7 from Invitrogen, the PerC6 system from Crucell, and Lentiviral based systems such as pLP1 from Invitrogen, and others. For example, in cases where an adenovirus is used as an expression vector, sequences encoding a polypeptide of interest may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain a viable virus which is capable of expressing the polypeptide in infected host cells (Logan & Shenk, PNAS USA. 81:3655-3659, 1984). In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

**[0170]** Examples of useful mammalian host cell lines include monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells sub-cloned for growth in suspension culture, Graham et al., J. Gen Virol. 36:59, 1977); baby hamster kidney cells (BHK, ATCC CCL 10); mouse sertoli cells (TM4, Mather, Biol. Reprod. 23:243-251, 1980); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TR1 cells (Mather et al., Annals N.Y. Acad. Sci. 383:44-68, 1982); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2). Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including DHFR-CHO cells (Urlaub et al., PNAS USA. 77:4216, 1980); and myeloma cell lines such as NSO and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, see, e.g., Yazaki and Wu, Methods in Molecular Biology, Vol. 248 (B. K.C Lo, ed., Humana Press, Totowa, N.J., 2003), pp.255-268. Certain preferred mammalian cell expression systems include CHO and HEK293-cell based expression systems. Mammalian expression systems can utilize attached cell lines, for example, in T-flasks, roller bottles, or cell factories, or suspension cultures, for example, in 1L and 5L spinners, 5L, 14L, 40L, 100L and 200L stir tank bioreactors, or 20/50L and 100/200L WAVE bioreactors, among others known in the art.

**[0171]** Also included are methods of cell-free protein expression. These and related embodiments of the present disclosure typically utilize purified RNA polymerase, ribosomes, tRNA, and ribonucleotides. Such reagents can be produced, for example, by extraction from cells or from a cell-based expression system.



**[0172]** In addition, a host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, post-translational modifications such as acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation, or the insertion of non-naturally occurring amino acids (see generally US Patent Nos. 7,939,496; 7,816,320; 7,947,473; 7,883,866; 7,838,265; 7,829,310; 7,820,766; 7,820,766; 7,7737,226, 7,736,872; 7,638,299; 7,632,924; and 7,230,068). In some embodiments of the present disclosure, such non-naturally occurring amino acids may be inserted at position Cys130. Post-translational processing which cleaves a "prepro" form of the protein may also be used to facilitate correct insertion, folding and/or function. Different host cells such as yeast, CHO, HeLa, MDCK, HEK293, and W138, in addition to bacterial cells, which have or even lack specific cellular machinery and characteristic mechanisms for such post-translational activities, may be chosen to ensure the correct modification and processing of the foreign protein.

**[0173]** The HRS polypeptides or HRS-Fc conjugates produced by a recombinant cell can be purified and characterized according to a variety of techniques known in the art. Exemplary systems for performing protein purification and analyzing protein purity include fast protein liquid chromatography (FPLC) (e.g., AKTA and Bio-Rad FPLC systems), high-pressure liquid chromatography (HPLC) (e.g., Beckman and Waters HPLC). Exemplary chemistries for purification include ion exchange chromatography (e.g., Q, S), size exclusion chromatography, salt gradients, affinity purification (e.g., Ni, Co, FLAG, maltose, glutathione, protein A/G), gel filtration, reverse-phase, ceramic HYPERD® ion exchange chromatography, and hydrophobic interaction columns (HIC), among others known in the art. Several exemplary methods are also disclosed in the Examples sections.

#### ***HRS-Fc Conjugates***

**[0174]** As noted above, embodiments of the present disclosure of the present disclosure relate to HRS-Fc conjugates, which comprise at least one Fc region that is covalently attached to one or more HRS polypeptides. Examples of HRS-Fc conjugates include fusion proteins and various forms of chemically cross-linked proteins. A wide variety of Fc region sequences may be employed in the HRS-Fc conjugates of the present disclosure, including wild-type sequences from any number of species, as well as variants, fragments, hybrids, and chemically modified forms thereof. The HRS-Fc polypeptides may also (optionally) comprise one or more linkers, which typically separate the Fc region(s) from the HRS polypeptide(s), including peptide linkers and chemical linkers, as described herein and known in the art. It will be appreciated that in any of these HRS-Fc conjugates the native N or C terminal amino acid of the HRS polypeptides, or native N or C-amino acid in the Fc domain, may be deleted and/or replaced with non native amino acid(s), for example, to facilitate expression and or cloning or to serve as a linker sequence between the two proteins.

**[0175]** HRS-Fc conjugate polypeptides can provide a variety of advantages relative to un-conjugated or unmodified HRS polypeptides, e.g., corresponding HRS polypeptides of the same or similar sequence having no Fc region(s) attached thereto. Merely by way of illustration, the covalent attachment of one or more Fc regions can alter (e.g., increase, decrease) the HRS polypeptide's solubility, half-life (e.g., in serum, in a selected tissue, in a test tube under storage conditions, for example, at room temperature or under refrigeration), dimerization or multimerization properties, biological activity or activities, for instance, by providing Fc-region-associated effector functions (e.g., activation of the classical complement cascade, interaction with immune effector cells via the Fc receptor (FcR), compartmentalization of immunoglobulins), cellular uptake, intracellular transport, tissue distribution, and/or bioavailability, relative to an unmodified HRS polypeptide having the same or similar sequence. In certain aspects of the present disclosure, Fc regions can confer effector functions relating to complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), and/or antibody-dependent cell-mediated phagocytosis (ADCP), which are believed to play a role in clearing specific target cells such as tumor cells and infected cells.



[0176] Certain embodiments of the present disclosure employ HRS-Fc fusion proteins. "Fusion proteins" are defined elsewhere herein and well known in the art, as are methods of making fusion proteins (see, e.g., U.S. Patent Nos. 5,116,964; 5,428,130; 5,455,165; 5,514,582; 6,406,697; 6,291,212; and 6,300,099 for general disclosure and methods related to Fc fusion proteins). In a HRS-Fc fusion protein, the Fc region can be fused to the N-terminus of the HRS polypeptide, the C-terminus, or both. In some embodiments of the present disclosure, one or more Fc regions can be fused internally relative to HRS sequences, for instance, by placing an Fc region between a first HRS sequence (e.g., domain) and a second HRS sequence (e.g., domain), where the first HRS sequence is fused to the N-terminus of the Fc region and the second HRS sequence is fused to the C-terminus of the Fc region. In specific embodiments of the present disclosure, the first and second HRS sequences are identical. In other embodiments of the present disclosure, the first and second HRS sequences are different (e.g., they include different functional domains of the HRS polypeptide). Certain HRS-Fc fusion proteins can also include additional heterologous protein sequences, that is, non-Fc region and non-HRS polypeptide sequences.

[0177] The term "HRS-Fc" can indicate, but does not necessarily indicate, the N-terminal or C-terminal attachment of the Fc region to the HRS polypeptide. For instance, in certain instances the term "Fc-HRS" indicates fusion of the Fc region to the N-terminus of the HRS polypeptide, and the term "HRS-Fc" indicates fusion of the Fc region to the C-terminus of the HRS polypeptide. However, either term can be used more generally to refer to any fusion protein or conjugate of an Fc region and a HRS polypeptide.

[0178] In some embodiments of the present disclosure the HRS-Fc fusion proteins may comprise tandemly repeated copies of the HRS polypeptide coupled to a single Fc domain, optionally separated by linker peptides. Exemplary tandemly repeated HRS-Fc fusion proteins are provided in **Table D10**. The preparation and sequences for specific tandemly repeated HRS-Fc conjugates are illustrated in the Examples.

| Table D10                                                                                 |
|-------------------------------------------------------------------------------------------|
| Exemplary Tandem HRS-Fc conjugates                                                        |
| HRS polypeptide-L-HRS-polypeptide-L-Fc                                                    |
| HRS-polypeptide-L-HRS-polypeptide-L-HRS-polypeptide-L-Fc                                  |
| HRS-polypeptide-L-HRS-polypeptide-L-HRS-polypeptide-L-HRS-polypeptide -L-Fc               |
|                                                                                           |
| Fc-L-HRS-polypeptide -L-HRS-polypeptide                                                   |
| Fc-L-HRS-polypeptide -L-HRS-L-HRS-polypeptide                                             |
| Fc-L-HRS-polypeptide -L-HRS-L-HRS-L-HRS-polypeptide                                       |
| Where:                                                                                    |
| "Fc" is an Fc domain as described herein.                                                 |
| "HRS-polypeptide" is any of the truncated HRS polypeptides described in <b>Table D5</b> . |
| "L" is an optional peptide linker.                                                        |

[0179] Certain embodiments of the present disclosure relate to HRS-Fc conjugates, where, for instance, one or more Fc regions are chemically conjugated or cross-linked to the HRS polypeptide(s). In these and related aspects of the present disclosure, the Fc region can be conjugated to the HRS polypeptide at the N-terminal region (e.g., within the first 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or so amino acids), the internal region (between the N-terminal and C-terminal regions), and/or the C-terminal region (e.g., within the last 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or so amino acids). Polypeptides can be conjugated or cross-linked to other polypeptides according to a variety of routine techniques in the art. For instance, certain techniques employ the carboxyl-reactive carbodiimide crosslinker EDC (or EDAC), which covalently attaches via D, E, and C-



terminal carboxyl groups. Other techniques employ activated EDC, which covalently attaches via K and N-terminal amino groups). Still other techniques employ *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester (MBS) or Sulfo-MBS, which covalently attach via the thiol group of a cysteine residue (see also U.S. Application No. 2007/0092940 for cysteine engineered Ig regions that can be used for thiol conjugation). Such cross-linked proteins can also comprise linkers, including cleavable or otherwise releasable linkers (e.g., enzymatically cleavable linkers, hydrolysable linkers), and non-cleavable linkers (*i.e.*, physiologically-stable linkers). Certain embodiments of the present disclosure may employ non-peptide polymers (e.g., PEG polymers; HRS-N-PEG-N-Fc conjugate) as a cross-linker between the Fc region(s) and the HRS polypeptide(s), as described, for example, in U.S. Application No. 2006/0269553. See also US Application No. 2007/0269369 for exemplary descriptions of Fc region conjugation sites.

**[0180]** In certain embodiments of the present disclosure, discussed in greater detail below, variant or otherwise modified Fc regions can be employed, including those having altered properties or biological activities relative to wild-type Fc region(s). Examples of modified Fc regions include those having mutated sequences, for instance, by substitution, insertion, deletion, or truncation of one or more amino acids relative to a wild-type sequence, hybrid Fc polypeptides composed of domains from different immunoglobulin classes/subclasses, Fc polypeptides having altered glycosylation/sialylation patterns, and Fc polypeptides that are modified or derivatized, for example, by biotinylation (see, e.g., US Application No. 2010/0209424), phosphorylation, sulfation, etc., or any combination of the foregoing. Such modifications can be employed to alter (e.g., increase, decrease) the binding properties of the Fc region to one or more particular FcRs (e.g., FcγRI, FcγRIIa, FcγRIIb, FcγRIIc, FcγRIIIa, FcγRIIIb, FcRn), its pharmacokinetic properties (e.g., stability or half-life, bioavailability, tissue distribution, volume of distribution, concentration, elimination rate constant, elimination rate, area under the curve (AUC), clearance,  $C_{max}$ ,  $t_{max}$ ,  $C_{min}$ , fluctuation), its immunogenicity, its complement fixation or activation, and/or the CDC/ADCC/ADCP-related activities of the Fc region, among other properties described herein, relative to a corresponding wild-type Fc sequence.

**[0181]** The "Fc region" of a HRS-Fc conjugate provided herein is usually derived from the heavy chain of an immunoglobulin (Ig) molecule. A typical Ig molecule is composed of two heavy chains and two light chains. The heavy chains can be divided into at least three functional regions: the Fd region, the Fc region (fragment crystallizable region), and the hinge region (see **Figure 1**), the latter being found only in IgG, IgA, and IgD immunoglobulins. The Fd region comprises the variable ( $V_H$ ) and constant ( $CH_1$ ) domains of the heavy chains, and together with the variable ( $V_L$ ) and constant ( $C_L$ ) domains of the light chains forms the antigen-binding fragment or Fab region.

**[0182]** The Fc region of IgG, IgA, and IgD immunoglobulins comprises the heavy chain constant domains 2 and 3, designated respectively as  $CH_2$  and  $CH_3$  regions; and the Fc region of IgE and IgM immunoglobulins comprises the heavy chain constant domains 2, 3, and 4, designated respectively as  $CH_2$ ,  $CH_3$ , and  $CH_4$  regions. The Fc region is mainly responsible for the immunoglobulin effector functions, which include, for example, complement fixation and binding to cognate Fc receptors of effector cells.

**[0183]** The hinge region (found in IgG, IgA, and IgD) acts as a flexible spacer that allows the Fab portion to move freely in space relative to the Fc region. In contrast to the constant regions, the hinge regions are structurally diverse, varying in both sequence and length among immunoglobulin classes and subclasses. The hinge region may also contain one or more glycosylation site(s), which include a number of structurally distinct types of sites for carbohydrate attachment. For example, IgA1 contains five glycosylation sites within a 17 amino acid segment of the hinge region, conferring significant resistance of the hinge region polypeptide to intestinal proteases. Residues in the hinge proximal region of the  $CH_2$  domain can also influence the specificity of the interaction between an immunoglobulin and its respective Fc receptor(s) (see, e.g., Shin et al., Intern. Rev. Immunol. 10:177-186, 1993).



**[0184]** The term "Fc region" or "Fc fragment" or "Fc" as used herein, thus refers to a protein that contains one or more of a CH<sub>2</sub> region, a CH<sub>3</sub> region, and/or a CH<sub>4</sub> region from one or more selected immunoglobulin(s), including fragments and variants and combinations thereof. An "Fc region" may also include one or more hinge region(s) of the heavy chain constant region of an immunoglobulin. In certain embodiments of the present disclosure, the Fc region does not contain one or more of the CH<sub>1</sub>, C<sub>L</sub>, V<sub>L</sub>, and/or V<sub>H</sub> regions of an immunoglobulin.

**[0185]** The Fc region can be derived from the CH<sub>2</sub> region, CH<sub>3</sub> region, CH<sub>4</sub> region, and/or hinge region(s) of any one or more immunoglobulin classes, including but not limited to IgA, IgD, IgE, IgG, IgM, including subclasses and combinations thereof. In some embodiments of the present disclosure, the Fc region is derived from an IgA immunoglobulin, including subclasses IgA1 and/or IgA2. In certain embodiments of the present disclosure, the Fc region is derived from an IgD immunoglobulin. In particular embodiments of the present disclosure, the Fc region is derived from an IgE immunoglobulin. In some embodiments of the present disclosure, the Fc region is derived from an IgG immunoglobulin, including subclasses IgG1, IgG2, IgG2, IgG3, and/or IgG4. In certain embodiments of the present disclosure, the Fc region is derived from an IgM immunoglobulin. **Figure 2** shows an alignment of Fc regions from human IgA1 (SEQ ID NO: 156), IgA2 (SEQ ID NO:157), IgM (SEQ ID NO:158), IgG1 (SEQ ID NO:159), IgG2 (SEQ ID NO:160), IgG3 (SEQ ID NO:161), IgG4(SEQ ID NO:162), and IgE (SEQ ID NO:163).

**[0186]** Certain Fc regions demonstrate specific binding for one or more Fc-receptors (FcRs). Examples of classes of Fc receptors include Fcγ receptors (FcγR), Fcα receptors (FcαR), Fcε receptors (FcεR), and the neonatal Fc receptor (FcRn). For instance, certain Fc regions have increased binding to (or affinity for) one or more FcγRs, relative to FcαRs, FcεRs, and/or FcRn. In some embodiments of the present disclosure, Fc regions have increased binding to FcαRs, relative to one or more FcγRs, FcεRs, and/or FcRn. In other embodiments of the present disclosure, Fc regions have increased binding to FcεRs (e.g., FcαRI), relative to one or more FcγRs, FcαRs, and/or FcRn. In particular embodiments of the present disclosure, Fc regions have increased binding to FcRn, relative to one or more FcγRs, FcαRs, and/or FcεRs. In certain embodiments of the present disclosure, the binding (or affinity) of an Fc region to one or more selected FcR(s) is increased relative to its binding to (or affinity for) one or more different FcR(s), typically by about 1.5x, 2x, 2.5x, 3x, 3.5x, 4x, 4.5x, 5x, 6x, 7x, 8x, 9x, 10x, 15x, 20x, 25x, 30x, 40x, 50x, 60x, 70x, 80x, 90x, 100x, 200x, 300x, 400x, 500x, 600x, 700x, 800x, 900x, 1000x or more (including all integers in between).

**[0187]** Examples of FcγRs include FcγRI, FcγRIIa, FcγRIIb, FcγRIIc, FcγRIIIa, and FcγRIIIb. FcγRI (CD64) is expressed on macrophages and dendritic cells and plays a role in phagocytosis, respiratory burst, cytokine stimulation, and dendritic cell endocytic transport. Expression of FcγRI is upregulated by both GM-CSF and γ-interferon (γ-IFN) and downregulated by interleukin-4 (IL-4). FcγRIIa is expressed on polymorphonuclear leukocytes (PMN), macrophages, dendritic cells, and mast cells. FcγRIIa plays a role in phagocytosis, respiratory burst, and cytokine stimulation. Expression of FcγRIIa is upregulated by GM-CSF and γ-IFN, and decreased by IL-4. FcγIIb is expressed on B cells, PMN, macrophages, and mast cells. FcγIIb inhibits immunoreceptor tyrosine-based activation motif (ITAM) mediated responses, and is thus an inhibitory receptor. Expression of FcγRIIc is upregulated by intravenous immunoglobulin (IVIG) and IL-4 and decreased by γ-IFN. FcγRIIc is expressed on NK cells. FcγRIIIa is expressed on natural killer (NK) cells, macrophages, mast cells, and platelets. This receptor participates in phagocytosis, respiratory burst, cytokine stimulation, platelet aggregation and degranulation, and NK-mediated ADCC. Expression of FcγRIII is upregulated by C5a, TGF-β, and γ-IFN and downregulated by IL-4. FcγRIIIb is a GPI-linked receptor expressed on PMN

**[0188]** Certain Fc regions have increased binding to FcγRI, relative to FcγRIIa, FcγRIIb, FcγRIIc, FcγRIIIa, and/or FcγRIIIb. Some embodiments of the present disclosure have increased binding to FcγRIIa, relative to FcγRI, FcγRIIb, FcγRIIc, FcγRIIIa, and/or FcγRIIIb. Particular Fc regions have increased binding to FcγRIIb, relative to FcγRI, FcγRIIa, FcγRIIc, FcγRIIIa, and/or FcγRIIIb. Certain Fc regions have increased binding to FcγRIIc, relative to FcγRI, FcγRIIa, FcγRIIb, FcγRIIIa, and/or FcγRIIIb. Some Fc regions have increased



binding to FcγRIIIa, relative to FcγRI, FcγRIIa, FcγRIIb, FcγRIIc, and/or FcγRIIIb. Specific Fc regions have increased binding to FcγRIIIb, relative to FcγRI, FcγRIIa, FcγRIIb, FcγRIIc, and/or FcγRIIIa.

[0189] FcαRs include FcαRI (CD89). FcαRI is found on the surface of neutrophils, eosinophils, monocytes, certain macrophages (e.g., Kupffer cells), and certain dendritic cells. FcαRI is composed of two extracellular Ig-like domains, is a member of both the immunoglobulin superfamily and the multi-chain immune recognition receptor (MIRR) family, and signals by associating with two FcRγ signaling chains.

[0190] FcεRs include FcεRI and FcεRII. The high-affinity receptor FcεRI is a member of the immunoglobulin superfamily, is expressed on epidermal Langerhans cells, eosinophils, mast cells and basophils, and plays a major role in controlling allergic responses. FcεRI is also expressed on antigen-presenting cells, and regulates the production pro-inflammatory cytokines. The low-affinity receptor FcεRII (CD23) is a C-type lectin that can function as a membrane-bound or soluble receptor. FcεRII regulates B cell growth and differentiation, and blocks IgE-binding of eosinophils, monocytes, and basophils. Certain Fc regions have increased binding to FcεRI, relative to FcεRII. Other Fc regions have increased binding to FcεRII, relative to FcεRI.

Table F1 below summarizes the characteristics of certain FcRs.

| Table F1               |                         |                                |                               |                                                                   |
|------------------------|-------------------------|--------------------------------|-------------------------------|-------------------------------------------------------------------|
| Exemplary Fc-Receptors |                         |                                |                               |                                                                   |
| Receptor               | Primary Antibody Ligand | Ligand Affinity                | Cell Distribution             | Exemplary Effects Following Binding to Fc Ligand                  |
| FcγRI (CD64)           | IgG1 and IgG3           | High (Kd ~ 10 <sup>-9</sup> M) | Macrophages                   | Phagocytosis                                                      |
|                        |                         |                                | Neutrophils                   | Cell activation                                                   |
|                        |                         |                                | Eosinophils                   | Activation of respiratory burst                                   |
|                        |                         |                                | Dendritic cells               | Induction of microbe killing                                      |
| FcγRIIa (CD32)         | IgG                     | Low (Kd > 10 <sup>-7</sup> M)  | Macrophages                   | Phagocytosis                                                      |
|                        |                         |                                | Neutrophils                   | Degranulation (eosinophils)                                       |
|                        |                         |                                | Eosinophils                   |                                                                   |
|                        |                         |                                | Platelets                     |                                                                   |
|                        |                         |                                | Langerhans cells              |                                                                   |
| FcγRIIb1 (CD32)        | IgG                     | Low (Kd > 10 <sup>-7</sup> M)  | B Cells                       | No phagocytosis                                                   |
|                        |                         |                                | Mast cells                    | Inhibition of cell activity                                       |
| FcγRIIb2 (CD32)        | IgG                     | Low (Kd > 10 <sup>-7</sup> M)  | Macrophages                   | Phagocytosis                                                      |
|                        |                         |                                | Neutrophils                   | Inhibition of cell activity                                       |
| FcγRIIIa (CD16a)       | IgG                     | Low (Kd > 10 <sup>-6</sup> M)  | Eosinophils                   | Induction of antibody-dependent cell-mediated cytotoxicity (ADCC) |
|                        |                         |                                | Macrophages (certain tissues) |                                                                   |
|                        |                         |                                |                               | Induction of cytokine release by macrophages                      |
| FcγRIIIb (CD16b)       | IgG                     | Low (Kd > 10 <sup>-6</sup> M)  | Eosinophils                   | Induction of microbe killing                                      |
|                        |                         |                                | Macrophages                   |                                                                   |
|                        |                         |                                | Neutrophils                   |                                                                   |
|                        |                         |                                | Mast cells                    |                                                                   |
|                        |                         |                                | Follicular                    |                                                                   |



| Table F1               |                         |                                 |                   |                                                           |
|------------------------|-------------------------|---------------------------------|-------------------|-----------------------------------------------------------|
| Exemplary Fc-Receptors |                         |                                 |                   |                                                           |
| Receptor               | Primary Antibody Ligand | Ligand Affinity                 | Cell Distribution | Exemplary Effects Following Binding to Fc Ligand          |
| FcεRI                  | IgE                     | High (Kd ~ 10 <sup>-10</sup> M) | dendritic cells   |                                                           |
|                        |                         |                                 | Mast cells        | Degranulation                                             |
|                        |                         |                                 | Eosinophils       |                                                           |
|                        |                         |                                 | Basophils         |                                                           |
|                        |                         |                                 | Langerhans cells  |                                                           |
| FcεRII (CD23)          | IgE                     | Low (Kd > 10 <sup>-7</sup> M)   | B cells           | Possible adhesion molecule                                |
|                        |                         |                                 | Eosinophils       |                                                           |
|                        |                         |                                 | Langerhans cells  |                                                           |
| FcαRI (CD89)           | IgA                     | Low (Kd > 10 <sup>-6</sup> M)   | Monocytes         | Phagocytosis                                              |
|                        |                         |                                 | Macrophages       | Induction of microbe killing                              |
|                        |                         |                                 | Neutrophils       |                                                           |
|                        |                         |                                 | Eosinophils       |                                                           |
| Fcα/μR                 | IgA and IgM             | High for IgM,                   | B cells           | Endocytosis                                               |
|                        |                         | Moderate for IgA                | Mesangial cells   | Induction of microbe killing                              |
|                        |                         |                                 | Macrophages       |                                                           |
| FcRn                   | IgG                     |                                 | Monocytes         | Transfers IgG from a mother to fetus through the placenta |
|                        |                         |                                 | Macrophages       |                                                           |
|                        |                         |                                 | Dendrite cells    | Transfers IgG from a mother to infant in milk             |
|                        |                         |                                 | Epithelial cells  |                                                           |
|                        |                         |                                 | Endothelial cells | Protects IgG from degradation                             |
|                        |                         |                                 | Hepatocytes       |                                                           |

[0191] Fc regions can be derived from the immunoglobulin molecules of any animal, including vertebrates such as mammals such cows, goats, swine, dogs, mice, rabbits, hamsters, rats, guinea pigs, non-human primates, and humans. The amino acid sequences of CH<sub>2</sub>, CH<sub>3</sub>, CH<sub>4</sub>, and hinge regions from exemplary, wild-type human IgA1, IgA2, IgD, IgE, IgG1, IgG2, IgG3, IgG4, and IgM immunoglobulins are shown below (SEQ ID NOS:128-154).

SEQ ID NO:128 is the amino acid sequence of a human IgA1 hinge region (VPSTPPTPSPSTPPTSPS).

SEQ ID NO:129 is the amino acid sequence of a human IgA1 CH<sub>2</sub> region (CCHPRLSLHRPALEDLLLSEANLTCTLTGLRDASGVFTFTWTPSSGKSAVQGPPERDLGCGYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKS).

SEQ ID NO:130 is the amino acid sequence of a human IgA1 CH<sub>3</sub> region (GNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLRWLQGSQELPREKYLTWASRQEPSQGTTFFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPTHVNVSVVM AEVDGTCY).

SEQ ID NO:131 is the amino acid sequence of a human IgA2 hinge region (VPPPPP).



SEQ ID NO:132 is the amino acid sequence of a human IgA2 CH2 region (CCHPRLSLHRPALEDLLLGSEANLTCTLTGLRDASGATFTWTPSSGKSAVQGPPERDLCGCY SVS SVLPGCAQPWNHGETFTCTAAHPELKTPLTANITKS).

SEQ ID NO:133 is the amino acid sequence of a human IgA2 CH3 region (GNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLRWLQGSQELPREKYLTWASRQE PSQGTTFFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPTHVNVSVVM AEVDGTCY).

SEQ ID NO:134 is the amino acid sequence of a human IgD hinge region (ESPKAQASSVPTAQPPQAEGSLAKATTAPATTRNTGRGGEEKKKEKEKEEQEERETKTP).

SEQ ID NO:135 is the amino acid sequence of a human IgD CH2 region (ECPSHTQPLGVYLLTPAVQDLWLRDKATFTCFVVGSDLKDAHLTWEVAGKVPTGGVEEGL LERHSNGSQSQHSRLTLPRSLWNAGTSVTCTLNHPSLPPQRLMALREP).

SEQ ID NO:136 is the amino acid sequence of a human IgD CH3 region (AAQAPVKLSLNLASSDPPEAASWLLCEVSGFSPPNILLMWLEDQREVNTSGFAPARPPPQP RSTTFWAWSVLRVPAPPSPQPATYTCVSHEDSRTLLNASRSLEVSYVTDHGPMK).

SEQ ID NO:137 is the amino acid sequence of a human IgE CH2 region (VCSRDFTPPTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDVDLSTASTT QEGELASTQSELTLSQKHWLSDRTYTCQVTYQGHTFEDSTKKCA).

SEQ ID NO:138 is the amino acid sequence of a human IgE CH3 region (DSNPRGVSAYLSPSPFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQ RNGTHLTVTSTLPVGTRDWIEGETYQCRVTHPHLPRALMRSTTKTS).

SEQ ID NO:139 is the amino acid sequence of a human IgE CH4 region (GPRAAPEVYAFATPEWPGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKT KGSGFFVFSRLEVTRAWEQKDEFICRAVHEAASPSQTVQRAVSVNPGK).

SEQ ID NO:140 is the amino acid sequence of a human IgG1 hinge region (EPKSCDKTHTCPPCP) .

SEQ ID NO:341 is the amino acid sequence of a modified human IgG1 hinge region derived sequence (SDKTHTCPPCP).

SEQ ID NO:141 is the amino acid sequence of a human IgG1 CH2 region (APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK).

SEQ ID NO:142 is the amino acid sequence of a human IgG1 CH3 region (GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDG SFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK).

SEQ ID NO:342 is the amino acid sequence of a human IgG1 heavy chain sequence (MSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSF FLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK). It will be appreciated that the Met residue in this human IgG1 heavy chain sequence can be deleted, for instance, upon N-terminal fusion to a HRS polypeptide (see SEQ ID NO:340).

SEQ ID NO:143 is the amino acid sequence of a human IgG2 hinge region (ERKCCVECPPCP).

SEQ ID NO:144 is the amino acid sequence of a human IgG2 CH2 region



(APPVAGPSVFLFPPKPKDTLMISRTPEVTCVWVDVSHEDPEVQFNWYVDGVEVHNAKTKPR  
EEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTK).

SEQ ID NO:145 is the amino acid sequence of a human IgG2 CH3 region  
(GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSD  
GSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK).

SEQ ID NO:146 is the amino acid sequence of a human IgG3 hinge region  
(ELKTPLGDTTHTCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCP).

SEQ ID NO:147 is the amino acid sequence of a human IgG3 CH2 region  
(APELLGGPSVFLFPPKPKDTLMISRTPEVTCVWVDVSHEDPEVQFKWYVDGVEVHNAKTKP  
REEQYNSTFRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKTK).

SEQ ID NO:148 is the amino acid sequence of a human IgG3 CH3 region  
(GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDG  
SFFLYSKLTVDKSRWQQGNIFCFSVMHEALHNRFTQKSLSLSPGK).

SEQ ID NO:149 is the amino acid sequence of a human IgG4 hinge region (ESKYGPPCPSCP).

SEQ ID NO:150 is the amino acid sequence of a human IgG4 CH2 region  
(APEFLGGPSVFLFPPKPKDTLMISRTPEVTCVWVDVSQEDPEVQFNWYVDGVEVHNAKTKP  
REEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK).

SEQ ID NO:151 is the amino acid sequence of a human IgG4 CH3 region  
(GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG  
SFFLYSRLTVDKSRWQEGNVFCFSVMHEALHNHYTQKSLSLGLGK).

SEQ ID NO:152 is the amino acid sequence of a human IgM CH2 region  
(VIAELPPKVSFVPPRDGFFGNPRKSKLICQATGFSPRQIQVSWLREGKQVGSGVTDDQVQA  
EAKESGPTTYKVTSTLTIKESDWLGQSMFTCRVDHRGLTFQQNASSMCVP).

SEQ ID NO:153 is the amino acid sequence of a human IgM CH3 region  
(DQDTAIRVFAIPPSFASIFLTSTKLTCLVTDLTYSVTISWTRQNGEAVKTHTNISESHRNA  
TFSAVGEASICEDDWNSGERFTCTVTHDLPSPKQTSRPK).

SEQ ID NO:154 is the amino acid sequence of a human IgM CH4 region  
(GVALHRPDVYLLPPAREQLNLRESATITCLVTGFSPADVQVWMQRGQPLSPEKYVTSAPM  
PEPQAPGRYFAHSILTVSEEEWNTGETYTCVVAHEALPNRVTERTVDKSTGKPTLYNVSLVM SDTAGTCY).

**[0192]** A HRS-Fc conjugate of the present disclosure can thus comprise, consist of, or consist essentially of one or more of the human Fc region amino acid sequences of SEQ ID NOS:128-163 or 339-342, including variants, fragments, homologs, orthologs, paralog, and combinations thereof. Certain illustrative embodiments of the present disclosure comprise an Fc region that ranges in size from about 20-50, 20-100, 20-150, 20-200, 20-250, 20-300, 20-400, 50-100, 50-150, 50-200, 50-250, 50-300, 50-400, 100-150, 100-200, 100-250, 100-300, 100-350, 100-400, 200-250, 200-300, 200-350, or 200-400 amino acids in length, and optionally comprises, consists of, or consists essentially of any one or more of SEQ ID NOS:128-154 or 341-342. Certain embodiments of the present disclosure comprise an Fc region of up to about 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 300, 350, 400 or more amino acids, which optionally comprises, consists of, or consists essentially of any one or more of SEQ ID NOS: 128-154 or 339-342.

**[0193]** Certain Fc regions comprise, consist of, or consist essentially of human IgA1 sequences set forth in



SEQ ID NOS:128-130 or 156, in any order reading from N-terminus to C-terminus, including combinations thereof (e.g., SEQ ID NOS:128 and 129 and 130, SEQ ID NOS:128 and 129; SEQ ID NOS:128 and 130; SEQ ID NOS:129 and 130), and variants and fragments thereof. Certain Fc regions comprise, consist of, or consist essentially of human the IgA1 sequence set forth in SEQ ID NOS:128. Certain Fc regions comprise, consist of, or consist essentially of the human IgA1 sequence set forth in SEQ ID NOS:129. Certain Fc regions comprise, consist of, or consist essentially of the human IgA1 sequence set forth in SEQ ID NOS:130.

**[0194]** Some Fc regions comprise, consist of, or consist essentially of human IgA2 sequences set forth in SEQ ID NOS:131-133 or 157, in any order reading from N-terminus to C-terminus, including combinations thereof (e.g., SEQ ID NOS:131 and 132 and 133, SEQ ID NOS:131 and 132; SEQ ID NOS:131 and 133; SEQ ID NOS:132 and 133), and variants and fragments thereof. Certain Fc regions comprise, consist of, or consist essentially of human the IgA2 sequence set forth in SEQ ID NOS:131. Certain Fc regions comprise, consist of, or consist essentially of the human IgA2 sequence set forth in SEQ ID NOS:132. Certain Fc regions comprise, consist of, or consist essentially of the human IgA2 sequence set forth in SEQ ID NOS:133.

**[0195]** Certain Fc regions comprise, consist of, or consist essentially of human IgD sequences set forth in SEQ ID NOS:134-136, in any order reading from N-terminus to C-terminus, including combinations thereof (e.g., SEQ ID NOS:134 and 135 and 136, SEQ ID NOS:134 and 135; SEQ ID NOS:134 and 136; SEQ ID NOS:135 and 136), and variants and fragments of these sequences and combinations. Certain Fc regions comprise, consist of, or consist essentially of human the IgD sequence set forth in SEQ ID NOS:134. Certain Fc regions comprise, consist of, or consist essentially of the human IgD sequence set forth in SEQ ID NOS:135. Certain Fc regions comprise, consist of, or consist essentially of the human IgD sequence set forth in SEQ ID NOS:136.

**[0196]** Certain Fc regions comprise, consist of, or consist essentially of human IgE sequences set forth in SEQ ID NOS: 137-139 or 163, in any order reading from N-terminus to C-terminus, including combinations thereof (e.g., SEQ ID NOS:137 and 138 and 139, SEQ ID NOS:137 and 138; SEQ ID NOS: 137 and 139; SEQ ID NOS: 138 and 139), and variants and fragments of these sequences and combinations. Certain Fc regions comprise, consist of, or consist essentially of human the IgE sequence set forth in SEQ ID NOS: 137. Certain Fc regions comprise, consist of, or consist essentially of the human IgE sequence set forth in SEQ ID NOS:138. Certain Fc regions comprise, consist of, or consist essentially of the human IgE sequence set forth in SEQ ID NOS: 139.

**[0197]** Certain Fc regions comprise, consist of, or consist essentially of human IgG1 sequences set forth in SEQ ID NOS:140-142 or 159 or 339-342, in any order reading from N-terminus to C-terminus, including combinations thereof (e.g., SEQ ID NOS:140 and 141 and 142, SEQ ID NOS:140 and 141; SEQ ID NOS:140 and 142; SEQ ID NOS:141 and 142), and variants and fragments of these sequences and combinations. Certain Fc regions comprise, consist of, or consist essentially of human the IgG1 sequence set forth in SEQ ID NOS:140. Certain Fc regions comprise, consist of, or consist essentially of the human IgG1 sequence set forth in SEQ ID NOS:141. Certain Fc regions comprise, consist of, or consist essentially of the human IgG1 sequence set forth in SEQ ID NOS:142. Certain Fc regions comprise, consist of, or consist essentially of the human IgG1 sequence set forth in SEQ ID NOS:339. Certain Fc regions comprise, consist of, or consist essentially of the human IgG1 sequence set forth in SEQ ID NOS:340. Certain Fc regions comprise, consist of, or consist essentially of the human IgG1 sequence set forth in SEQ ID NOS:341. Certain Fc regions comprise, consist of, or consist essentially of the human IgG1 sequence set forth in SEQ ID NOS:342.

**[0198]** Certain Fc regions comprise, consist of, or consist essentially of human IgG2 sequences set forth in SEQ ID NOS: 143-145 or 160, in any order reading from N-terminus to C-terminus, including combinations thereof (e.g., SEQ ID NOS:143 and 144 and 145, SEQ ID NOS:143 and 144; SEQ ID NOS:143 and 145; SEQ ID NOS:144 and 145), and variants and fragments of these sequences and combinations. Certain Fc regions comprise, consist of, or consist essentially of human the IgG2 sequence set forth in SEQ ID NOS: 143. Certain



Fc regions comprise, consist of, or consist essentially of the human IgG2 sequence set forth in SEQ ID NOS:144. Certain Fc regions comprise, consist of, or consist essentially of the human IgG2 sequence set forth in SEQ ID NOS: 145.

**[0199]** Certain Fc regions comprise, consist of, or consist essentially of human IgG3 sequences set forth in SEQ ID NOS: 146-148 or 161, in any order reading from N-terminus to C-terminus, including combinations thereof (e.g., SEQ ID NOS:146 and 147 and 148, SEQ ID NOS:146 and 147; SEQ ID NOS:146 and 148; SEQ ID NOS:147 and 148), and variants and fragments of these sequences and combinations. Certain Fc regions comprise, consist of, or consist essentially of human the IgG3 sequence set forth in SEQ ID NOS: 146. Certain Fc regions comprise, consist of, or consist essentially of the human IgG3 sequence set forth in SEQ ID NOS:147. Certain Fc regions comprise, consist of, or consist essentially of the human IgG3 sequence set forth in SEQ ID NOS: 148.

**[0200]** Certain Fc regions comprise, consist of, or consist essentially of human IgG4 sequences set forth in SEQ ID NOS:149-151 or 162, in any order reading from N-terminus to C-terminus, including combinations thereof (e.g., SEQ ID NOS:149 and 150 and 151, SEQ ID NOS:149 and 150; SEQ ID NOS:149 and 151; SEQ ID NOS:150 and 151), and variants and fragments of these sequences and combinations. Certain Fc regions comprise, consist of, or consist essentially of human the IgG4 sequence set forth in SEQ ID NOS:149. Certain Fc regions comprise, consist of, or consist essentially of the human IgG4 sequence set forth in SEQ ID NOS:150. Certain Fc regions comprise, consist of, or consist essentially of the human IgG4 sequence set forth in SEQ ID NOS:151.

**[0201]** Certain Fc regions comprise, consist of, or consist essentially of human IgM sequences set forth in SEQ ID NOS:152-154 or 158, in any order reading from N-terminus to C-terminus, including combinations thereof (e.g., SEQ ID NOS:152 and 153 and 154, SEQ ID NOS:152 and 153; SEQ ID NOS:152 and 154; SEQ ID NOS:153 and 154), and variants and fragments of these sequences and combinations. Certain Fc regions comprise, consist of, or consist essentially of human the IgM sequence set forth in SEQ ID NOS:152. Certain Fc regions comprise, consist of, or consist essentially of the human IgM sequence set forth in SEQ ID NOS:153. Certain Fc regions comprise, consist of, or consist essentially of the human IgM sequence set forth in SEQ ID NOS:154.

**[0202]** As noted above, certain embodiments of the present disclosure employ variants, fragments, hybrids, and/or otherwise modified forms an Fc region described herein and known in the art (e.g., the human Ig sequences of SEQ ID NOS:128-163).

**[0203]** Included are variants having one or more amino acid substitutions, insertions, deletions, and/or truncations relative to a reference sequence, such as any one or more of the reference sequences set forth in SEQ ID NOS:128-163. In certain embodiments of the present disclosure, a variant Fc region includes an amino acid sequence having at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or more sequence identity or similarity or homology to any one or more of SEQ ID NOS:128-163. Also included are Fc regions differing from one or more of SEQ ID NOS:128-163 by the addition, deletion, insertion, or substitution of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150 or more amino acids. In certain embodiments of the present disclosure, the amino acid additions or deletions occur at the C-terminal end and/or the N-terminal end of the Fc reference sequence.

**[0204]** In particular embodiments of the present disclosure, a variant Fc region comprises an amino acid sequence that can be optimally aligned with any one or more of SEQ ID NOS:128-163 to generate a BLAST bit scores or sequence similarity scores of at least about 50, 60, 70, 80, 90, 100, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570,



580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, or more, including all integers and ranges in between, wherein the BLAST alignment used the BLOSUM62 matrix, a gap existence penalty of 11, and a gap extension penalty of 1.

**[0205]** Also included are hybrid Fc regions, for example, Fc regions that comprise a combination of Fc domains (e.g., hinge, CH<sub>2</sub>, CH<sub>3</sub>, CH<sub>4</sub>) from immunoglobulins of different species, different Ig classes, and/or different Ig subclasses. General examples include hybrid Fc regions that comprise, consist of, or consist essentially of the following combination of CH<sub>2</sub>/CH<sub>3</sub> domains: IgA1/IgA1, IgA1/IgA2, IgA1/IgD, IgA1/IgE, IgA1/IgG1, IgA1/IgG2, IgA1/IgG3, IgA1/IgG4, IgA1/IgM, IgA2/IgA1, IgA2/IgA2, IgA2/IgD, IgA2/IgE, IgA2/IgG1, IgA2/IgG2, IgA2/IgG3, IgA2/IgG4, IgA2/IgM, IgD/IgA1, IgD/IgA2, IgD/IgD, IgD/IgE, IgD/IgG1, IgD/IgG2, IgD/IgG3, IgD/IgG4, IgD/IgM, IgE/IgA1, IgE/IgA2, IgE/IgD, IgE/IgE, IgE/IgG1, IgE/IgG2, IgE/IgG3, IgE/IgG4, IgE/IgM, IgG1/IgA1, IgG1/IgA2, IgG1/IgD, IgG1/IgE, IgG1/IgG1, IgG1/IgG2, IgG1/IgG3, IgG1/IgG4, IgG1/IgM, IgG2/IgA1, IgG2/IgA2, IgG2/IgD, IgG2/IgE, IgG2/IgG1, IgG2/IgG2, IgG2/IgG3, IgG2/IgG4, IgG2/IgM, IgG3/IgA1, IgG3/IgA2, IgG3/IgD, IgG3/IgE, IgG3/IgG1, IgG3/IgG2, IgG3/IgG3, IgG3/IgG4, IgG3/IgM, IgG4/IgA1, IgG4/IgA2, IgG4/IgD, IgG4/IgE, IgG4/IgG1, IgG4/IgG2, IgG4/IgG3, IgG4/IgG4, IgG4/IgM, IgM/IgA1, IgM/IgA2, IgM/IgD, IgM/IgE, IgM/IgG1, IgM/IgG2, IgM/IgG3, IgM/IgG4, IgM/IgM (or fragments or variants thereof), and optionally include a hinge from one or more of IgA1, IgA2, IgD, IgG1, IgG2, IgG3, or IgG4, and/or a CH<sub>4</sub> domain from IgE and/or IgM. In specific embodiments of the present disclosure, the hinge, CH<sub>2</sub>, CH<sub>3</sub>, and CH<sub>4</sub> domains are from human Ig.

**[0206]** Additional examples include hybrid Fc regions that comprise, consist of, or consist essentially of the following combination of CH<sub>2</sub>/CH<sub>4</sub> domains: IgA1/IgE, IgA2/IgE, IgD/IgE, IgE/IgE, IgG1/IgE, IgG2/IgE, IgG3/IgE, IgG4/IgE, IgM/IgE, IgA1/IgM, IgA2/IgM, IgD/IgM, IgE/IgM, IgG1/IgM, IgG2/IgM, IgG3/IgM, IgG4/IgM, IgM/IgM (or fragments or variants thereof), and optionally include a hinge from one or more of IgA1, IgA2, IgD, IgG1, IgG2, IgG3, IgG4, and/or a CH<sub>3</sub> domain from one or more of IgA1, IgA2, IgD, IgE, IgG1, IgG2, IgG3, IgG4, or IgM. In specific embodiments of the present disclosure, the hinge, CH<sub>2</sub>, CH<sub>3</sub>, and CH<sub>4</sub> domains are from human Ig.

**[0207]** Certain examples include hybrid Fc regions that comprise, consist of, or consist essentially of the following combination of CH<sub>3</sub>/CH<sub>4</sub> domains: IgA1/IgE, IgA2/IgE, IgD/IgE, IgE/IgE, IgG1/IgE, IgG2/IgE, IgG3/IgE, IgG4/IgE, IgM/IgE, IgA1/IgM, IgA2/IgM, IgD/IgM, IgE/IgM, IgG1/IgM, IgG2/IgM, IgG3/IgM, IgG4/IgM, IgM/IgM (or fragments or variants thereof), and optionally include a hinge from one or more of IgA1, IgA2, IgD, IgG1, IgG2, IgG3, IgG4, and/or a CH<sub>2</sub> domain from one or more of IgA1, IgA2, IgD, IgE, IgG1, IgG2, IgG3, IgG4, or IgM. In specific embodiments of the present disclosure, the hinge, CH<sub>2</sub>, CH<sub>3</sub>, and CH<sub>4</sub> domains are from human Ig.

**[0208]** Particular examples include hybrid Fc regions that comprise, consist of, or consist essentially of the following combination of hinge/CH<sub>2</sub> domains: IgA1/IgA1, IgA1/IgA2, IgA1/IgD, IgA1/IgE, IgA1/IgG1, IgA1/IgG2, IgA1/IgG3, IgA1/IgG4, IgA1/IgM, IgA2/IgA1, IgA2/IgA2, IgA2/IgD, IgA2/IgE, IgA2/IgG1, IgA2/IgG2, IgA2/IgG3, IgA2/IgG4, IgA2/IgM, IgD/IgA1, IgD/IgA2, IgD/IgD, IgD/IgE, IgD/IgG1, IgD/IgG2, IgD/IgG3, IgD/IgG4, IgD/IgM, IgG1/IgA1, IgG1/IgA2, IgG1/IgD, IgG1/IgE, IgG1/IgG1, IgG1/IgG2, IgG1/IgG3, IgG1/IgG4, IgG1/IgM, IgG2/IgA1, IgG2/IgA2, IgG2/IgD, IgG2/IgE, IgG2/IgG1, IgG2/IgG2, IgG2/IgG3, IgG2/IgG4, IgG2/IgM, IgG3/IgA1, IgG3/IgA2, IgG3/IgD, IgG3/IgE, IgG3/IgG1, IgG3/IgG2, IgG3/IgG3, IgG3/IgG4, IgG3/IgM, IgG4/IgA1, IgG4/IgA2, IgG4/IgD, IgG4/IgE, IgG4/IgG1, IgG4/IgG2, IgG4/IgG3, IgG4/IgG4, IgG4/IgM (or fragments or variants thereof), and optionally include a CH<sub>3</sub> domain from one or more of IgA1, IgA2, IgD, IgE, IgG1, IgG2, IgG3, IgG4, or IgM, and/or a CH<sub>4</sub> domain from IgE and/or IgM. In specific embodiments of the present disclosure, the hinge, CH<sub>2</sub>, CH<sub>3</sub>, and CH<sub>4</sub> domains are from human Ig.



**[0209]** Certain examples include hybrid Fc regions that comprise, consist of, or consist essentially of the following combination of hinge/CH<sub>3</sub> domains: IgA1/IgA1, IgA1/IgA2, IgA1/IgD, IgA1/IgE, IgA1/IgG1, IgA1/IgG2, IgA1/IgG3, IgA1/IgG4, IgA1/IgM, IgA2/IgA1, IgA2/IgA2, IgA2/IgD, IgA2/IgE, IgA2/IgG1, IgA2/IgG2, IgA2/IgG3, IgA2/IgG4, IgA2/IgM, IgD/IgA1, IgD/IgA2, IgD/IgD, IgD/IgE, IgD/IgG1, IgD/IgG2, IgD/IgG3, IgD/IgG4, IgD/IgM, IgG1/IgA1, IgG1/IgA2, IgG1/IgD, IgG1/IgE, IgG1/IgG1, IgG1/IgG2, IgG1/IgG3, IgG1/IgG4, IgG1/IgM, IgG2/IgA1, IgG2/IgA2, IgG2/IgD, IgG2/IgE, IgG2/IgG1, IgG2/IgG2, IgG2/IgG3, IgG2/IgG4, IgG2/IgM, IgG3/IgA1, IgG3/IgA2, IgG3/IgD, IgG3/IgE, IgG3/IgG1, IgG3/IgG2, IgG3/IgG3, IgG3/IgG4, IgG3/IgM, IgG4/IgA1, IgG4/IgA2, IgG4/IgD, IgG4/IgE, IgG4/IgG1, IgG4/IgG2, IgG4/IgG3, IgG4/IgG4, IgG4/IgM (or fragments or variants thereof), and optionally include a CH<sub>2</sub> domain from one or more of IgA1, IgA2, IgD, IgE, IgG1, IgG2, IgG3, IgG4, or IgM, and/or a CH<sub>4</sub> domain from IgE and/or IgM. In specific embodiments of the present disclosure, the hinge, CH<sub>2</sub>, CH<sub>3</sub>, and CH<sub>4</sub> domains are from human Ig.

**[0210]** Some examples include hybrid Fc regions that comprise, consist of, or consist essentially of the following combination of hinge/CH<sub>4</sub> domains: IgA1/IgE, IgA1/IgM, IgA2/IgE, IgA2/IgM, IgD/IgE, IgD/IgM, IgG1/IgE, IgG1/IgM, IgG2/IgE, IgG2/IgM, IgG3/IgE, IgG3/IgM, IgG4/IgE, IgG4/IgM (or fragments or variants thereof), and optionally include a CH<sub>2</sub> domain from one or more of IgA1, IgA2, IgD, IgE, IgG1, IgG2, IgG3, IgG4, or IgM, and/or a CH<sub>3</sub> domain from one or more of IgA1, IgA2, IgD, IgE, IgG1, IgG2, IgG3, IgG4, or IgM.

**[0211]** Specific examples of hybrid Fc regions can be found, for example, in WO 2008/147143, which are derived from combinations of IgG subclasses or combinations of human IgD and IgG.

**[0212]** Also included are derivatized or otherwise modified Fc regions. In certain aspects of the present disclosure, the Fc region may be modified by phosphorylation, sulfation, acrylation, glycosylation, methylation, farnesylation, acetylation, amidation, and the like, for instance, relative to a wild-type or naturally-occurring Fc region. In certain embodiments of the present disclosure, the Fc region may comprise wild-type or native glycosylation patterns, or alternatively, it may comprise increased glycosylation relative to a native form, decreased glycosylation relative to a native form, or it may be entirely deglycosylated. As one example of a modified Fc glycoform, decreased glycosylation of an Fc region reduces binding to the C1q region of the first complement component C1, a decrease in ADCC-related activity, and/or a decrease in CDC-related activity. Certain embodiments of the present disclosure thus employ a deglycosylated or aglycosylated Fc region. See, e.g., WO 2005/047337 for the production of exemplary aglycosylated Fc regions. Another example of an Fc region glycoform can be generated by substituting the Q295 position with a cysteine residue (see, e.g., U.S. Application No. 2010/0080794), according to the Kabat *et al.* numbering system. Certain embodiments of the present disclosure may include Fc regions where about 80-100% of the glycoprotein in Fc region comprises a mature core carbohydrate structure that lacks fructose (see, e.g., U.S. Application No. 2010/0255013). Some embodiments of the present disclosure may include Fc regions that are optimized by substitution or deletion to reduce the level of fucosylation, for instance, to increase affinity for FcγRI, FcγRIa, or FcγRMIa, and/or to improve phagocytosis by FcγRIIa-expressing cells (see U.S. Application Nos. 2010/0249382 and 2007/0148170).

**[0213]** As another example of a modified Fc glycoform, an Fc region may comprise oligomannose-type N-glycans, and optionally have one or more of the following: increased ADCC activity, increased binding affinity for FcγRIIIA (and certain other FcRs), similar or increased binding specificity for the target of the HRS polypeptide, similar or higher binding affinity for the target of the HRS polypeptide, and/or similar or lower binding affinity for mannose receptor, relative to a corresponding Fc region or HRS-Fc conjugate that contains complex-type N-glycans (see, e.g., U.S. Application No. 2007/0092521 and U.S. Patent No. 7,700,321). As another example, enhanced affinity of Fc regions for FcγRs has been achieved using engineered glycoforms generated by expression of antibodies in engineered or variant cell lines (see, e.g., Umana *et al.*, *Nat Biotechnol.* 17:176-180, 1999; Davies *et al.*, *Biotechnol Bioeng.* 74:288-294, 2001; Shields *et al.*, *J Biol Chem.* 277:26733-26740, 2002; Shinkawa *et al.*, 2003, *J Biol Chem.* 278:3466-3473, 2003; and U.S. Application No.



2007/0111281). Certain Fc region glycoforms comprise an increased proportion of N-glycoside bond type complex sugar chains, which do not have the 1-position of fucose bound to the 6-position of N-acetylglucosamine at the reducing end of the sugar chain (see, e.g., U.S. Application No. 2010/0092997). Particular embodiments of the present disclosure may include IgG Fc region that is glycosylated with at least one galactose moiety connected to a respective terminal sialic acid moiety by an  $\alpha$ -2,6 linkage, optionally where the Fc region has a higher anti-inflammatory activity relative to a corresponding, wild-type Fc region (see U.S. Application No. 2008/0206246). Certain of these and related altered glycosylation approaches have generated substantial enhancements of the capacity of Fc regions to selectively bind FcRs such as Fc $\gamma$ RIII, to mediate ADCC, and to alter other properties of Fc regions, as described herein.

**[0214]** Certain variant, fragment, hybrid, or otherwise modified Fc regions may have altered binding to one or more FcRs, relative to a corresponding, wild-type Fc sequence (e.g., same species, same Ig class, same Ig subclass). For instance, such Fc regions may have increased binding to one or more of Fc $\gamma$  receptors, Fc $\alpha$  receptors, Fc $\epsilon$  receptors, and/or the neonatal Fc receptor, relative to a corresponding, wild-type Fc sequence. In other embodiments of the present disclosure, variant, fragment, hybrid, or modified Fc regions may have decreased binding to one or more of Fc $\gamma$  receptors, Fc $\alpha$  receptors, Fc $\epsilon$  receptors, and/or the neonatal Fc receptor, relative to a corresponding, wild-type Fc sequence. Specific FcRs are described elsewhere herein.

**[0215]** Specific examples of Fc variants having altered (e.g., increased, decreased) FcR binding can be found, for example, in U.S. Pat. Nos. 5,624,821 and 7,425,619; U.S. Application Nos. 2009/0017023, 2009/0010921, and 2010/0203046; and WO 2000/42072 and WO 2004/016750. Certain examples include human Fc regions having a one or more substitutions at position 298, 333, and/or 334, for example, S298A, E333A, and/or K334A (based on the numbering of the EU index of Kabat *et al.*), which have been shown to increase binding to the activating receptor Fc $\gamma$ RIIIa and reduce binding to the inhibitory receptor Fc $\gamma$ RIIb. These mutations can be combined to obtain double and triple mutation variants that have further improvements in binding to FcRs. Certain embodiments of the present disclosure include a S298A/E333A/K334A triple mutant, which has increased binding to Fc $\gamma$ RIIIa, decreased binding to Fc $\gamma$ RIIb, and increased ADCC (see, e.g., Shields *et al.*, J Biol Chem. 276:6591-6604, 2001; and Presta *et al.*, Biochem Soc Trans. 30:487-490, 2002). See also engineered Fc glycoforms that have increased binding to FcRs, as disclosed in Umana *et al.*, *supra*; and U.S. Patent No. 7,662,925. Some embodiments of the present disclosure include Fc regions that comprise one or more substitutions selected from 434S, 252Y/428L, 252Y/434S, and 428L/434S (see U.S. Application Nos. 2009/0163699 and 20060173170), based on the EU index of Kabat *et al.*

**[0216]** Certain variant, fragment, hybrid, or modified Fc regions may have altered effector functions, relative to a corresponding, wild-type Fc sequence. For example, such Fc regions may have increased complement fixation or activation, increased C1q binding affinity, increased CDC-related activity, increased ADCC-related activity, and/or increased ADCP-related activity, relative to a corresponding, wild-type Fc sequence. In other embodiments of the present disclosure, such Fc regions may have decreased complement fixation or activation, decreased C1q binding affinity, decreased CDC-related activity, decreased ADCC-related activity, and/or decreased ADCP-related activity, relative to a corresponding, wild-type Fc sequence. As merely one illustrative example, an Fc region may comprise a deletion or substitution in a complement-binding site, such as a C1q-binding site, and/or a deletion or substitution in an ADCC site. Examples of such deletions/substitutions are described, for example, in U.S. Patent No. 7,030,226. Many Fc effector functions, such as ADCC, can be assayed according to routine techniques in the art. (see, e.g., Zuckerman *et al.*, CRC Crit Rev Microbiol. 7:1-26, 1978). Useful effector cells for such assays includes, but are not limited to, natural killer (NK) cells, macrophages, and other peripheral blood mononuclear cells (PBMC). Alternatively, or additionally, certain Fc effector functions may be assessed *in vivo*, for example, by employing an animal model described in Clynes *et al.* PNAS. 95:652-656, 1998.

**[0217]** Certain variant hybrid, or modified Fc regions may have altered stability or half-life relative to a corresponding, wild-type Fc sequence. In certain embodiments of the present disclosure, such Fc regions may



have increased half-life relative to a corresponding, wild-type Fc sequence. In other embodiments of the present disclosure, variant hybrid, or modified Fc regions may have decreased half-life relative to a corresponding, wild-type Fc sequence. Half-life can be measured *in vitro* (e.g., under physiological conditions) or *in vivo*, according to routine techniques in the art, such as radiolabeling, ELISA, or other methods. *In vivo* measurements of stability or half-life can be measured in one or more bodily fluids, including blood, serum, plasma, urine, or cerebrospinal fluid, or a given tissue, such as the liver, kidneys, muscle, central nervous system tissues, bone, etc. As one example, modifications to an Fc region that alter its ability to bind the FcRn can alter its half-life *in vivo*. Assays for measuring the *in vivo* pharmacokinetic properties (e.g., *in vivo* mean elimination half-life) and non-limiting examples of Fc modifications that alter its binding to the FcRn are described, for example, in U.S. Pat. Nos. 7,217,797 and 7,732,570; and U.S. Application Nos. US 2010/0143254 and 2010/0143254.

**[0218]** Additional non-limiting examples of modifications to alter stability or half-life include substitutions/deletions at one or more of amino acid residues selected from 251-256, 285-290, and 308-314 in the CH<sub>2</sub> domain, and 385-389 and 428-436 in the CH<sub>3</sub> domain, according to the numbering system of Kabat *et al.* See U.S. Application No. 2003/0190311. Specific examples include substitution with leucine at position 251, substitution with tyrosine, tryptophan or phenylalanine at position 252, substitution with threonine or serine at position 254, substitution with arginine at position 255, substitution with glutamine, arginine, serine, threonine, or glutamate at position 256, substitution with threonine at position 308, substitution with proline at position 309, substitution with serine at position 311, substitution with aspartate at position 312, substitution with leucine at position 314, substitution with arginine, aspartate or serine at position 385, substitution with threonine or proline at position 386, substitution with arginine or proline at position 387, substitution with proline, asparagine or serine at position 389, substitution with methionine or threonine at position 428, substitution with tyrosine or phenylalanine at position 434, substitution with histidine, arginine, lysine or serine at position 433, and/or substitution with histidine, tyrosine, arginine or threonine at position 436, including any combination thereof. Such modifications optionally increase affinity of the Fc region for the FcRn and thereby increase half-life, relative to a corresponding, wild-type Fc region.

**[0219]** Certain variant hybrid, or modified Fc regions may have altered solubility relative to a corresponding, wild-type Fc sequence. In certain embodiments of the present disclosure, such Fc regions may have increased solubility relative to a corresponding, wild-type Fc sequence. In other embodiments of the present disclosure, variant hybrid, or modified Fc regions may have decreased solubility relative to a corresponding, wild-type Fc sequence. Solubility can be measured, for example, *in vitro* (e.g., under physiological conditions) according to routine techniques in the art. Exemplary solubility measurements are described elsewhere herein.

**[0220]** Additional examples of variants include IgG Fc regions having conservative or non-conservative substitutions (as described elsewhere herein) at one or more of positions 250, 314, or 428 of the heavy chain, or in any combination thereof, such as at positions 250 and 428, or at positions 250 and 314, or at positions 314 and 428, or at positions 250, 314, and 428 (see, e.g., U.S. Application No. 2011/0183412). In specific embodiments of the present disclosure, the residue at position 250 is substituted with glutamic acid or glutamine, and/or the residue at position 428 is substituted with leucine or phenylalanine. As another illustrative example of an IgG Fc variant, any one or more of the amino acid residues at positions 214 to 238, 297 to 299, 318 to 322, and/or 327 to 331 may be used as a suitable target for modification (e.g., conservative or non-conservative substitution, deletion). In particular embodiments of the present disclosure, the IgG Fc variant CH<sub>2</sub> domain contains amino acid substitutions at positions 228, 234, 235, and/or 331 (e.g., human IgG4 with Ser228Pro and Leu235Ala mutations) to attenuate the effector functions of the Fc region (see U.S. Patent No. 7,030,226). Here, the numbering of the residues in the heavy chain is that of the EU index (see Kabat *et al.*, "Sequences of Proteins of Immunological Interest," 5th Ed., National Institutes of Health, Bethesda, Md. (1991)). Certain of these and related embodiments of the present disclosure have altered (e.g., increased, decreased) FcRn binding and/or serum half-life, optionally without reduced effector functions such



as ADCC or CDC-related activities.

**[0221]** Additional examples include variant Fc regions that comprise one or more amino acid substitutions at positions 279, 341, 343 or 373 of a wild-type Fc region, or any combination thereof (see, e.g., U.S. Application No. 2007/0224188). The wild-type amino acid residues at these positions for human IgG are valine (279), glycine (341), proline (343) and tyrosine (373). The substitution(s) can be conservative or non-conservative, or can include non-naturally occurring amino acids or mimetics, as described herein. Alone or in combination with these substitutions, certain embodiments of the present disclosure may also employ a variant Fc region that comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acid substitutions selected from the following: 235G, 235R, 236F, 236R, 236Y, 237K, 237N, 237R, 238E, 238G, 238H, 238I, 238L, 238V, 238W, 238Y, 244L, 245R, 247A, 247D, 247E, 247F, 247M, 247N, 247Q, 247R, 247S, 247T, 247W, 247Y, 248F, 248P, 248Q, 248W, 249L, 249M, 249N, 249P, 249Y, 251H, 251I, 251W, 254D, 254E, 254F, 254G, 254H, 254I, 254K, 254L, 254M, 254N, 254P, 254Q, 254R, 254V, 254W, 254Y, 255K, 255N, 256H, 256I, 256K, 256L, 256V, 256W, 256Y, 257A, 257I, 257M, 257N, 257S, 258D, 260S, 262L, 264S, 265K, 265S, 267H, 267I, 267K, 268K, 269N, 269Q, 271T, 272H, 272K, 272L, 272R, 279A, 279D, 279F, 279G, 279H, 279I, 279K, 279L, 279M, 279N, 279Q, 279R, 279S, 279T, 279W, 279Y, 280T, 283F, 283G, 283H, 283I, 283K, 283L, 283M, 283P, 283R, 283T, 283W, 283Y, 285N, 286F, 288N, 288P, 292E, 292F, 292G, 292I, 292L, 293S, 293V, 301W, 304E, 307E, 307M, 312P, 315F, 315K, 315L, 315P, 315R, 316F, 316K, 317P, 317T, 318N, 318P, 318T, 332F, 332G, 332L, 332M, 332S, 332V, 332W, 339D, 339E, 339F, 339G, 339H, 339I, 339K, 339L, 339M, 339N, 339Q, 339R, 339S, 339W, 339Y, 341D, 341E, 341F, 341H, 341I, 341K, 341L, 341M, 341N, 341P, 341Q, 341R, 341S, 341T, 341V, 341W, 341Y, 343A, 343D, 343E, 343F, 343G, 343H, 343I, 343K, 343L, 343M, 343N, 343Q, 343R, 343S, 343T, 343V, 343W, 343Y, 373D, 373E, 373F, 373G, 373H, 373I, 373K, 373L, 373M, 373N, 373Q, 373R, 373S, 373T, 373V, 373W, 375R, 376E, 376F, 376G, 376H, 376I, 376L, 376M, 376N, 376P, 376Q, 376R, 376S, 376T, 376V, 376W, 376Y, 377G, 377K, 377P, 378N, 379N, 379Q, 379S, 379T, 380D, 380N, 380S, 380T, 382D, 382F, 382H, 382I, 382K, 382L, 382M, 382N, 382P, 382Q, 382R, 382S, 382T, 382V, 382W, 382Y, 385E, 385P, 386K, 423N, 424H, 424M, 424V, 426D, 426L, 427N, 429A, 429F, 429M, 430A, 430D, 430F, 430G, 430H, 430I, 430K, 430L, 430M, 430N, 430P, 430Q, 430R, 430S, 430T, 430V, 430W, 430Y, 431H, 431K, 431P, 432R, 432S, 438G, 438K, 438L, 438T, 438W, 439E, 439H, 439Q, 440D, 440E, 440F, 440G, 440H, 440I, 440K, 440L, 440M, 440Q, 440T, 440V or 442K. As above, the numbering of the residues in the heavy chain is that of the EU index (see Kabat *et al.*, *supra*). Such variant Fc regions typically confer an altered effector function or altered serum half-life upon HRS polypeptide to which the variant Fc region is operably attached. Preferably the altered effector function is an increase in ADCC, a decrease in ADCC, an increase in CDC, a decrease in CDC, an increase in Clq binding affinity, a decrease in Clq binding affinity, an increase in FcR (preferably FcRn) binding affinity or a decrease in FcR (preferably FcRn) binding affinity as compared to a corresponding Fc region that lacks such amino acid substitution(s).

**[0222]** Additional examples include variant Fc regions that comprise an amino acid substitution at one or more of position(s) 221, 222, 224, 227, 228, 230, 231, 223, 233, 234, 235, 236, 237, 238, 239, 240, 241, 243, 244, 245, 246, 247, 249, 250, 258, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 278, 280, 281, 283, 285, 286, 288, 290, 291, 293, 294, 295, 296, 297, 298, 299, 300, 302, 313, 317, 318, 320, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336 and/or 428 (see, e.g., U.S. Patent No. 7,662,925). In specific embodiments of the present disclosure, the variant Fc region comprises at least one amino acid substitution selected from the group consisting of: P230A, E233D, L234E, L234Y, L234I, L235D, L235S, L235Y, L235I, S239D, S239E, S239N, S239Q, S239T, V240I, V240M, F243L, V264I, V264T, V264Y, V266I, E272Y, K274T, K274E, K274R, K274L, K274Y, F275W, N276L, Y278T, V302I, E318R, S324D, S324I, S324V, N325T, K326I, K326T, L328M, L328I, L328Q, L328D, L328V, L328T, A330Y, A330L, A330I, I332D, I332E, I332N, I332Q, T335D, T335R, and T335Y. In other specific embodiments of the present disclosure, the variant Fc region comprises at least one amino acid substitution selected from the group consisting of: V264I, F243L/V264I, L328M, I332E, L328M/I332E, V264I/I332E, S298A/I332E, S239E/I332E, S239Q/I332E, S239E, A330Y, I332D, L328I/I332E, L328Q/I332E, V264T, V240I, V266I, S239D, S239D/I332D, S239D/I332E, S239D/I332N, S239D/I332Q, S239E/I332D, S239E/I332N, S239E/I332Q, S239N/I332D,



S239N/I332E, S239Q/I332D, A330Y/I332E, V264I/A330Y/I332E, A330L/I332E, V264I/A330L/I332E, L234E, L234Y, L234I, L235D, L235S, L235Y, L235I, S239T, V240M, V264Y, A330I, N325T, L328D/I332E, L328V/I332E, L328T/I332E, L328I/I332E, S239E/V264I/I332E, S239Q/V264I/I332E, S239E/V264I/A330Y/I332E, S239D/A330Y/I332E, S239N/A330Y/I332E, S239D/A330L/I332E, S239N/A330L/I332E, V264I/S298A/I332E, S239D/S298A/I332E, S239N/S298A/I332E, S239D/V264I/I332E, S239D/V264I/S298A/I332E, S239D/V264I/A330L/I332E, S239D/I332E/A330I, P230A, P230A/E233D/I332E, E272Y, K274T, K274E, K274R, K274L, K274Y, F275W, N276L, Y278T, V302I, E318R, S324D, S324I, S324V, K326I, K326T, T335D, T335R, T335Y, V240I/V266I, S239D/A330Y/I332E/L234I, S239D/A330Y/I332E/L235D, S239D/A330Y/I332E/V240I, S239D/A330Y/I332E/V264T, S239D/A330Y/I332E/K326E, and S239D/A330Y/I332E/K326T. In more specific embodiments of the present disclosure, the variant Fc region comprises a series of substitutions selected from the group consisting of: N297D/I332E, F241Y/F243Y/V262T/V264T/N297D/I332E, S239D/N297D/I332E, S239E/N297D/I332E, S239D/D265Y/N297D/I332E, S239D/D265H/N297D/I332E, V264E/N297D/I332E, Y296N/N297D/I332E, N297D/A330Y/I332E, S239D/D265V/N297D/I332E, S239D/D265I/N297D/I332E, and N297D/S298A/A330Y/I332E. In specific embodiments of the present disclosure, the variant Fc region comprises an amino acid substitution at position 332 (using the numbering of the EU index, Kabat *et al.*, *supra*). Examples of substitutions include 332A, 332D, 332E, 332F, 332G, 332H, 332K, 332L, 332M, 332N, 332P, 332Q, 332R, 332S, 332T, 332V, 332W and 332Y. The numbering of the residues in the Fc region is that of the EU index of Kabat *et al.* Among other properties described herein, such variant Fc regions may have increased affinity for an FcγR, increased stability, and/or increased solubility, relative to a corresponding, wild-type Fc region.

**[0223]** Further examples include variant Fc regions that comprise one or more of the following amino acid substitutions: 224N/Y, 225A, 228L, 230S, 239P, 240A, 241L, 243S/L/G/H/I, 244L, 246E, 247L/A, 252T, 254T/P, 258K, 261Y, 265V, 266A, 267G/N, 268N, 269K/G, 273A, 276D, 278H, 279M, 280N, 283G, 285R, 288R, 289A, 290E, 291L, 292Q, 297D, 299A, 300H, 301C, 304G, 305A, 306I/F, 311R, 312N, 315D/K/S, 320R, 322E, 323A, 324T, 325S, 326E/R, 332T, 333D/G, 335I, 338R, 339T, 340Q, 341E, 342R, 344Q, 347R, 351S, 352A, 354A, 355W, 356G, 358T, 361D/Y, 362L, 364C, 365Q/P, 370R, 372L, 377V, 378T, 383N, 389S, 390D, 391C, 393A, 394A, 399G, 404S, 408G, 409R, 411I, 412A, 414M, 421S, 422I, 426F/P, 428T, 430K, 431S, 432P, 433P, 438L, 439E/R, 440G, 441F, 442T, 445R, 446A, 447E, optionally where the variant has altered recognition of an Fc ligand and/or altered effector function compared with a parent Fc polypeptide, and wherein the numbering of the residues is that of the EU index as in Kabat *et al.* Specific examples of these and related embodiments of the present disclosure include variant Fc regions that comprise or consist of the following sets of substitutions: (1) N276D, R292Q, V305A, I377V, T394A, V412A and K439E; (2) P244L, K246E, D399G and K409R; (3) S304G, K320R, S324T, K326E and M358T; (4) F243S, P247L, D265V, V266A, S383N and T411I; (5) H224N, F243L, T393A and H433P; (6) V240A, S267G, G341E and E356G; (7) M252T, P291L, P352A, R355W, N390D, S408G, S426F and A431S; (8) P228L, T289A, L365Q, N389S and S440G; (9) F241L, V273A, K340Q and L441F; (10) F241L, T299A, I332T and M428T; (11) E269K, Y300H, Q342R, V422I and G446A; (12) T225A, R301C, S304G, D312N, N315D, L351S and N421S; (13) S254T, L306I, K326R and Q362L; (14) H224Y, P230S, V323A, E333D, K338R and S364C; (15) T335I, K414M and P445R; (16) T335I and K414M; (17) P247A, E258K, D280N, K288R, N297D, T299A, K322E, Q342R, S354A and L365P; (18) H268N, V279M, A339T, N361D and S426P; (19) C261Y, K290E, L306F, Q311R, E333G and Q438L; (20) E283G, N315K, E333G, R344Q, L365P and S442T; (21) Q347R, N361Y and K439R; (22) S239P, S254P, S267N, H285R, N315S, F372L, A378T, N390D, Y391C, F404S, E430K, L432P and K447E; and (23) E269G, Y278H, N325S and K370R, wherein the numbering of the residues is that of the EU index as in Kabat *et al.* (see, e.g., U.S. Application No. 2010/0184959).

**[0224]** Another specific example of an Fc variant comprises the sequence of SEQ ID NO:155, wherein Xaa at position 1 is Ala or absent; Xaa at position 16 is Pro or Glu; Xaa at position 17 is Phe, Val, or Ala; Xaa at position 18 is Leu, Glu, or Ala; Xaa at position 80 is Asn or Ala; and/or Xaa at position 230 is Lys or is absent (see, e.g., U.S. Application No. 2007/0253966). Certain of these Fc regions, and related HRS-Fc conjugates,



have increased half-life, reduced effector activity, and/or are significantly less immunogenic than wild-type Fc sequences.

**[0225]** Variant Fc regions can also have one or more mutated hinge regions, as described, for example, in U.S. Application No. 2003/0118592. For instance, one or more cysteines in a hinge region can be deleted or substituted with a different amino acid. The mutated hinge region can comprise no cysteine residues, or it can comprise 1, 2, or 3 fewer cysteine residues than a corresponding, wild-type hinge region. In some embodiments of the present disclosure, an Fc region having a mutated hinge region of this type exhibits a reduced ability to dimerize, relative to a wild-type Ig hinge region.

**[0226]** As noted above, HRS-Fc conjugates such as HRS-Fc fusion proteins typically have altered (e.g., improved, increased, decreased) pharmacokinetic properties relative to corresponding HRS polypeptides. Examples of pharmacokinetic properties include stability or half-life, bioavailability (the fraction of a drug that is absorbed), tissue distribution, volume of distribution (apparent volume in which a drug is distributed immediately after it has been injected intravenously and equilibrated between plasma and the surrounding tissues), concentration (initial or steady-state concentration of drug in plasma), elimination rate constant (rate at which drugs are removed from the body), elimination rate (rate of infusion required to balance elimination), area under the curve (AUC or exposure; integral of the concentration-time curve, after a single dose or in steady state), clearance (volume of plasma cleared of the drug per unit time),  $C_{max}$  (peak plasma concentration of a drug after oral administration),  $t_{max}$  (time to reach  $C_{max}$ ),  $C_{min}$  (lowest concentration that a drug reaches before the next dose is administered), and fluctuation (peak trough fluctuation within one dosing interval at steady state). In some aspects of the present disclosure, these improved properties are achieved without significantly altering the secondary structure and/or reducing the non-canonical biological activity of the HRS polypeptide. Indeed, some HRS-Fc conjugates have increased non-canonical biological activity.

**[0227]** Hence, in some embodiments of the present disclosure, the HRS-Fc conjugate or HRS-Fc fusion polypeptide has a plasma or sera pharmacokinetic AUC profile at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 50, 100, 200, 300, 400, or 500-fold greater than a corresponding unmodified or differently modified HRS polypeptide when administered to a mammal under the same or comparable conditions. In certain embodiments of the present disclosure, the HRS-Fc conjugate or HRS-Fc fusion polypeptide has a stability (e.g., as measured by half-life) which is at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, or 500% greater than a corresponding unmodified or differently modified HRS polypeptide when compared under similar conditions at room temperature, for example, in PBS at pH 7.4 for about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days, or 1, 2, 3, 4 weeks or so.

**[0228]** In particular embodiments of the present disclosure, a HRS-Fc conjugate or HRS-Fc fusion polypeptide has a biological half life at pH 7.4, 25°C, e.g., a physiological pH, human body temperature (e.g., *in vivo*, in serum, in a given tissue, in a given species such as rat, mouse, monkey, or human), of about or at least about 30 minutes, about 1 hour, about 2 hour, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 12 hours, about 18 hours, about 20 hours, about 24 hours, about 30 hours, about 36 hours, about 40 hours, about 48 hours, about 50 hours, about 60 hours, about 70 hours, about 72 hours, about 80 hours, about 84 hours, about 90 hours, about 96 hours, about 120 hours, or about 144 hours or more or any intervening half-life.

**[0229]** In certain embodiments of the present disclosure, the HRS-Fc conjugate or HRS-Fc fusion polypeptide has greater bioavailability after subcutaneous (SC) administration compared to a corresponding unmodified HRS-polypeptide. In certain embodiments of the present disclosure, the HRS-Fc conjugate or HRS-Fc fusion polypeptide has at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 100 %, or more bioavailability compared to the corresponding unmodified HRS polypeptide.



**[0230]** In certain embodiments of the present disclosure, the HRS-Fc fusion polypeptide has substantially the same secondary structure as a corresponding unmodified or differently modified HRS polypeptide, as determined via UV circular dichroism analysis. In certain embodiments of the present disclosure, the HRS-Fc fusion polypeptide has substantially the same activity of a corresponding unmodified or differently modified HRS polypeptide in an assay of anti-inflammatory activity. In other embodiments of the present disclosure, the HRS-Fc fusion polypeptide has greater than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20-fold the activity of a corresponding unmodified or differently modified HRS polypeptide in an assay of anti-inflammatory activity.

### ***Peptide Linkers***

**[0231]** In certain embodiments of the present disclosure, a peptide linker sequence may be employed to separate the HRS polypeptide(s) and the Fc region(s) by a distance sufficient to ensure that each polypeptide folds into its desired secondary and tertiary structures. Such a peptide linker sequence can be incorporated into the fusion protein using standard techniques well known in the art.

**[0232]** Certain peptide linker sequences may be chosen based on the following exemplary factors: (1) their ability to adopt a flexible extended conformation; (2) their inability to adopt a secondary structure that could interact with functional epitopes on the first and second polypeptides; (3) their physiological stability; and (4) the lack of hydrophobic or charged residues that might react with the polypeptide functional epitopes, or other features. See, e.g., George and Heringa, J Protein Eng. 15:871-879, 2002.

**[0233]** The linker sequence may generally be from 1 to about 200 amino acids in length. Particular linkers can have an overall amino acid length of about 1-200 amino acids, 1-150 amino acids, 1-100 amino acids, 1-90 amino acids, 1-80 amino acids, 1-70 amino acids, 1-60 amino acids, 1-50 amino acids, 1-40 amino acids, 1-30 amino acids, 1-20 amino acids, 1-10 amino acids, 1-5 amino acids, 1-4 amino acids, 1-3 amino acids, or about 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, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 60, 70, 80, 90, 100 or more amino acids.

**[0234]** A peptide linker may employ any one or more naturally-occurring amino acids, non-naturally occurring amino acid(s), amino acid analogs, and/or amino acid mimetics as described elsewhere herein and known in the art. Certain amino acid sequences which may be usefully employed as linkers include those disclosed in Maratea et al., Gene 40:39-46, 1985; Murphy et al., PNAS USA. 83:8258-8262, 1986; U.S. Pat. No. 4,935,233 and U.S. Pat. No. 4,751,180. Particular peptide linker sequences contain Gly, Ser, and/or Asn residues. Other near neutral amino acids, such as Thr and Ala may also be employed in the peptide linker sequence, if desired.

**[0235]** Certain exemplary linkers include Gly, Ser and/or Asn-containing linkers, as follows: [G]<sub>x</sub>, [S]<sub>x</sub>, [N]<sub>x</sub>, [GS]<sub>x</sub>, [GGS]<sub>x</sub>, [GSS]<sub>x</sub>, [GSGS]<sub>x</sub> (SEQ ID NO:200), [GGSG]<sub>x</sub> (SEQ ID NO:201), [GGGS]<sub>x</sub> (SEQ ID NO:202), [GGGGS]<sub>x</sub> (SEQ ID NO:203), [GN]<sub>x</sub>, [GGN]<sub>x</sub>, [GNN]<sub>x</sub>, [GNGN]<sub>x</sub> (SEQ ID NO: 204), [GGNG]<sub>x</sub> (SEQ ID NO:205), [GGGN]<sub>x</sub> (SEQ ID NO:206), [GGGGN]<sub>x</sub> (SEQ ID NO:207) linkers, where <sub>x</sub> is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 or more. Other combinations of these and related amino acids will be apparent to persons skilled in the art.

**[0236]** Additional examples of linker peptides include, but are not limited to the following amino acid sequences: Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-(SEQ ID NO:208); Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-(SEQ ID NO:209); Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-(SEQ ID NO:210); Asp-Ala-Ala-Ala-Lys-Glu-Ala-Ala-Ala-Lys-Asp-Ala-Ala-Ala-Arg-Glu-Ala-Ala-Ala-Arg-Asp-Ala-



Ala-Ala-Lys-(SEQ ID NO:211); and Asn-Val-Asp-His-Lys-Pro-Ser-Asn-Thr-Lys-Val-Asp-Lys-Arg-(SEQ ID NO:212). Further non-limiting examples of linker peptides include DGGGS (SEQ ID NO:213); TGEKP (SEQ ID NO:214) (see, e.g., Liu et al., PNAS. 94:5525-5530, 1997); GGRR (SEQ ID NO:215) (Pomerantz *et al.* 1995); (GGGS)<sub>n</sub> (SEQ ID NO:203) (Kim et al., PNAS. 93:1156-1160, 1996); EGKSSGSGSESKVD (SEQ ID NO:216) (Chaudhary et al., PNAS. 87:1066-1070, 1990); KESGSVSSEQLAQFRSLD (SEQ ID NO:217) (Bird et al., Science. 242:423-426, 1988), GGRRGGGS (SEQ ID NO:218); LRQRDGERP (SEQ ID NO:219); LRQKDGGGSERP (SEQ ID NO:220); LRQKd(GGGS)<sub>2</sub> ERP (SEQ ID NO:221). In specific embodiments of the present disclosure, the linker sequence comprises a Gly3 linker sequence, which includes three glycine residues. In particular embodiments of the present disclosure, flexible linkers can be rationally designed using a computer program capable of modeling both DNA-binding sites and the peptides themselves (Desjarlais & Berg, PNAS. 90:2256-2260, 1993; and PNAS. 91:11099-11103, 1994) or by phage display methods.

**[0237]** The peptide linkers may be physiologically stable or may include a releasable linker such as a physiologically degradable or enzymatically cleavable linker (e.g., proteolytically cleavable linker). In certain embodiments of the present disclosure, one or more releasable linkers can result in a shorter half-life and more rapid clearance of the conjugate. These and related embodiments of the present disclosure can be used, for example, to enhance the solubility and blood circulation lifetime of HRS polypeptides in the bloodstream, while also delivering a HRS polypeptide into the bloodstream that, subsequent to linker degradation, is substantially free of the Fc region(s). These aspects of the present disclosure are especially useful in those cases where HRS polypeptides, when permanently conjugated to an Fc region, demonstrate reduced activity. By using the linkers as provided herein, such HRS polypeptides can maintain their therapeutic activity when in conjugated form. As another example, a large and relatively inert HRS-Fc conjugate polypeptide may be administered, which is then degraded *in vivo* (via the degradable linker) to generate a bioactive HRS polypeptide possessing a portion of the Fc region or lacking the Fc region entirely. In these and other ways, the properties of the HRS-Fc conjugate polypeptide can be more effectively tailored to balance the bioactivity and circulating half-life of the HRS polypeptide overtime.

**[0238]** In particular embodiments of the present disclosure, the linker peptide comprises an autocatalytic or self-cleaving peptide cleavage site. In a particular embodiment of the present disclosure, self-cleaving peptides include those polypeptide sequences obtained from potyvirus and cardiovirus 2A peptides, FMDV (foot-and-mouth disease virus), equine rhinitis A virus, Thosa asigna virus and porcine teschovirus. In certain embodiments of the present disclosure, the self-cleaving polypeptide site comprises a 2A or 2A-like site, sequence or domain (Donnelly et al., J. Gen. Virol. 82:1027-1041, 2001). Exemplary 2A sites include the following sequences: LLNFDLLKLAGDVESNPGP (SEQ ID NO:222); TLNFDLLKLAGDVESNPGP (SEQ ID NO:223); LLKLAGDVESNPGP (SEQ ID NO:224); NFDLLKLAGDVESNPGP (SEQ ID NO:225); QLLNFDLLKLAGDVESNPGP (SEQ ID NO:226); APVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO:227); VTELLYRMKRAETYCPRLAIHPTEARHKQKIVAPVKQT (SEQ ID NO:228); LNFDLLKLAGDVESNPGP (SEQ ID NO:229); LLAIHPTEARHKQKIVAPVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO:230); and EARHKQKIVAPVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO:231). In one embodiment of the present disclosure, the autocatalytic peptide cleavage site comprises a translational 2A signal sequence, such as, e.g., the 2A region of the aphthovirus foot-and-mouth disease virus (FMDV) polyprotein, which is an 18 amino acid sequence. Additional examples of 2A-like sequences that may be used include insect virus polyproteins, the NS34 protein of type C rotaviruses, and repeated sequences in *Trypanosoma* spp., as described, for example, in Donnelly et al., Journal of General Virology. 82:1027-1041, 2001.

**[0239]** Suitable protease cleavages sites and self-cleaving peptides are known to the skilled person (see, e.g., Ryan et al., J. Gener. Virol. 78:699-722, 1997; and Scymczak et al., Nature Biotech. 5:589-594, 2004). Exemplary protease cleavage sites include, but are not limited to the cleavage sites of potyvirus Nla proteases (e.g., tobacco etch virus protease), potyvirus HC proteases, potyvirus PI (P35) proteases, byovirus Nla proteases, byovirus RNA-2-encoded proteases, aphthovirus L proteases, enterovirus 2A proteases, rhinovirus 2A proteases, picorna 3C proteases, comovirus 24K proteases, nepovirus 24K proteases, RTSV (rice tungro



spherical virus) 3C-like protease, PYVF (parsnip yellow fleck virus) 3C-like protease, heparin, thrombin, factor Xa and enterokinase. Due to its high cleavage stringency, TEV (tobacco etch virus) protease cleavage sites are included in some embodiments of the present disclosure, e.g., EXXYXQ(G/S) (SEQ ID NO:232), for example, ENLYFQG (SEQ ID NO:233) and ENLYFQS (SEQ ID NO:234), wherein X represents any amino acid (cleavage by TEV occurs between Q and G or Q and S).

**[0240]** Further examples of enzymatically degradable linkers suitable for use in particular embodiments of the present disclosure include, but are not limited to: an amino acid sequence cleaved by a serine protease such as thrombin, chymotrypsin, trypsin, elastase, kallikrein, or subtilisin. Illustrative examples of thrombin-cleavable amino acid sequences include, but are not limited to: -Gly-Arg-Gly-Asp-(SEQ ID NO:235), -Gly-Gly-Arg-, -Gly-Arg-Gly-Asp-Asn-Pro-(SEQ ID NO: 236), -Gly-Arg-Gly-Asp-Ser-(SEQ ID NO:237), -Gly-Arg-Gly-Asp-Ser-Pro-Lys-(SEQ ID NO: 238), -Gly-Pro-Arg-, -Val-Pro-Arg-, and -Phe-Val-Arg-. Illustrative examples of elastase-cleavable amino acid sequences include, but are not limited to: -Ala-Ala-Ala-, -Ala-Ala-Pro-Val-(SEQ ID NO:239), -Ala-Ala-Pro-Leu-(SEQ ID NO:240), -Ala-Ala-Pro-Phe-(SEQ ID NO:241), -Ala-Ala-Pro-Ala-(SEQ ID NO:242), and -Ala-Tyr-Leu-Val-(SEQ ID NO:243).

**[0241]** Enzymatically degradable linkers also include amino acid sequences that can be cleaved by a matrix metalloproteinase such as collagenase, stromelysin, and gelatinase. Illustrative examples of matrix metalloproteinase-cleavable amino acid sequences include, but are not limited to: -Gly-Pro-Y-Gly-Pro-Z-(SEQ ID NO:244), -Gly-Pro-, Leu-Gly-Pro-Z-(SEQ ID NO:245), -Gly-Pro-Ile-Gly-Pro-Z-(SEQ ID NO:246), and -Ala-Pro-Gly-Leu-Z-(SEQ ID NO:247), where Y and Z are amino acids. Illustrative examples of collagenase-cleavable amino acid sequences include, but are not limited to: -Pro-Leu-Gly-Pro-D-Arg-Z-(SEQ ID NO:248), -Pro-Leu-Gly-Leu-Leu-Gly-Z-(SEQ ID NO:249), -Pro-Gln-Gly-Ile-Ala-Gly-Trp-(SEQ ID NO:250), -Pro-Leu-Gly-Cys(Me)-His-(SEQ ID NO:251), -Pro-Leu-Gly-Leu-Tyr-Ala-(SEQ ID NO:252), -Pro-Leu-Ala-Leu-Trp-Ala-Arg-(SEQ ID NO:253), and -Pro-Leu-Ala-Tyr-Trp-Ala-Arg-(SEQ ID NO:254), where Z is an amino acid. An illustrative example of a stromelysin-cleavable amino acid sequence is -Pro-Tyr-Ala-Tyr-Tyr-Met-Arg-(SEQ ID NO:255); and an example of a gelatinase-cleavable amino acid sequence is -Pro-Leu-Gly-Met-Tyr-Ser-Arg-(SEQ ID NO:256).

**[0242]** Enzymatically degradable linkers suitable for use in particular embodiments of the present disclosure also include amino acid sequences that can be cleaved by an angiotensin converting enzyme, such as, for example, -Asp-Lys-Pro-, -Gly-Asp-Lys-Pro-(SEQ ID NO:257), and -Gly-Ser-Asp-Lys-Pro-(SEQ ID NO:258).

**[0243]** Enzymatically degradable linkers suitable for use in particular embodiments of the present disclosure also include amino acid sequences that can be degraded by cathepsin B, such as, for example, Val-Cit, Ala-Leu-Ala-Leu-(SEQ ID NO:259), Gly-Phe-Leu-Gly-(SEQ ID NO:260) and Phe-Lys.

**[0244]** In particular embodiments of the present disclosure, a releasable linker has a half life at pH 7.4, 25°C, e.g., a physiological pH, human body temperature (e.g., *in vivo*, in serum, in a given tissue), of about 30 minutes, about 1 hour, about 2 hour, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 12 hours, about 18 hours, about 24 hours, about 36 hours, about 48 hours, about 72 hours, or about 96 hours or more or any intervening half-life. One having skill in the art would appreciate that the half life of a HRS-Fc conjugate polypeptide can be finely tailored by using a particular releasable linker.

**[0245]** In certain embodiments of the present disclosure, however, any one or more of the peptide linkers are optional. For instance, linker sequences may not required when the first and second polypeptides have non-essential N-terminal and/or C-terminal amino acid regions that can be used to separate the functional domains and prevent steric interference.

#### **Methods for use**



**[0246]** Embodiments of the present disclosure relate to the discovery that Fc region-histidyl-tRNA synthetase (HRS-Fc) conjugate polypeptides, and fragments and variants thereof, offer improved methods of modulating inflammatory responses in a variety of useful ways, both *in vitro* and *in vivo*. The compositions of the disclosure may thus be useful as immunomodulators for treating a broad range of pro-inflammatory, inflammatory, and/or autoimmune indications, including inflammatory responses, chronic inflammation, acute inflammation, and immune diseases, by modulating the cells that mediate, either directly or indirectly, such inflammatory and/or autoimmune diseases, conditions and disorders. The utility of the compositions of the disclosure as immunomodulators can be monitored using any of a number of known and available techniques in the art including, for example, migration assays (*e.g.*, using leukocytes or lymphocytes), cytokine production assays, or cell viability or cell differentiation assays (*e.g.*, using B-cells, T-cells, monocytes or NK cells).

**[0247]** "Inflammation" refers generally to the biological response of tissues to harmful stimuli, such as pathogens, damaged cells (*e.g.*, wounds), and irritants. The term "inflammatory response" refers to the specific mechanisms by which inflammation is achieved and regulated, including, merely by way of illustration, immune cell activation or migration, migration, autoimmunity and autoimmune disease, cytokine production, vasodilation, including kinin release, fibrinolysis, and coagulation, among others described herein and known in the art. Ideally, inflammation is a protective attempt by the body to both remove the injurious stimuli and initiate the healing process for the affected tissue or tissues. In the absence of inflammation, wounds and infections would never heal, creating a situation in which progressive destruction of the tissue would threaten survival. On the other hand, excessive or chronic inflammation may associate with a variety of diseases, such as hay fever, atherosclerosis, and rheumatoid arthritis, among others described herein and known in the art.

**[0248]** Clinical signs of chronic inflammation are dependent upon duration of the illness, inflammatory lesions, cause and anatomical area affected, (see, *e.g.*, Kumar et al., Robbins Basic Pathology-8th Ed., 2009 Elsevier, London; Miller, LM, Pathology Lecture Notes, Atlantic Veterinary College, Charlottetown, PEI, Canada). Chronic inflammation is associated with a variety of pathological conditions or diseases, including, for example, allergies, Alzheimer's disease, anemia, aortic valve stenosis, arthritis such as rheumatoid arthritis and osteoarthritis, cancer, congestive heart failure, fibromyalgia, fibrosis, heart attack, kidney failure, lupus, pancreatitis, stroke, surgical complications, inflammatory lung disease, inflammatory bowel diseases including Crohn's disease (CD) and ulcerative colitis (UC), atherosclerosis, neurological disorders, diabetes, metabolic disorders, obesity, and psoriasis, among others described herein and known in the art. Many other chronic diseases may also include an inflammatory component, and thus may be treated with the HRS-Fc conjugates of the disclosure including, for example, muscular dystrophies and rhabdomyolysis. Hence, HRS-Fc conjugates may be used to treat or manage chronic inflammation, modulate any of one or more of the individual chronic inflammatory responses, or treat any one or more diseases or conditions associated with chronic inflammation.

**[0249]** Certain specific inflammatory responses include cytokine production and activity, and related pathways. For instance, certain exemplary embodiments of the present disclosure relate to modulating cell-signaling through nuclear factor-kB (NF- $\kappa$ B), such as by increasing the downstream activities of this transcription factor. In certain instances, increases in NF- $\kappa$ B activity can lead to increases in cytokine signaling or activity, such as pro-inflammatory cytokines (*e.g.*, TNF-alpha or beta), and anti-inflammatory cytokines (*e.g.*, IL-10).

**[0250]** Criteria for assessing the signs and symptoms of inflammatory and other conditions, including for purposes of making differential diagnosis and also for monitoring treatments such as determining whether a therapeutically effective dose has been administered in the course of treatment, *e.g.*, by determining improvement according to accepted clinical criteria, will be apparent to those skilled in the art and are exemplified by the teachings of *e.g.*, Berkow et al., eds., The Merck Manual, 16th edition, Merck and Co., Rahway, N.J., 1992; Goodman et al., eds., Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10th edition, Pergamon Press, Inc., Elmsford, N.Y., (2001); Avery's Drug Treatment: Principles



and Practice of Clinical Pharmacology and Therapeutics, 3rd edition, ADIS Press, Ltd., Williams and Wilkins, Baltimore, MD. (1987); Ebadi, Pharmacology, Little, Brown and Co., Boston, (1985); Osolci al., eds., Remington's Pharmaceutical Sciences, 18th edition, Mack Publishing Co., Easton, PA (1990); Katzung, Basic and Clinical Pharmacology, Appleton and Lange, Norwalk, CT (1992).

**[0251]** Also included are methods of modulating an immune response, such as an innate or adaptive immune response via the use of any of the HRS-Fc conjugates described herein. As used herein, the term "immune response" includes a measurable or observable reaction to an antigen, vaccine composition, or immunomodulatory molecule mediated by one or more cells of the immune system. An immune response typically begins with an antigen or immunomodulatory molecule binding to an immune system cell. A reaction to an antigen or immunomodulatory molecule may be mediated by many cell types, including a cell that initially binds to an antigen or immunomodulatory molecule and cells that participate in mediating an innate, humoral, cell-mediated immune response.

**[0252]** Also included are methods of treating immune diseases. Illustrative immune system diseases, disorders or conditions that may be treated according to the present disclosure include, but are not limited to, primary immunodeficiencies, immune-mediated thrombocytopenia, Kawasaki syndrome, bone marrow transplant (for example, recent bone marrow transplant in adults or children), chronic B cell lymphocytic leukemia, HIV infection (for example, adult or pediatric HIV infection), chronic inflammatory demyelinating polyneuropathy, post-transfusion purpura, and the like.

**[0253]** Additionally, further diseases, disorders and conditions which may be treated with any of the HRS-Fc conjugates described herein include Guillain-Barre syndrome, anemia (for example, anemia associated with parvovirus B19, patients with stable multiple myeloma who are at high risk for infection (for example, recurrent infection), autoimmune hemolytic anemia (for example, warm-type autoimmune hemolytic anemia), thrombocytopenia (for example, neonatal thrombocytopenia), and immune-mediated neutropenia), transplantation (for example, cytomegalovirus (CMV)-negative recipients of CMV-positive organs), hypogammaglobulinemia (for example, hypogammaglobulinemic neonates with risk factor for infection or morbidity), epilepsy (for example, intractable epilepsy), systemic vasculitic syndromes, myasthenia gravis (for example, decompensation in myasthenia gravis), dermatomyositis, and polymyositis.

**[0254]** Further autoimmune diseases, disorders and conditions which may be treated with any of the HRS-Fc conjugates described herein include but are not limited to, autoimmune hemolytic anemia, autoimmune neonatal thrombocytopenia, idiopathic thrombocytopenia purpura, autoimmunocytopenia, hemolytic anemia, antiphospholipid syndrome, dermatitis, allergic encephalomyelitis, myocarditis, relapsing polychondritis, rheumatic heart disease, glomerulonephritis (for example, IgA nephropathy), multiple sclerosis, neuritis, uveitis, ophthalmia, polyendocrinopathies, purpura (for example, Henoch-Schoenlein purpura), Reiter's disease, stiff-man syndrome, autoimmune pulmonary inflammation, Guillain-Barre Syndrome, insulin dependent diabetes mellitus, and autoimmune inflammatory eye disease.

**[0255]** Additional autoimmune diseases, disorders or conditions which may be treated with any of the HRS-Fc conjugates described herein include, but are not limited to, autoimmune thyroiditis; hypothyroidism, including Hashimoto's thyroiditis and thyroiditis characterized, for example, by cell-mediated and humoral thyroid cytotoxicity; SLE (which is often characterized, for example, by circulating and locally generated immune complexes); Goodpasture's syndrome (which is often characterized, for example, by anti-basement membrane antibodies); pemphigus (which is often characterized, for example, by epidermal acantholytic antibodies); receptor autoimmunities such as, for example, Graves' disease (which is often characterized, for example, by antibodies to a thyroid stimulating hormone receptor; myasthenia gravis, which is often characterized, for example, by acetylcholine receptor antibodies); insulin resistance (which is often characterized, for example, by insulin receptor antibodies); autoimmune hemolytic anemia (which is often characterized, for example, by phagocytosis of antibody-sensitized red blood cells); and autoimmune



thrombocytopenic purpura (which is often characterized, for example, by phagocytosis of antibody-sensitized platelets).

**[0256]** Further autoimmune diseases, disorders or conditions which may be treated with any of the HRS-Fc conjugates described herein include, but are not limited to, rheumatoid arthritis (which is often characterized, for example, by immune complexes in joints); scleroderma with anti-collagen antibodies (which is often characterized, for example, by nucleolar and other nuclear antibodies); mixed connective tissue disease, (which is often characterized, for example, by antibodies to extractable nuclear antigens, for example, ribonucleoprotein); polymyositis/dermatomyositis (which is often characterized, for example, by nonhistone anti-nuclear antibodies); pernicious anemia (which is often characterized, for example, by antiparietal cell, antimicrosome, and anti-intrinsic factor antibodies); idiopathic Addison's disease (which is often characterized, for example, by humoral and cell-mediated adrenal cytotoxicity); infertility (which is often characterized, for example, by antispermatozoal antibodies); glomerulonephritis (which is often characterized, for example, by glomerular basement membrane antibodies or immune complexes); by primary glomerulonephritis, by IgA nephropathy; bullous pemphigoid (which is often characterized, for example, by IgG and complement in the basement membrane); Sjogren's syndrome (which is often characterized, for example, by multiple tissue antibodies and/or the specific nonhistone antinuclear antibody (SS-B)); diabetes mellitus (which is often characterized, for example, by cell-mediated and humoral islet cell antibodies); and adrenergic drug resistance, including adrenergic drug resistance with asthma or cystic fibrosis (which is often characterized, for example, by beta-adrenergic receptor antibodies).

**[0257]** Still further autoimmune diseases, disorders or conditions which may be treated with any of the HRS-Fc conjugates described herein include, but are not limited to chronic active hepatitis (which is often characterized, for example by smooth muscle antibodies); primary biliary cirrhosis (which is often characterized, for example, by anti-mitochondrial antibodies); other endocrine gland failure (which is characterized, for example, by specific tissue antibodies in some cases); vitiligo (which is often characterized, for example, by anti-melanocyte antibodies); vasculitis (which is often characterized, for example, by immunoglobulin and complement in vessel walls and/or low serum complement); post-myocardial infarction conditions (which are often characterized, for example, by anti-myocardial antibodies); cardiomyopathy syndrome (which is often characterized, for example, by anti-myocardial antibodies); urticaria (which is often characterized, for example, by IgG and IgM antibodies to IgE); atopic dermatitis (which is often characterized, for example, by IgG and IgM antibodies to IgE); asthma (which is often characterized, for example, by IgG and IgM antibodies to IgE); inflammatory myopathies; and other inflammatory, granulomatous, degenerative, and atrophic disorders.

**[0258]** Additional diseases and disorders which may be treated with any of the HRS-Fc conjugates described herein include those that result from or associate with an imbalance of Th17 or other Th cell subtypes. Examples include psoriasis, psoriatic arthritis, atopic dermatitis (eczema), Balo concentric sclerosis, Schilder's diffuse sclerosis, Marburg MS, IBD, Crohn's, ulcerative colitis, collagenous colitis, lymphocytic colitis, ischaemic colitis, diversion colitis, Behçet's disease, indeterminate colitis, asthma, autoimmune myocarditis, endometriosis, Adult onset Still's disorder (AOSD), Henoch-Schonlein purpura (HSP), Vogt-Koyanagi-Harada (VKH), periodontal disease, organ transplantation failure, graft versus host disease, and Devic's disease (neuromyelitis optica).

**[0259]** In some aspects of the present disclosure, the present disclosure includes a method of reducing muscle or lung inflammation associated with an autoimmune disease comprising administering to a subject in need thereof a composition comprising any of the HRS-Fc conjugates described herein. Exemplary muscular inflammatory diseases and disorders include muscular dystrophies, exercise-induced muscle inflammation, inflammation associated with muscle injury or surgery, rhabdomyolysis, and related diseases and disorders as described herein.



**[0260]** Also included are methods of treating a disease associated with an autoantibody comprising administering to a subject in need thereof a therapeutic composition comprising any of the HRS-Fc conjugates described herein, wherein the HRS polypeptide comprises at least one epitope specifically recognized by the autoantibody.

**[0261]** Certain embodiments of the present disclosure include methods of inducing tolerance to a histidyl-tRNA synthetase (HisRS) antigen, said method comprising administering to a subject a composition comprising any of the HRS-Fc conjugates described herein, wherein the HRS polypeptide comprises at least one epitope specifically recognized by the autoantibody, and wherein administration of the composition causes tolerization to the autoantigen.

**[0262]** Also included are methods for eliminating a set or subset of T cells involved in an autoimmune response to a histidyl tRNA synthetase (HisRS) autoantigen, the method comprising administering to a subject a composition comprising any of the HRS-Fc conjugates described herein, wherein the HRS polypeptide comprises at least one epitope specifically recognized by the autoantibody, or auto-reactive T cell, and wherein administration of the composition causes clonal deletion of auto-reactive T-cells.

**[0263]** In another embodiment of the present disclosure, the present disclosure includes a method for inducing anergy in T cells involved in an autoimmune response to a histidyl tRNA synthetase (HRS) autoantigen, the method comprising administering to a subject a composition comprising any of the HRS-Fc conjugates described herein, wherein the HRS polypeptide comprises at least one epitope specifically recognized by the autoantibody, or T cell, and wherein administration of the composition causes functional inactivation of the T cells involved in the autoimmune response.

**[0264]** In another embodiment of the present disclosure, the present disclosure includes a replacement therapy for treating a disease associated with an insufficiency of histidyl tRNA synthetase comprising administering to a subject in need thereof a therapeutic composition comprising any of the HRS-Fc conjugates described herein, wherein the HRS polypeptide functionally compensates for the histidyl tRNA synthetase insufficiency.

**[0265]** In one aspect of the present disclosure of this replacement therapy, the histidyl tRNA synthetase insufficiency is caused by the presence of anti-Jo-1 antibodies. In one aspect of the present disclosure of this replacement therapy, the histidyl tRNA synthetase insufficiency is caused by mutations in an endogenous histidyl tRNA synthetase which modulate the activity, expression or cellular distribution of the endogenous histidyl tRNA synthetase. In one aspect of the present disclosure the histidyl tRNA synthetase insufficiency is associated with Perrault syndrome or Usher syndrome.

**[0266]** In any of these methods, the term "tolerance" refers to the sustained reduction or absence of an immune response to a specific antigen in a mammal, particularly a human. Tolerance is distinct from generalized immunosuppression, in which all, or all of a specific class of immune cells, such as B cell mediated immune responses, of an immune responses are diminished, or eliminated. The development of tolerance may be routinely monitored by the absence, or a decrease, in the concentration of antibodies to HRS polypeptides in the serum of the host subject after administration, in single or successive doses of the treating HRS-Fc conjugate. The development of tolerance will typically be sufficient to decrease the symptoms of the autoimmune disease in the patient, for example a patient may be sufficiently improved so as to maintain normal activities in the absence, or in the presence of reduced amounts, of general immunosuppressants, e.g. corticosteroids.

**[0267]** In any of these methods, and compositions tolerance will typically be sustained, meaning that it will have a duration of about one month, about two months, about three months, about 4 months, about 5 months, or about 6 months or longer. Tolerance may result in selective B-cell anergy, or T-cell anergy or both.



**[0268]** In any of these methods, treatments and therapeutic compositions, the term "a disease associated with autoantibodies specific for histidyl tRNA synthetase" refers to any disease or disorder in which antibodies to histidyl tRNA synthetase are detected, or detectable, irrespective of whether other autoantibodies are also detected, or thought to play a role in disease progression or cause. Methods for detecting antibodies in patient samples may be carried out by any standard procedure including for example, by RIA, ELISA, by immunoprecipitation, by staining of tissues or cells (including transfected cells), antigen microarrays, mass spec analysis, specific neutralization assays or one of a number of other methods known in the art for identifying desired antigen specificity. In some aspects of the present disclosure, antibody specificity can be further characterized by determining the ability of the antibodies to selectively bind to different splice variants and truncated or proteolytic forms of histidyl tRNA synthetase. A relatively well known human auto-antibody to histidyl tRNA synthetase includes for example antibodies to Jo-1.

**[0269]** In some embodiments of the present disclosure of any of the disclosed methods, and compositions, the HRS polypeptide or HRS-Fc conjugate comprises an epitope from histidyl tRNA synthetase which specifically cross reacts with a disease associated auto-antibody to histidyl-tRNA synthetase. In some embodiments of the present disclosure of any of the disclosed methods, and compositions, the HRS polypeptide or HRS-Fc conjugate comprises an epitope from histidyl tRNA synthetase which specifically cross reacts with a disease associated auto-reactive T cell to histidyl-tRNA synthetase. In some embodiments of the present disclosure of any of the disclosed methods, and compositions, the HRS polypeptide or HRS-Fc conjugate comprises an epitope which specifically cross reacts with a disease associated auto-antibody to either another tRNA synthetase, or to a non tRNA synthetase auto antibody.

**[0270]** In some embodiments of the present disclosure of any of the disclosed methods the HRS polypeptide or HRS-Fc conjugate comprises an immunodominant epitope which is specifically recognized by the majority of antibodies from the sera of a patient with a disease associated with auto antibodies to histidyl- tRNA synthetase. In some embodiments of the present disclosure of any of the disclosed methods the HRS polypeptide or HRS-Fc conjugate comprises an immunodominant epitope which is specifically recognized by the majority of autoreactive T cells from the sera of a patient with a disease associated with auto antibodies to histidyl-tRNA synthetase.

**[0271]** In some embodiments of the present disclosure, the epitope is comprised within the WHEP domain of the HRS polypeptide (approximately amino acids 1-43 of SEQ ID NO:1); the aminoacylation domain (approximately amino acids 54-398 of SEQ ID NO:1); or the anticodon binding domain (approximately amino acids 406-501 of SEQ ID NO:1) or any combination thereof.

**[0272]** In some embodiments of the present disclosure, the HRS polypeptide does not comprise an epitope which specifically cross reacts with a disease associated auto-antibody to histidyl-tRNA synthetase. In some embodiments of the present disclosure, the HRS polypeptide does not significantly compete for disease associated auto-antibody binding to histidyl-tRNA synthetase in a competitive ELISA up to a concentration of about  $1 \times 10^{-7}$ M. In some embodiments of the present disclosure, the HRS polypeptide does not significantly compete for disease associated auto-antibody binding to histidyl-tRNA synthetase in a competitive ELISA up to a concentration of about  $5 \times 10^{-7}$ M. In some embodiments of the present disclosure, the HRS polypeptide does not significantly compete for disease associated auto-antibody binding to histidyl-tRNA synthetase in a competitive ELISA up to a concentration of about  $1 \times 10^{-6}$ M.

**[0273]** Accordingly in some embodiments of the present disclosure, the HRS polypeptide has a lower affinity to a disease associated auto-antibody than wild type histidyl-tRNA synthetase (SEQ ID NO:1) as measured in a competitive ELISA. In some embodiments of the present disclosure, the HRS polypeptide has an apparent affinity for the disease associated auto-antibody which is at least about 10 fold less, or at least about 20 fold less, or at least about 50 fold less, or at least about 100 fold less than the affinity of the disease associated



auto-antibody to wild type human (SEQ ID NO:1). In one aspect of the present disclosure, the auto-antibody to histidyl- tRNA synthetase is directed to the Jo-1 antigen.

**[0274]** Examples of diseases associated with autoantibodies specific for histidyl-tRNA synthetase (as well as diseases associated with an insufficiency of histidyl-tRNA synthetase) include without limitation, autoimmune diseases, inflammatory diseases, and inflammatory myopathies, including idiopathic inflammatory myopathies, polymyositis, statin induced myopathies, dermatomyositis, interstitial lung disease (and other pulmonary fibrotic conditions) and related disorders, such as polymyositis-scleroderma overlap and inclusion body myositis (IBM) and conditions such as those found in anti-synthetase syndromes, including for example, interstitial lung disease, arthritis, esophageal dysmotility, cardiovascular disease and other vascular manifestations such as Reynaud's phenomenon; other examples of diseases associated with an insufficiency of histidyl-tRNA synthetase include genetic disorders that result in an insufficiency of active histidyl-tRNA synthetase including Usher syndrome and Perrault syndrome.

**[0275]** Polymyositis affects skeletal muscles (involved with making movement) on both sides of the body. It is rarely seen in persons under age 18; most cases are in people between the ages of 31 and 60. In addition to symptoms listed above, progressive muscle weakness leads to difficulty swallowing, speaking, rising from a sitting position, climbing stairs, lifting objects, or reaching overhead. People with polymyositis may also experience arthritis, shortness of breath, and heart arrhythmias. Polymyositis is often associated with antibodies to synthetases, including HisRS, resulting in immune cell invasion into the damaged muscle cells. HRS-Fc conjugates may thus be used to reduce immune cell activation and invasion, and to treat polymyositis.

**[0276] Dermatomyositis** is characterized by a skin rash that precedes or accompanies progressive muscle weakness. The rash looks patchy, with purple or red discolorations, and characteristically develops on the eyelids and on muscles used to extend or straighten joints, including knuckles, elbows, knees, and toes. Red rashes may also occur on the face, neck, shoulders, upper chest, back, and other locations, and there may be swelling in the affected areas. The rash sometimes occurs without obvious muscle involvement. Adults with dermatomyositis may experience weight loss or a low-grade fever, have inflamed lungs, and be sensitive to light. Adult dermatomyositis, unlike polymyositis, may accompany tumors of the breast, lung, female genitalia, or bowel. Children and adults with dermatomyositis may develop calcium deposits, which appear as hard bumps under the skin or in the muscle (called calcinosis). Calcinosis most often occurs 1-3 years after disease onset but may occur many years later. These deposits are seen more often in childhood dermatomyositis than in dermatomyositis that begins in adults. Dermatomyositis may be associated with collagen-vascular or autoimmune diseases.

**[0277]** In some cases of polymyositis and dermatomyositis, distal muscles (away from the trunk of the body, such as those in the forearms and around the ankles and wrists) may be affected as the disease progresses. Polymyositis and dermatomyositis may be associated with collagen-vascular or autoimmune diseases resulting in immune cell invasion into the damaged muscle cells. HRS-Fc conjugates may thus be used to reduce immune cell activation and invasion, and to treat dermatomyositis.

**[0278] Inclusion body myositis (IBM)** is characterized by progressive muscle weakness and wasting. The onset of muscle weakness is generally gradual (over months or years) and affects both proximal and distal muscles. Muscle weakness may affect only one side of the body. Small holes called vacuoles are sometimes seen in the cells of affected muscle fibers. Falling and tripping are usually the first noticeable symptoms of IBM. For some individuals the disorder begins with weakness in the wrists and fingers that causes difficulty with pinching, buttoning, and gripping objects. There may be weakness of the wrist and finger muscles and atrophy (thinning or loss of muscle bulk) of the forearm muscles and quadricep muscles in the legs. Difficulty swallowing occurs in approximately half of IBM cases. Symptoms of the disease usually begin after the age of 50, although the disease can occur earlier. Unlike polymyositis and dermatomyositis, IBM occurs more frequently in men than in women. As with other muscular dystrophies, IBM also results in progressive immune



cell invasion into the damaged muscle cells. HRS-Fc conjugates may thus be used to reduce immune cell activation and invasion, and to treat IBM.

**[0279] Juvenile myositis** has some similarities to adult dermatomyositis and polymyositis. It typically affects children ages 2 to 15 years, with symptoms that include proximal muscle weakness and inflammation, edema (an abnormal collection of fluids within body tissues that causes swelling), muscle pain, fatigue, skin rashes, abdominal pain, fever, and contractures (chronic shortening of muscles or tendons around joints, caused by inflammation in the muscle tendons, which prevents the joints from moving freely). Children with juvenile myositis may also have difficulty swallowing and breathing, and the heart may be affected. Approximately 20 to 30 percent of children with juvenile dermatomyositis develop calcinosis. Affected children may not show higher than normal levels of the muscle enzyme creatine kinase in their blood but have higher than normal levels of other muscle enzymes. Juvenile myositis also results in progressive immune cell invasion into the damaged muscle cells. HRS-Fc conjugates may thus be used to reduce immune cell activation and invasion, and to treat juvenile myositis.

**[0280] Statin Induced Myopathies** are associated with the long term use of statins which act via the inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR). Generally well-tolerated, these medications have been described as inducers of myotoxicity. More recently, there have been reports of patients in whom statin myopathies persist even after drug cessation, which are hypothesized to have an autoimmune cause. The benefits of statins are undisputed in reducing the risk of coronary heart disease and the progression of coronary atherosclerosis. Nevertheless, associated complications can be life-threatening. More than 38 million people in the U.S. are currently estimated to be taking statins and up to 7% (>2.6 million) of these are predicted to develop muscle symptoms with up to 0.5% (>190,000) of these potentially going on to develop life-threatening myopathies.

**[0281]** All the statins can cause muscle problems and the risk increases along with increases in their lipophilicity, cholesterol-lowering potency, and dosage. Cerivastatin in particular has been implicated as having a higher risk and it has been withdrawn from the US market. Of the remaining statins, atorvastatin and simvastatin have higher myotoxicity rates. Other nonstatin lipid-lowering agents such as niacin and fibrates also carry risks of muscle problems, particularly when combined with statins. While it is not possible to predict what patients will have statin-induced muscle problems, prior muscle problems may be a risk factor and should be considered when initiating statin treatment. A family history of myopathy is relevant if a patient might be a carrier of a genetic myopathy because it could be unmasked by the added stress of statin treatment. Other risk factors may include age over 80 years, low body weight, female sex, hypothyroidism, certain genetic defects and Asian descent, as well as concomitant use of certain medications, including calcium channel blockers, macrolide antibiotics, omeprazole, amiodarone, azole antifungals, histamine H<sub>2</sub> receptor antagonists, nefazodone, cyclosporin, HIV protease inhibitors, warfarin, and grapefruit juice.

**[0282]** The most common muscle symptom caused by statins is muscle pain or myalgia and it occurs in about 7% of statin users. The myalgia can be anywhere from mild to severe and is often worsened by muscle activity. If the symptom is tolerable and the indication for statin treatment strong, for example, in a patient with hypercholesterolemia and a recent myocardial infarction, continued statin treatment may be appropriate.

**[0283]** Baseline creatine kinase (CK) levels are not uniformly recommended before initiation of statin treatment by the organizations guiding statin treatment, but CK levels can provide very useful information if muscle symptoms later develop. Muscle weakness can also occur, and it is often fatigable in quality and combined with pain and elevated CK. Like most myopathies, the weakness is most pronounced proximally. Rare episodes of rhabdomyolysis have also occurred with statin therapy; these are far less frequent but can possibly be fatal. The changes that can be seen on muscle histology that are most typical of a statin myopathy are cytochrome oxidase negative fibers, increased lipid content, and ragged red fibers. Autoimmune necrotizing myopathy is a rare form of statin myopathy. In these patients, discontinuation of the statin drug



does not translate into recovery even after several months off the drug. Patients have a predominantly proximal, often painless weakness.

**[0284]** Diagnosis is based on the individual's medical history, results of a physical exam and tests of muscle strength, and blood samples that show elevated levels of various muscle enzymes and autoantibodies. Diagnostic tools include electromyography to record the electrical activity that controls muscles during contraction and at rest, ultrasound to look for muscle inflammation, and magnetic resonance imaging to reveal abnormal muscle and evaluate muscle disease. A muscle biopsy can be examined by microscopy for signs of chronic inflammation, muscle fiber death, vascular deformities, or the changes specific to the diagnosis of IBM. HRS-Fc conjugates may thus be used to reduce immune cell activation and invasion into damaged muscle, and to treat statin induced myopathies and rhabdomyolysis.

**[0285] Interstitial lung disease (ILD)** is a broad category of lung diseases that includes more than 130 disorders characterized by scarring (*i.e.*, "fibrosis") and/or inflammation of the lungs. ILD accounts for 15 percent of the cases seen by pulmonologists. Interstitial lung disease (ILD) can develop from a variety of sources, ranging from other diseases to environmental factors. Some of the known causes of ILD include: connective tissue or autoimmune disease, including for example, scleroderma/progressive systemic sclerosis, lupus (systemic lupus erythematosus), rheumatoid arthritis and polymyositis/dermatomyositis; and occupational and environmental exposures, including for example, exposure to dust and certain gases, poisons, chemotherapy and radiation therapy.

**[0286]** In ILD, the tissue in the lungs becomes inflamed and/or scarred. The interstitium of the lung includes the area in and around the small blood vessels and alveoli (air sacs) where the exchange of oxygen and carbon dioxide takes place. Inflammation and scarring of the interstitium disrupts this tissue and leads to a decrease in the ability of the lungs to extract oxygen from the air. HRS-Fc conjugates may thus be used to reduce immune cell activation and invasion into damaged lung, and to treat ILD.

**[0287]** The progression of ILD varies from disease to disease and from person to person. Because interstitial lung disease disrupts the transfer of oxygen and carbon dioxide in the lungs, its symptoms typically manifest as problems with breathing. The two most common symptoms of ILD are shortness of breath with exercise and a non-productive cough.

**[0288] Usher Syndrome** is the most common condition that affects both hearing and vision. The major symptoms of Usher syndrome are hearing loss and retinitis pigmentosa (RP). RP causes night-blindness and a loss of peripheral vision (side vision) through the progressive degeneration of the retina. As RP progresses, the field of vision narrows until only central vision remains. Many people with Usher syndrome also have severe balance problems. Approximately 3 to 6 percent of all children who are deaf and another 3 to 6 percent of children who are hard-of-hearing have Usher syndrome. In developed countries such as the United States, about four babies in every 100,000 births have Usher syndrome. Usher syndrome is inherited as an autosomal recessive trait. Several genetic loci have been associated with Usher syndrome including histidyl t-RNA synthetase (Puffenberger et al., (2012) PLoS ONE 7(1) e28936 doi: 10.1371 / journal. pone.0028936).

**[0289]** There are three clinical types of Usher syndrome: type 1, type 2, and type 3. In the United States, types 1 and 2 are the most common types. Together, they account for approximately 90 to 95 percent of all cases of children who have Usher syndrome.

**[0290]** Children with type 1 Usher syndrome are profoundly deaf at birth and have severe balance problems. Because of the balance problems associated with type 1 Usher syndrome, children with this disorder are slow to sit without support and typically don't walk independently before they are 18 months old. These children usually begin to develop vision problems in early childhood, almost always by the time they reach age 10. Vision problems most often begin with difficulty seeing at night, but tend to progress rapidly until the person is



completely blind.

**[0291]** Children with type 2 Usher syndrome are born with moderate to severe hearing loss and normal balance. Although the severity of hearing loss varies, most of these children can benefit from hearing aids and can communicate orally. The vision problems in type 2 Usher syndrome tend to progress more slowly than those in type 1, with the onset of RP often not apparent until the teens.

**[0292]** Children with type 3 Usher syndrome have normal hearing at birth. Although most children with the disorder have normal to near-normal balance, some may develop balance problems later on. Hearing and sight worsen over time, but the rate at which they decline can vary from person to person, even within the same family. A person with type 3 Usher syndrome may develop hearing loss by the teens, and he or she will usually require hearing aids by mid- to late adulthood. Night blindness usually begins sometime during puberty. Blind spots appear by the late teens to early adulthood, and, by mid-adulthood, the person is usually legally blind.

**[0293] Perrault syndrome (PS)** is characterized by the association of ovarian dysgenesis in females with sensorineural hearing impairment, and in some subjects, neurologic abnormalities, including progressive cerebellar ataxia and intellectual deficit. The exact prevalence for Perrault syndrome is unknown, and is probably underdiagnosed, particularly in males where hypogonadism is not a feature and the syndrome remains undetected. Mean age at diagnosis is 22 years following presentation with delayed puberty in females with sensorineural deafness. Hearing defects were noted in all but one of the reported cases (mean age at diagnosis of 8 years). The hearing loss is always sensorineural and bilateral but the severity is variable (mild to profound), even in affected patients from the same family. Ovarian dysgenesis has been reported in all female cases but no gonad defects are detected in males. Amenorrhea is generally primary but secondary amenorrhea has also been reported. Delayed growth (height below the third percentile) was reported in half the documented cases. The exact frequency of the neurological abnormalities is unknown, but nine females and two males (16-37 years old) lacking neurological abnormalities have been reported. Neurological signs are progressive and generally appear later in life, however, walking delay or early frequent falls have been noted in young PS patients. Common neurological signs are ataxia, dyspraxia, limited extraocular movements, and polyneuropathy. Some cases with scoliosis have also been reported. Transmission of PS is autosomal recessive and mutations in mitochondrial histidyl tRNA synthetase have recently been identified to cause the ovarian dysgenesis and sensorineural hearing loss associated with Perrault syndrome. (Pierce et al., PNAS USA. 108(16) 6543-6548, 2011).

**[0294] Muscular dystrophy** refers to a group of inherited disorders in which strength and muscle bulk gradually decline. All of the muscular dystrophies are marked by muscle weakness that is driven by a primary genetic defect in one or more muscle specific genes. Additionally muscular dystrophies, typically have a variable inflammatory component that drives muscular inflammation and ultimately enhances the degeneration of muscular tissues. Accordingly HRS-Fc conjugates may be used to reduce immune cell activation and invasion into damaged muscle, and to treat muscular dystrophies. At least nine types of muscular dystrophies are generally recognized. In some aspects of the present disclosure, the muscular dystrophy is selected from Duchenne muscular dystrophy, Becker muscular dystrophy, Emery-Dreifuss muscular dystrophy, Limb-girdle muscular dystrophy, facioscapulohumeral muscular dystrophy, myotonic dystrophy, oculopharyngeal muscular dystrophy, distal muscular dystrophy and congenital muscular dystrophy.

**[0295] Duchenne muscular dystrophy (DMD):** DMD affects young boys, causing progressive muscle weakness, usually beginning in the legs. It is the most severe form of muscular dystrophy. DMD occurs in about 1 in 3,500 male births, and affects approximately 8,000 boys and young men in the United States. A milder form occurs in very few female carriers.

**[0296]** DMD is caused by mutations in the gene encoding dystrophin, a subsarcolemmal protein functioning



within the dystrophin-associated glycoprotein complex (DGC) which prevent the production of functional protein. The amount of dystrophin correlates with the severity of the disease (i.e., the less dystrophin present, the more severe the phenotype). The DGC complex connects the intracellular cytoskeleton to the extracellular matrix. The DGC is concentrated at the Z-lines of the sarcomere and confers the transmission of force across the muscle fibre. Disruption of this link results in membrane instability, which eventually leads to sarcolemmal ruptures. Influx of extracellular calcium alters molecular processes like muscle contraction and activates proteolytic activity. Affected muscle fibres become necrotic or apoptotic, and release mitogenic chemoattractants, which initiate inflammatory processes. Cycles of degeneration and regeneration eventually lead to irreversible muscle wasting and replacement by fibrotic and adipose tissue.

**[0297]** A boy with Duchenne muscular dystrophy usually begins to show symptoms as a preschooler. The legs are affected first, making walking difficult and causing balance problems. Most patients walk three to six months later than expected and have difficulty running. Contractures (permanent muscle tightening) usually begin by age five or six, most severely in the calf muscles. Frequent falls and broken bones are common beginning at this age. Climbing stairs and rising unaided may become impossible by age nine or ten, and most boys use a wheelchair for mobility by the age of 12. Weakening of the trunk muscles around this age often leads to scoliosis (a side-to-side spine curvature) and kyphosis (a front-to back curvature).

**[0298]** One of the most serious weakness of DMD is weakness of the diaphragm, the sheet of muscles at the top of the abdomen that perform the main work of breathing and coughing. Diaphragm weakness leads to reduced energy and stamina, and increased lung infection because of the inability to cough effectively. Young men with DMD can live into their twenties and beyond, provided they have mechanical ventilation assistance and good respiratory hygiene.

**[0299]** In some embodiments of the present disclosure, a subject having DMD is characterized by one or more of the following: a positive Gower's sign, reflecting impairment of the lower extremity muscles; high levels of creatine kinase (CPK-MM) in the blood; genetic errors in the Xp21 gene; or reduced levels of absence of dystrophin, for instance, as measured by muscle biopsy.

**[0300]** HRS-Fc conjugates may be used in the treatment of DMD, either alone or in combination with other therapies, such as antisense oligonucleotides (e.g., exon-skipping therapies such as Eteplirsen), corticosteroids, beta2-agonists, physical therapy, respiratory support, stem cell therapies, and gene replacement therapies. In some embodiments of the present disclosure, administration of HRS-Fc conjugates leads to statistically significant improvements in the 6-minute walk test.

**[0301] Becker muscular dystrophy (BMD):** BMD affects older boys and young men, following a milder course than DMD. BMD occurs in about 1 in 30,000 male births. Becker muscular dystrophy is a less severe variant of Duchenne muscular dystrophy and is caused by the production of a truncated, but partially functional form of dystrophin.

**[0302]** The symptoms of BMD usually appear in late childhood to early adulthood. Though the progression of symptoms may parallel that of DMD, the symptoms are usually milder and the course more variable. Scoliosis may occur, but is usually milder and progresses more slowly. Heart muscle disease (cardiomyopathy), occurs more commonly in BMD. Problems may include irregular heartbeats (arrhythmias) and congestive heart failure. Symptoms may include fatigue, shortness of breath, chest pain, and dizziness. Respiratory weakness also occurs, and may lead to the need for mechanical ventilation. HRS-Fc conjugates may be used in the treatment of BMD, either alone or in combination with other therapies.

**[0303] Emery-Dreifuss muscular dystrophy (EDMD):** EDMD affects young boys, causing contractures and weakness in the calves, weakness in the shoulders and upper arms, and problems in the way electrical impulses travel through the heart to make it beat (heart conduction defects). There are three subtypes of



Emery-Dreifuss Muscular Dystrophy, distinguishable by their pattern of inheritance: X-Linked, autosomal dominant and autosomal recessive. The X-linked form is the most common. Each type varies in prevalence and symptoms. The disease is caused by mutations in the LMNA gene, or more commonly, the EMD gene. Both genes encode for protein components of the nuclear envelope.

**[0304]** EDMD usually begins in early childhood, often with contractures preceding muscle weakness. Weakness affects the shoulder and upper arm originally, along with the calf muscles, leading to foot-drop. Most men with EDMD survive into middle age, although a defect in the heart's rhythm (heart block) may be fatal if not treated with a pacemaker. HRS-Fc conjugates may be used in the treatment of EDMD, either alone or in combination with other therapies.

**[0305] Limb-girdle muscular dystrophy (LGMD):** LGMD begins in late childhood to early adulthood and affects both men and women, causing weakness in the muscles around the hips and shoulders. It is the most variable of the muscular dystrophies, and there are several different forms of the disease now recognized. Many people with suspected LGMD have probably been misdiagnosed in the past, and therefore the prevalence of the disease is difficult to estimate. The number of people affected in the United States may be in the low thousands.

**[0306]** While there are at least a half-dozen genes that cause the various types of LGMD, two major clinical forms of LGMD are usually recognized. A severe childhood form is similar in appearance to DMD, but is inherited as an autosomal recessive trait.

**[0307]** Limb Girdle Muscular Dystrophy type 2B (LGMD2B) is caused by the loss of function mutations in the dysferlin gene. Dysferlin is primarily expressed in skeletal and cardiac muscle, but also in monocytes, macrophages, and other tissues where it is localized to cytoplasmic vesicles and the cell membrane. Dysferlin appears to be involved in membrane fusion and trafficking, as well as repair processes. LGMD2B is a late onset (teens/young adults) muscle disease that is characterized by progressive symmetrical muscle weakness, and notably aggressive immune/inflammatory pathology. Muscle biopsies typically show marked inflammatory cell infiltration, consisting primarily of macrophages/macrophage activation markers (HLA-DR, HLA-ABC, CD86), CD8<sup>+</sup> cytotoxic T cells, and CD4<sup>+</sup> T cells, together with muscle fiber degeneration/regeneration. Accordingly, HRS-Fc conjugates may be used to reduce immune cell activation and invasion into damaged muscle, and to treat Limb Girdle Muscular Dystrophy.

**[0308]** Symptoms of adult-onset LGMD usually appear in a person's teens or twenties, and are marked by progressive weakness and wasting of the muscles closest to the trunk. Contractures may occur, and the ability to walk is usually lost about 20 years after onset. Some people with LGMD develop respiratory weakness that requires use of a ventilator. Lifespan may be somewhat shortened.

**(Autosomal dominant forms usually occur later in life and progress relatively slowly.)**

**[0309] Facioscapulohumeral muscular dystrophy (FSH):** FSH, also known as Landouzy-Dejerine disease, begins in late childhood to early adulthood and affects both men and women, causing weakness in the muscles of the face, shoulders, and upper arms. The hips and legs may also be affected. FSH occurs in about 1 out of every 20,000 people, and affects approximately 13,000 people in the United States.

**[0310]** FSH varies in its severity and age of onset, even among members of the same family. Symptoms most commonly begin in the teens or early twenties, though infant or childhood onset is possible. Symptoms tend to be more severe in those with earlier onset. The disease is named for the regions of the body most severely affected by the disease: muscles of the face (facio-), shoulders (scapulo-), and upper arms (humeral). Hips and legs may be affected as well. Children with FSH often develop partial or complete deafness.



**[0311]** Two defects are needed for FSHD, the first is the deletion of D4Z4 repeats and the second is a "toxic gain of function" of the DUX4 gene. The first symptom noticed is often difficulty lifting objects above the shoulders. The weakness may be greater on one side than the other. Shoulder weakness also causes the shoulder blades to jut backward, called scapular winging. FSHD is associated with inflammatory invasion is specific muscle groups, and accordingly HRS-Fc conjugates may thus be used to reduce immune cell activation and invasion into damaged muscles, and to treat FSHD.

**[0312] Myotonic dystrophy:** Myotonic dystrophy, also known as Steinert's disease, affects both men and women, causing generalized weakness first seen in the face, feet, and hands. It is accompanied by the inability to relax the affected muscles (myotonia). Symptoms may begin from birth through adulthood. Myotonic muscular dystrophy type 1 (DM1) is the most common form of muscular dystrophy, affecting more than 30,000 people in the United States. It results from the expansion of a short (CTG) repeat in the DNA sequence of the DMPK (myotonic dystrophy protein kinase) gene. Myotonic muscular dystrophy type 2 (DM2) is much rarer and is a result of the expansion of the CCTG repeat in the ZNF9 (zinc finger protein 9) gene.

**[0313]** Symptoms of myotonic dystrophy include facial weakness and a slack jaw, drooping eyelids (ptosis), and muscle wasting in the forearms and calves. A person with this dystrophy has difficulty relaxing his grasp, especially if the object is cold. Myotonic dystrophy affects heart muscle, causing arrhythmias and heart block, and the muscles of the digestive system, leading to motility disorders and constipation. Other body systems are affected as well: Myotonic dystrophy may cause cataracts, retinal degeneration, low IQ, frontal balding, skin disorders, testicular atrophy, sleep apnea, and insulin resistance. An increased need or desire for sleep is common, as is diminished motivation. Severe disability affects most people with this type of dystrophy within 20 years of onset, although most do not require a wheelchair even late in life. HRS-Fc conjugates can thus be used to treat myotonic dystrophy, for instance, by reducing inflammation associated with muscle tissue, including skeletal muscle (e.g., quadricep muscles) and/or heart tissue, among other tissues.

**[0314] Oculopharyngeal muscular dystrophy (OPMD):** OPMD affects adults of both sexes, causing weakness in the eye muscles and throat. It is most common among French Canadian families in Quebec, and in Spanish-American families in the southwestern United States.

**[0315]** OPMD usually begins in a person's thirties or forties, with weakness in the muscles controlling the eyes and throat. Symptoms include drooping eyelids, difficulty swallowing (dysphagia), and weakness progresses to other muscles of the face, neck, and occasionally the upper limbs. Swallowing difficulty may cause aspiration, or the introduction of food or saliva into the airways. Pneumonia may follow. HRS-Fc conjugates can thus be used to treat OPMD, for instance, by reducing inflammation associated with muscle tissue.

**[0316] Distal muscular dystrophy (DD):** DD begins in middle age or later, causing weakness in the muscles of the feet and hands. It is most common in Sweden, and rare in other parts of the world. DD usually begins in the twenties or thirties, with weakness in the hands, forearms, and lower legs. Difficulty with fine movements such as typing or fastening buttons may be the first symptoms. Symptoms progress slowly, and the disease usually does not affect life span. HRS-Fc conjugates can thus be used to treat DD, by reducing inflammation associated with muscle tissue inflammation.

**[0317] Congenital muscular dystrophy (CMD):** CMD is present from birth, results in generalized weakness, and usually progresses slowly. A subtype, called Fukuyama CMD, also involves mental retardation. Both are rare; Fukuyama CMD is more common in Japan.

**[0318]** CMD is marked by severe muscle weakness from birth, with infants displaying "floppiness" and very little voluntary movement. Nonetheless, a child with CMD may learn to walk, either with or without some assistive device, and live into young adulthood or beyond. In contrast, children with Fukuyama CMD are rarely



able to walk, and have severe mental retardation. Most children with this type of CMD die in childhood. As with the other muscular dystrophies, HRS-Fc conjugates can thus be used to treat CMD, for example, by reducing inflammation associated with muscle tissue inflammation.

**[0319] Cachexia:** Cachexia (or wasting syndrome) is typically characterized by loss of weight, muscle atrophy, fatigue, weakness, and significant loss of appetite in someone who is not actively trying to lose weight. The formal definition of cachexia is the loss of body mass that cannot be reversed nutritionally. Even if the affected patient consumes more calories, lean body mass is lost, indicating the existence of a primary pathology.

**[0320]** Cachexia is experienced by patients with cancer, AIDS, chronic obstructive lung disease, multiple sclerosis, congestive heart failure, tuberculosis, familial amyloid polyneuropathy, mercury poisoning (acrodynia), and hormonal deficiency, among other disease.

**[0321]** Cachexia can also be a sign of various underlying disorders, including cancer, metabolic acidosis (i.e., decreased protein synthesis and increased protein catabolism), certain infectious diseases (e.g., tuberculosis, AIDS), chronic pancreatitis, autoimmune disorders, or addiction to amphetamines. Cachexia physically weakens patients to a state of immobility stemming from loss of appetite, asthenia, and anemia, and response to standard treatment is usually poor.

**[0322]** About 50% of all cancer patients suffer from cachexia. Those with upper gastrointestinal and pancreatic cancers have the highest frequency of developing a cachexic symptom. In addition to increasing morbidity and mortality, aggravating the side effects of chemotherapy, and reducing quality of life, cachexia is considered the immediate cause of death of a large proportion of cancer patients, ranging from 22% to 40% of the patients. Symptoms of cancer cachexia include progressive weight loss and depletion of host reserves of adipose tissue and skeletal muscle. Traditional treatment approaches include the use of appetite stimulants, 5-HT<sub>3</sub> antagonists, nutrient supplementation, and COX-2 inhibitors.

**[0323]** Although the pathogenesis of cachexia is poorly understood, multiple biologic pathways are expected to be involved, including pro-inflammatory cytokines such as TNF- $\alpha$ , neuroendocrine hormones, IGF-1, and tumor-specific factors such as proteolysis-inducing factor.

**[0324]** HRS-Fc conjugates may thus be used to treat cachexia and any of its related, underlying, or secondary disorders or complications. HRS-Fc conjugates can be used alone or in combination with other therapies, such as dietary supplementation with a combination of high protein, leucine and fish oil, antioxidants, progestogen (megestrol acetate, medroxyprogesterone acetate), and anticyclooxygenase-2 drugs, appetite stimulants, and 5-HT<sub>3</sub> antagonists, among others.

**[0325] Rhabdomyolysis:** Rhabdomyolysis is the breakdown of muscle fibers in skeletal muscle tissue. The breakdown products are released into the bloodstream, and certain some of these products, such as myoglobin, are harmful to the kidneys and may lead to kidney failure.

**[0326]** Symptoms include muscle pain, vomiting, confusion, coma, or abnormal heart rate and rhythm and their severity usually depends on the extent of muscle damage and whether kidney failure develops. Damage to the kidneys may cause decreased or absent urine production, usually about 12 to 24 hours after the initial muscle damage. Swelling of the damaged muscle can cause compartment syndrome, or compression of surrounding tissues, such as nerves and blood vessels, in the same fascial compartment, and lead to blood loss and damage to (e.g., loss of function) the affected body parts. Symptoms of this complication include pain or reduced sensation in the affected limb. Other complications include disseminated intravascular coagulation (DIC), a severe disruption in blood clotting that may lead to uncontrollable bleeding.

**[0327]** The initial muscle damage may be caused, for instance, by physical factors (e.g. crush injury,



strenuous exercise), altered blood supply (e.g., arterial thrombosis, embolism), altered metabolism (e.g., hyperglycemic hyperosmolar state, hyper- and hyponatremia, hypokalemia, hypocalcemia, hypophosphatemia, ketoacidosis, hypothyroidism), altered body temperature (hyperthermia, hypothermia), medications and toxins (e.g., statins, anti-psychotic medications, neuromuscular blocking agents, diuretics, heavy metals, hemlock, insect or snake venoms), drug abuse (e.g., alcohol, amphetamine, cocaine, heroin, ketamine, LDS, MDMA), infections (e.g., Coxsackie virus, influenza A virus, influenza B virus, Epstein-Barr virus, primary HIV infection, *Plasmodium falciparum*, herpes viruses, *Legionella pneumophila*, salmonella), and autoimmune muscle damage (e.g., polymyositis, dermatomyositis). Also, certain hereditary conditions increase the risk of rhabdomyolysis, including glycolysis and glycogenolysis defects (e.g., McArdle's disease, phosphofructokinase deficiency, glycogen storage diseases VIII, IX, X and XI), lipid metabolism defects (e.g., carnitine palmitoyltransferase I and II deficiency, deficiency of subtypes of acyl CoA dehydrogenase (e.g., LCAD, SCAD, MCAD, VLCAD, 3-hydroxyacyl-coenzyme A dehydrogenase deficiency), thiolase deficiency), mitochondrial myopathies (e.g., deficiency of succinate dehydrogenase, cytochrome c oxidase and coenzyme Q10), and others such as glucose-6-phosphate dehydrogenase deficiency, myoadenylate deaminase deficiency, and muscular dystrophies.

**[0328]** Rhabdomyolysis is usually diagnosed with blood tests and urinalysis, and can be indicated by abnormally raised or increasing creatinine and urea levels, falling urine output, or reddish-brown discoloration of the urine. The primary treatments include intravenous fluids, dialysis, and hemofiltration.

**[0329]** HRS-Fc conjugates may thus be used to treat rhabdomyolysis and any of its related, secondary, or underlying disorders or complications. HRS-Fc conjugates can be used alone or in combination with other therapies, including those meant to treat shock and preserve kidney function. Exemplary therapies include administration of intravenous fluids, usually isotonic saline (0.9% weight per volume sodium chloride solution) and renal replacement therapies (RRT) such as hemodialysis, continuous hemofiltration and peritoneal dialysis.

**[0330]** More generally, the HRS-Fc conjugates described herein can reduce an inflammatory response, such as by reducing the activation, differentiation, migration, or infiltration of immune cells into selected tissues, increasing the production of anti-inflammatory cytokines, or reducing the production or activity of pro-inflammatory cytokines, among other mechanisms. Moreover, certain of the present methods, by blocking the binding, action, or production of anti-histidyl-tRNA synthetase antibodies or auto-reactive T cells, have utility to treat a broad range of auto-immune and inflammatory diseases and disorders associated with anti-histidyl-tRNA synthetase antibodies, other auto-antibodies, as well as other causes of histidyl-tRNA synthetase insufficiency.

#### ***Pharmaceutical Formulations, Administration, and Kits***

**[0331]** Embodiments of the present disclosure include compositions comprising HRS-Fc conjugate polypeptides formulated in pharmaceutically-acceptable or physiologically-acceptable solutions for administration to a cell, subject, or an animal, either alone, or in combination with one or more other modalities of therapy. It will also be understood that, if desired, the compositions of the disclosure may be administered in combination with other agents as well, for example, other proteins or polypeptides or various pharmaceutically-active agents. There is virtually no limit to other components that may also be included in the compositions, provided that the additional agents do not adversely affect the modulatory or other effects desired to be achieved.

**[0332]** For pharmaceutical production, HRS-Fc conjugate therapeutic compositions will typically be substantially endotoxin free. Endotoxins are toxins associated with certain bacteria, typically gram-negative bacteria, although endotoxins may be found in gram-positive bacteria, such as *Listeria monocytogenes*. The



most prevalent endotoxins are lipopolysaccharides (LPS) or lipo-oligosaccharides (LOS) found in the outer membrane of various Gram-negative bacteria, and which represent a central pathogenic feature in the ability of these bacteria to cause disease. Small amounts of endotoxin in humans may produce fever, a lowering of the blood pressure, and activation of inflammation and coagulation, among other adverse physiological effects.

**[0333]** Endotoxins can be detected using routine techniques known in the art. For example, the Limulus Amoebocyte Lysate assay, which utilizes blood from the horseshoe crab, is a very sensitive assay for detecting presence of endotoxin. In this test, very low levels of LPS can cause detectable coagulation of the limulus lysate due a powerful enzymatic cascade that amplifies this reaction. Endotoxins can also be quantitated by enzyme-linked immunosorbent assay (ELISA).

**[0334]** To be substantially endotoxin free, endotoxin levels may be less than about 0.001, 0.005, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.08, 0.09, 0.1, 0.5, 1.0, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, or 10 EU/mg of protein. Typically, 1 ng lipopolysaccharide (LPS) corresponds to about 1-10 EU.

**[0335]** In certain embodiments of the present disclosure, as noted herein, the HRS-Fc conjugate compositions have an endotoxin content of less than about 10 EU / mg of HRS-Fc conjugate, or less than about 5 EU / mg of HRS-Fc conjugate, less than about 3 EU / mg of HRS-Fc conjugate, or less than about 1 EU / mg of HRS-Fc conjugate or less than about 0.1 EU/ mg of HRS-Fc conjugate, or less than about 0.01EU / mg of HRS-Fc conjugate. In certain embodiments of the present disclosure, as noted above, the HRS-Fc conjugate pharmaceutical compositions are about 95% endotoxin free, preferably about 99% endotoxin free, and more preferably about 99.99% endotoxin free on wt/wt protein basis.

**[0336]** Pharmaceutical compositions comprising a therapeutic dose of a HRS-Fc conjugate polypeptide include all homologues, orthologs, and naturally-occurring isoforms of histidyl-tRNA synthetase.

**[0337]** In some embodiments of the present disclosure such pharmaceutical compositions may comprise a histidine buffer, which may be present in any of the pharmaceutical compositions within the range of about 1 mM to about 100 mM. In some embodiments of the present disclosure, the histidine buffer may be present at a concentration of about 1 mM, about 2 mM, about 3 mM, about 4 mM, about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, about 10 mM, about 11 mM, about 12 mM, about 13 mM, about 14 mM, about 15 mM, about 16 mM, about 17 mM, about 18 mM, about 19 mM, about 20 mM, about 25 mM, about 30 mM, about 40 mM, about 50 mM, about 60 mM, about 70 mM, about 80 mM, about 90 mM, or about 100 mM, including all integers and ranges in between said concentrations.

**[0338]** In one aspect of the present disclosure such compositions may comprises HRS-Fc conjugate polypeptides that are substantially monodisperse, meaning that the HRS-Fc conjugate compositions exist primarily (*i.e.*, at least about 90%, or greater) in one apparent molecular weight form when assessed for example, by size exclusion chromatography, dynamic light scattering, or analytical ultracentrifugation.

**[0339]** In another aspect of the present disclosure, such compositions have a purity (on a protein basis) of at least about 90%, or in some aspects of the present disclosure at least about 95% purity, or in some embodiments of the present disclosure, at least 98% purity. Purity may be determined via any routine analytical method as known in the art.

**[0340]** In another aspect of the present disclosure, such compositions have a high molecular weight aggregate content of less than about 10%, compared to the total amount of protein present, or in some embodiments of the present disclosure such compositions have a high molecular weight aggregate content of less than about 5%, or in some aspects of the present disclosure such compositions have a high molecular weight aggregate content of less than about 3%, or in some embodiments of the present disclosure a high



molecular weight aggregate content of less than about 1%. High molecular weight aggregate content may be determined via a variety of analytical techniques including for example, by size exclusion chromatography, dynamic light scattering, or analytical ultracentrifugation.

**[0341]** Pharmaceutical compositions may include pharmaceutically acceptable salts of a HRS-Fc conjugate polypeptide. For a review on suitable salts, see Handbook of Pharmaceutical Salts: Properties, Selection, and Use by Stahl and Wermuth (Wiley-VCH, 2002). Suitable base salts are formed from bases which form non-toxic salts. Representative examples include the aluminum, arginine, benzathine, calcium, choline, diethylamine, diolamine, glycine, lysine, magnesium, meglumine, olamine, potassium, sodium, tromethamine, and zinc salts. Hemisalts of acids and bases may also be formed, e.g., hemisulphate and hemicalcium salts. Compositions to be used in the disclosure suitable for parenteral administration may comprise sterile aqueous solutions and / or suspensions of the pharmaceutically active ingredients preferably made isotonic with the blood of the recipient, generally using sodium chloride, glycerin, glucose, mannitol, sorbitol, and the like. Organic acids suitable for forming pharmaceutically acceptable acid addition salts include, by way of example and not limitation, acetic acid, trifluoroacetic acid, propionic acid, hexanoic acid, cyclopentanepropionic acid, glycolic acid, oxalic acid, pyruvic acid, lactic acid, malonic acid, succinic acid, malic acid, maleic acid, fumaric acid, tartaric acid, citric acid, palmitic acid, benzoic acid, 3-(4-hydroxybenzoyl) benzoic acid, cinnamic acid, mandelic acid, alkylsulfonic acids (e.g., methanesulfonic acid, ethanesulfonic acid, 1,2-ethane-disulfonic acid, 2-hydroxyethanesulfonic acid), arylsulfonic acids (e.g., benzenesulfonic acid, 4-chlorobenzenesulfonic acid, 2-naphthalenesulfonic acid, 4-toluenesulfonic acid, camphorsulfonic acid), 4-methylbicyclo(2.2.2)-oct-2-ene-1-carboxylic acid, glucoheptonic acid, 3-phenylpropionic acid, trimethylacetic acid, tertiary butylacetic acid, lauryl sulfuric acid, gluconic acid, glutamic acid, hydroxynaphthoic acid, salicylic acid, stearic acid, muconic acid, and the like.

**[0342]** In particular embodiments of the present disclosure, the carrier may include water. In some embodiments of the present disclosure, the carrier may be an aqueous solution of saline, for example, water containing physiological concentrations of sodium, potassium, calcium, magnesium, and chloride at a physiological pH. In some embodiments of the present disclosure, the carrier may be water and the formulation may further include NaCl. In some embodiments of the present disclosure, the formulation may be isotonic. In some embodiments of the present disclosure, the formulation may be hypotonic. In other embodiments of the present disclosure, the formulation may be hypertonic. In some embodiments of the present disclosure, the formulation may be isosmotic. In some embodiments of the present disclosure, the formulation is substantially free of polymers (e.g., gel-forming polymers, polymeric viscosity-enhancing agents). In some embodiments of the present disclosure, the formulation is substantially free of viscosity-increasing agents (e.g., carboxymethylcellulose, polyanionic polymers). In some embodiments of the present disclosure, the formulation is substantially free of gel-forming polymers. In some embodiments of the present disclosure, the viscosity of the formulation is about the same as the viscosity of a saline solution containing the same concentration of a HRS-Fc conjugate (or a pharmaceutically acceptable salt thereof).

**[0343]** In the pharmaceutical compositions of the disclosure, formulation of pharmaceutically-acceptable excipients and carrier solutions is well-known to those of skill in the art, as is the development of suitable dosing and treatment regimens for using the particular compositions described herein in a variety of treatment regimens, including e.g., oral, parenteral, intravenous, intranasal, and intramuscular administration and formulation.

**[0344]** In certain embodiments of the present disclosure, the HRS-Fc conjugate polypeptide have a solubility that is desirable for the particular mode of administration, such intravenous administration. Examples of desirable solubility's include at least about 1 mg/ml, at least about 10 mg/ml, at least about 25 mg/ml, and at least about 50 mg/ml.

**[0345]** In certain applications, the pharmaceutical compositions disclosed herein may be delivered via oral



administration to a subject. As such, these compositions may be formulated with an inert diluent or with an edible carrier, or they may be enclosed in hard- or soft-shell gelatin capsule, or they may be compressed into tablets, or they may be incorporated directly with the food of the diet.

**[0346]** Pharmaceutical compositions suitable for the delivery of HRS-Fc conjugates and methods for their preparation will be readily apparent to those skilled in the art. Such compositions and methods for their preparation may be found, for example, in Remington's Pharmaceutical Sciences, 19th Edition (Mack Publishing Company, 1995).

**[0347]** Administration of a therapeutic dose of a HRS-Fc conjugate may be by any suitable method known in the medicinal arts, including for example, oral, intranasal, parenteral administration include intravitreal, subconjunctival, sub-tenon, retrobulbar, suprachoroidal intravenous, intra-arterial, intraperitoneal, intrathecal, intraventricular, intraurethral, intrasternal, intracranial, intramuscular, intrasynovial, intraocular, topical and subcutaneous. Suitable devices for parenteral administration include needle (including microneedle) injectors, needle-free injectors, and infusion techniques.

**[0348]** Parenteral formulations are typically aqueous solutions which may contain excipients such as salts, carbohydrates, and buffering agents (preferably to a pH of from 3 to 9), but, for some applications, they may be more suitably formulated as a sterile non-aqueous solution or as a dried form to be used in conjunction with a suitable vehicle such as sterile, pyrogen-free water. The preparation of parenteral formulations under sterile conditions, for instance, by lyophilization, may readily be accomplished using standard pharmaceutical techniques well-known to those skilled in the art.

**[0349]** Formulations for parenteral administration may be formulated to be immediate and / or sustained release. Sustained release compositions include delayed, modified, pulsed, controlled, targeted and programmed release. Thus a HRS-Fc conjugate may be formulated as a suspension or as a solid, semi-solid, or thixotropic liquid for administration as an implanted depot providing sustained release of HRS-Fc conjugates. Examples of such formulations include without limitation, drug-coated stents and semi-solids and suspensions comprising drug-loaded poly(DL-lactic-co-glycolic)acid (PGLA), poly(DL-lactide-co-glycolide) (PLG) or poly(lactide) (PLA) lamellar vesicles or microparticles, hydrogels (Hoffman AS: Ann. N.Y. Acad. Sci. 944: 62-73 (2001)), poly-amino acid nanoparticles systems, such as the Medusa system developed by Flamel Technologies Inc., non aqueous gel systems such as Atrigel developed by Atrix, Inc., and SABER (Sucrose Acetate Isobutyrate Extended Release) developed by Durect Corporation, and lipid-based systems such as DepoFoam developed by SkyePharma.

**[0350]** Solutions of the active compounds as free base or pharmacologically acceptable salts may be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions may also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

**[0351]** The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions (U.S. Pat. No. 5,466,468). In all cases the form 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 should 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 (e.g., glycerol, propylene glycol, liquid polyethylene glycol, and the like), suitable mixtures thereof, and/or vegetable oils. Proper fluidity may be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be facilitated by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for



example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

**[0352]** For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, a sterile aqueous medium that can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage may be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion (see, e.g., Remington's Pharmaceutical Sciences, 15th Edition, pp. 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, and the general safety and purity standards as required by FDA Office of Biologics standards.

**[0353]** Sterile injectable solutions can be prepared by incorporating the active compounds in the required amount in the appropriate solvent with the various other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the 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 techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

**[0354]** The compositions disclosed herein may be formulated in a neutral or salt form. Pharmaceutically-acceptable salts, include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms such as injectable solutions, drug-release capsules, and the like.

**[0355]** As used herein, "carrier" includes any and all solvents, dispersion media, vehicles, coatings, diluents, antibacterial and antifungal agents, isotonic and absorption delaying agents, buffers, carrier solutions, suspensions, colloids, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

**[0356]** The phrase "pharmaceutically-acceptable" refers to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a human. The preparation of an aqueous composition that contains a protein as an active ingredient is well understood in the art. Typically, such compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection can also be prepared. The preparation can also be emulsified.

**[0357]** HRS-Fc conjugate polypeptides for use in the present disclosure may also be administered topically, (intra)dermally, or transdermally to the skin, mucosa, or surface of the eye, either alone or in combination with



one or more antihistamines, one or more antibiotics, one or more antifungal agents, one or more beta blockers, one or more anti-inflammatory agents, one or more antineoplastic agents, one or more immunosuppressive agents, one or more antiviral agents, one or more antioxidant agents, or other active agents. Formulations for topical and ocular administration may be formulated to be immediate and/or modified release. Modified release formulations include delayed, sustained, pulsed, controlled, targeted and programmed release.

**[0358]** Typical formulations for this purpose include gels, hydrogels, lotions, solutions, eye drops, creams, ointments, dusting powders, dressings, foams, films, skin patches, wafers, implants, sponges, fibers, bandages, and microemulsions. Liposomes may also be used. Typical carriers include alcohol, water, mineral oil, liquid petrolatum, white petrolatum, glycerin, polyethylene glycol, and propylene glycol. Penetration enhancers may be incorporated-see, e.g., Finnin and Morgan: J. Pharm. Sci. 88(10): 955-958, (1999). Other means of topical administration include delivery by electroporation, iontophoresis, phonophoresis, sonophoresis, and microneedle or needle-free injection (e.g., the systems sold under the trademarks POWDERJECT™, BIOJECT™).

**[0359]** Examples of antihistamines include, but are not limited to, loradatine, hydroxyzine, diphenhydramine, chlorpheniramine, brompheniramine, cyproheptadine, terfenadine, clemastine, triprolidine, carbinoxamine, diphenylpyraline, phenindamine, azatadine, tripeleminamine, dexchlorpheniramine, dexbrompheniramine, methdilazine, and trimprazine doxylamine, pheniramine, pyrilamine, chlorcyclizine, thonzylamine, and derivatives thereof.

**[0360]** Examples of antibiotics include, but are not limited to, aminoglycosides (e.g., amikacin, apramycin, arbekacin, bambarmycins, butirosin, dibekacin, dihydrostreptomycin, fortimicin(s), gentamicin, isepamicin, kanamycin, micromomicin, neomycin, neomycin undecylenate, netilmicin, paromomycin, ribostamycin, sisomicin, spectinomycin, streptomycin, tobramycin, trospectomycin), amphenicols (e.g., azidamfenicol, chloramphenicol, florfenicol, thiamphenicol), ansamycins (e.g., rifamide, rifampin, rifamycin sv, rifapentine, rifaximin), lactams (e.g., carbacephems (e.g., loracarbef), carbapenems (e.g., biapenem, imipenem, meropenem, panipenem), cephalosporins (e.g., cefaclor, cefadroxil, cefamandole, cefatrizine, cefazedone, cefazolin, cefcapene pivoxil, cefclidin, cefdinir, cefditoren, cefepime, cefetamet, cefixime, cefmenoxime, cefodizime, cefonicid, cefoperazone, ceforanide, cefotaxime, cefotiam, cefozopran, cefpimizole, cefpiramide, cefpirome, cefpodoxime proxetil, cefprozil, cefroxadine, cefsulodin, ceftazidime, cefteram, ceftazole, ceftibuten, ceftizoxime, ceftriaxone, cefuroxime, cefuzonam, cephradine sodium, cephalixin, cephaloglycin, cephaloridine, cephalosporin, cephalothin, cephalixin sodium, cephradine, pivcefalexin), cephamycins (e.g., cefbuperazone, cefmetazole, cefminox, cefotetan, cefoxitin), monobactams (e.g., aztreonam, carumonam, tigemonam), oxacephems, flomoxef, moxalactam), penicillins (e.g., amdinocillin, amdinocillin pivoxil, amoxicillin, ampicillin, apalcillin, aspoxicillin, azidocillin, azlocillin, bacampicillin, benzylpenicillinic acid, benzylpenicillin sodium, carbenicillin, carindacillin, clometocillin, cloxacillin, cyclacillin, dicloxacillin, epicillin, fenbenicillin, floxacillin, hetacillin, lenampicillin, metampicillin, methicillin sodium, mezlocillin, nafcillin sodium, oxacillin, penamecillin, penethamate hydriodide, penicillin g benethamine, penicillin g benzathine, penicillin g benzhydrylamine, penicillin g calcium, penicillin g hydrabamine, penicillin g potassium, penicillin g procaine, penicillin n, penicillin o, penicillin v, penicillin v benzathine, penicillin v hydrabamine, penimepicycline, phenethicillin potassium, piperacillin, pivampicillin, propicillin, quinacillin, sulbenicillin, sultamicillin, talampicillin, temocillin, ticarcillin), other (e.g., ritipenem), lincosamides (e.g., clindamycin, lincomycin), macrolides (e.g., azithromycin, carbomycin, clarithromycin, dirithromycin, erythromycin, erythromycin acistrate, erythromycin estolate, erythromycin glucoheptonate, erythromycin lactobionate, erythromycin propionate, erythromycin stearate, josamycin, leucomycins, midecamycins, miokamycin, oleandomycin, primycin, rokitamycin, rosaramicin, roxithromycin, spiramycin, troleandomycin), polypeptides (e.g., amphomycin, bacitracin, capreomycin, colistin, enduracidin, enviomycin, fusafungine, gramicidin s, gramicidin(s), mikamycin, polymyxin, pristnamycin, ristocetin, teicoplanin, thiostrepton, tuberactinomycin, tyrocidine, tyrothricin, vancomycin, viomycin, virginiamycin, zinc bacitracin), tetracyclines (e.g., apicycline, chlortetracycline,



clomocycline, demeclocycline, doxycycline, guamecycline, lymecycline, meclocycline, methacycline, minocycline, oxytetracycline, penimepicycline, pipacycline, rolitetracycline, sancycline, tetracycline), and others (e.g., cycloserine, mupirocin, tuberin). 2.4-Diaminopyrimidines (e.g., brodimoprim, tetroxoprim, trimethoprim), nitrofurans (e.g., furaltadone, furazolium chloride, nifuradene, nifuratel, nifurfoline, nifurpirinol, nifurprazine, nifurtinol, nitrofurantoin), quinolones and analogs (e.g., cinoxacin, ciprofloxacin, clinafloxacin, difloxacin, enoxacin, fleroxacin, flumequine, grepafloxacin, lomefloxacin, miloxacin, nadifloxacin, nalidixic acid, norfloxacin, ofloxacin, oxolinic acid, pazufloxacin, pefloxacin, pipemidic acid, piromidic acid, rosoxacin, rufloxacin, sparfloxacin, temafloxacin, tosufloxacin, trovafloxacin), sulfonamides (e.g., acetyl sulfamethoxypyrazine, benzylsulfamide, chloramine-b, chloramine-t, dichloramine t, n<sup>2</sup>-formylsulfisomidine, mafenide, 4'-(methylsulfamoyl)sulfanilamide, noprilsulfamide, phthalylsulfacetamide, phthalylsulfathiazole, salazosulfadimidine, succinylsulfathiazole, sulfabenzamide, sulfacetamide, sulfachlorpyridazine, sulfachrysoidine, sulfacytine, sulfadiazine, sulfadicramide, sulfadimethoxine, sulfadoxine, sulfaethidole, sulfaguanidine, sulfaguanol, sulfalene, sulfaloxic acid, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethomidine, sulfamethoxazole, sulfamethoxypyridazine, sulfametrole, sulfamidochrysoidine, sulfamoxole, sulfanilamide, 4-sulfanilamidosalicylic acid, n<sup>4</sup>-sulfanilylsulfanilamide, sulfanilylurea, n-sulfanilyl-3,4-xylamide, sulfanitran, sulfaperine, sulfaphenazole, sulfaproxyline, sulfapyrazine, sulfapyridine, sulfasomizole, sulfasymazine, sulfathiazole, sulfathiourea, sulfatolamide, sulfisomidine, sulfisoxazole) sulfones (e.g., acedapsone, acediasulfone, acetosulfone sodium, dapsone, diathymosulfone, glucosulfone sodium, solasulfone, succisulfone, sulfanilic acid, p-sulfanilylbenzylamine, sulfoxone sodium, thiazolsulfone), and others (e.g., clofoctol, hexedine, methenamine, methenamine anhydromethylene-citrate, methenamine hippurate, methenamine mandelate, methenamine sulfosalicylate, nitroxoline, taurolidine, xibornol).

**[0361]** Examples of antifungal agents include, but are not limited to Polyenes (e.g., amphotericin b, candicidin, dermostatin, filipin, fungichromin, hachimycin, hamycin, lucensomycin, mepartricin, natamycin, nystatin, pecilocin, perimycin), others (e.g., azaserine, griseofulvin, oligomycins, neomycin undecylenate, pyrrolnitrin, siccanin, tubercidin, viridin), Allylamines (e.g., butenafine, naftifine, terbinafine), imidazoles (e.g., bifonazole, butoconazole, chlordanol, chlormidazole, cloconazole, clotrimazole, econazole, enilconazole, fenticonazole, flutrimazole, isoconazole, ketoconazole, lanconazole, miconazole, omoconazole, oxiconazole nitrate, sertaconazole, sulconazole, tioconazole), thiocarbamates (e.g., tolclate, tolindate, tolnaftate), triazoles (e.g., fluconazole, itraconazole, saperconazole, terconazole) others (e.g., acrisorcin, amorolfine, biphenamine, bromosalicylchloranilide, buclosamide, calcium propionate, chlorphenesin, ciclopirox, cloxyquin, coparaffinate, diamthazole dihydrochloride, exalamide, flucytosine, halethazole, hexetidine, loflucarban, nifuratel, potassium iodide, propionic acid, pyrithione, salicylanilide, sodium propionate, sulbentine, tenonitroazole, triacetin, ujothion, undecylenic acid, zinc propionate).

**[0362]** Examples of beta blockers include but are not limited to acebutolol, atenolol, labetalol, metoprolol, propranolol, timolol, and derivatives thereof.

**[0363]** Examples of antineoplastic agents include, but are not limited to antibiotics and analogs (e.g., aclacinomycins, actinomycin f<sub>1</sub>, anthramycin, azaserine, bleomycins, cactinomycin, carubicin, carzinophilin, chromomycins, dactinomycin, daunorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, idarubicin, menogaril, mitomycins, mycophenolic acid, nogalamycin, olivomycines, peplomycin, pirarubicin, plicamycin, porfiromycin, puromycin, streptonigrin, streptozocin, tubercidin, zinostatin, zorubicin), antimetabolites (e.g. folic acid analogs (e.g., denopterin, edatrexate, methotrexate, piritrexim, pteropterin, Tomudex®, trimetrexate), purine analogs (e.g., cladribine, fludarabine, 6-mercaptopurine, thiamiprine, thioguanine), pyrimidine analogs (e.g., ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, doxifluridine, emitefur, enocitabine, floxuridine, fluorouracil, gemcitabine, tagafur).

**[0364]** Examples of anti-inflammatory agents include but are not limited to steroidal anti-inflammatory agents and non-steroidal anti-inflammatory agents. Exemplary steroidal anti-inflammatory agents include



acetoxypregnenolone, alclometasone, algestone, amcinonide, beclomethasone, betamethasone, budesonide, chloroprednisone, clobetasol, clobetasone, clocortolone, cloprednol, corticosterone, cortisone, cortivazol, deflazacort, desonide, desoximetasone, dexamethasone, diflorasone, diflucortolone, difluprednate, enoxolone, fluazacort, flucoronide, flumethasone, flunisolide, fluocinolone acetonide, fluocinonide, fluocortin butyl, fluocortolone, fluorometholone, fluperolone acetate, fluprednidene acetate, fluprednisolone, flurandrenolide, fluticasone propionate, formocortal, halcinonide, halobetasol propionate, halometasone, halopredone acetate, hydrocortamate, hydrocortisone, loteprednol etabonate, mazipredone, medrysone, meprednisone, methylprednisolone, mometasone furoate, paramethasone, prednicarbate, prednisolone, prednisolone 25-diethylamino-acetate, prednisolone sodium phosphate, prednisone, prednival, prednylidene, rimexolone, tixocortol, triamcinolone, triamcinolone acetonide, triamcinolone benetonide, and triamcinolone hexacetonide.

**[0365]** Exemplary non-steroidal anti-inflammatory agents include aminoarylcarboxylic acid derivatives (e.g., enfenamic acid, etofenamate, flufenamic acid, isonixin, meclofenamic acid, mefenamic acid, niflumic acid, talniflumate, terofenamate, tolfenamic acid), arylacetic acid derivatives (e.g., aceclofenac, acemetacin, alclofenac, amfenac, amtolmetin guacil, bromfenac, bufexamac, cinmetacin, clopirac, diclofenac sodium, etodolac, felbinac, fenclozic acid, fentiazac, glucametacin, ibufenac, indomethacin, isofezolac, isoxepac, lonazolac, metiazinic acid, mofezolac, oxametacine, pirazolac, proglumetacin, sulindac, tiaramide, tolmetin, tropesin, zomepirac), arylbutyric acid derivatives (e.g., bumadizon, butibufen, fenbufen, xenbucin), arylcarboxylic acids (e.g., clidanac, ketorolac, tinoridine), arylpropionic acid derivatives (e.g., alminoprofen, benoxaprofen, bermoprofen, bucloxic acid, carprofen, fenoprofen, flunoxaprofen, flurbiprofen, ibuprofen, ibuproxam, indoprofen, ketoprofen, loxoprofen, naproxen, oxaprozin, piketoprolen, pirprofen, pranoprofen, protizinic acid, suprofen, tiaprofenic acid, ximoprofen, zaltoprofen), pyrazoles (e.g., difenamizole, epirizole), pyrazolones (e.g., apazone, benzpiperylon, feprazone, mofebutazone, morazone, oxyphenbutazone, phenylbutazone, pipebuzone, propyphenazone, ramifenazone, suxibuzone, thiazolinobutazone), salicylic acid derivatives (e.g., acetaminosalol, aspirin, benorylate, bromosaligenin, calcium acetylsalicylate, diflunisal, etersalate, fendosal, gentisic acid, glycol salicylate, imidazole salicylate, lysine acetylsalicylate, mesalamine, morpholine salicylate, 1-naphthyl salicylate, olsalazine, parsalmide, phenyl acetylsalicylate, phenyl salicylate, salacetamide, salicylamide o-acetic acid, salicylsulfuric acid, salsalate, sulfasalazine), thiazinecarboxamides (e.g., ampiroxicam, droxicam, isoxicam, lornoxicam, piroxicam, tenoxicam),  $\epsilon$ -acetamidocaproic acid, s-adenosylmethionine, 3-amino-4-hydroxybutyric acid, amixetrine, bendazac, benzydamine, bucolome, difenpiramide, ditazol, emorfazone, fepradinol, guaiazulene, nabumetone, nimesulide, oxaceprol, paranyline, perisoxal, proquazone, superoxide dismutase, tenidap, and zileuton.

**[0366]** Examples of antiviral agents include interferon gamma, zidovudine, amantadine hydrochloride, ribavirin, acyclovir, valciclovir, dideoxycytidine, phosphonoformic acid, ganciclovir, and derivatives thereof

**[0367]** Examples of antioxidant agents include ascorbate, alpha-tocopherol, mannitol, reduced glutathione, various carotenoids, cysteine, uric acid, taurine, tyrosine, superoxide dismutase, lutein, zeaxanthin, cryotpxanthin, astaxanthin, lycopene, N-acetyl-cysteine, carnosine, gamma-glutamylcysteine, quercitin, lactoferrin, dihydrolipoic acid, citrate, Ginkgo Biloba extract, tea catechins, bilberry extract, vitamins E or esters of vitamin E, retinyl palmitate, and derivatives thereof. Other therapeutic agents include squalamine, carbonic anhydrase inhibitors, alpha-2 adrenergic receptor agonists, antiparasitics, antifungals, and derivatives thereof.

**[0368]** The exact dose of each component administered will, of course, differ depending on the specific components prescribed, on the subject being treated, on the severity of the disease, for example, severity of the inflammatory reaction, on the manner of administration and on the judgment of the prescribing physician. Thus, because of patient-to-patient variability, the dosages given above are a guideline and the physician may adjust doses of the compounds to achieve the treatment that the physician considers appropriate.

**[0369]** As will be understood by the skilled artisan, for HRS-Fc conjugate formulations where the carrier includes a gel-forming polymer, in certain formulations the inclusion of salt(s), in particular saline solution, is



contraindicated as inclusion of salt may either cause the solution to gel prior to topical administration, as with certain in situ gel-forming polymers (e.g., gellan gel), or the inclusion of salts may inhibit the gelling properties of the gel-forming polymer. The skilled artisan will be able to select appropriate combinations based on the desired properties of the formulation and characteristics of gel-forming polymers known in the art.

**[0370]** Suitable aqueous saline solutions will be understood by those of skill in the art and may include, for example, solutions at a pH of from about pH 4.5 to about pH 8.0. In further variations of aqueous solutions (where water is included in the carrier), the pH of the formulation is between any of about 6 and about 8.0; between about 6 and about 7.5; between about 6 and about 7.0; between about 6.2 and about 8; between about 6.2 and about 7.5; between about 7 and about 8; between about 6.2 and about 7.2; between about 5.0 and about 8.0; between about 5 and about 7.5; between about 5.5 and about 8.0; between about 6.1 and about 7.7; between about 6.2 and about 7.6; between about 7.3 and about 7.4; about 6.0; about 7.1; about 6.2; about 7.3; about 6.4; about 6.5; about 6.6; about 6.7; about 6.8; about 6.9; about 7.0; about 7.1; about 7.2; about 7.3; about 7.4; about 7.5; about 7.6; or about 8.0. In some variations, the HRS-Fc conjugate formulation has a pH of about 6.0 to about 7.0. In some variations, the formulation has a pH of about 7.4. In particular variations, the formulation has a pH of about 6.2 to about 7.5.

**[0371]** In certain embodiments of the present disclosure the concentration of the salt (e.g., NaCl) will be, for example, from about 0% to about 0.9% (w/v). For example, the concentration of salt may be from about 0.01 to about 0.9%, from about 0.02% to about 0.9%, from about 0.03% to about 9%, from about 0.05% to about 0.9% from about 0.07% to about 0.9%, from about 0.09% to about 0.9%, from about 0.1% to about 0.9% from about 0.2% to about 0.9%, from about 0.3% to about 0.9%, from about 0.4% to about 0.9% from about 0.5% to about 0.9%, from about 0.6% to about 0.9%, from about 0.7% to about 0.9%, from about 0.8% to about 0.9%, about 0.9%, about 0%, about 0.05%, about 0.01%, about 0.09%, about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, or about 0.8%. In certain embodiments of the present disclosure, the aqueous saline solution will be isotonic (e.g., NaCl concentration of about 0.9% NaCl (w/v)). In certain embodiments of the present disclosure, the aqueous solution will contain a NaCl concentration of about 0.5%, about 0.7%, about 0.8%, about 0.85, or about 0.75%. As will be appreciated the skilled artisan, depending on the concentrations of other components, for example where the HRS-Fc conjugates are present as salts of, the concentration of NaCl or other salt needed to achieve an formulation suitable for administration may vary.

**[0372]** In some embodiments of the present disclosure, where the formulation is substantially free of viscosity-increasing agents, the formulation may be substantially free of viscosity-increasing agents such as, but not limited to polyanionic polymers, water soluble cellulose derivatives (e.g., hypromellose (also known as HPMC, hydroxypropylmethyl cellulose, and hydroxypropylcellulose), hydroxyethylcellulose, carboxymethylcellulose, etc.), polyvinyl alcohol, polyvinyl pyrrolidone, chondroitin sulfate, hyaluronic acid, soluble starches, etc. In some variations, the formulation does not incorporate a hydrogel or other retention agent (e.g., such as those disclosed in U.S. Pat. Pub. No. 2005/0255144), e.g., where the hydrogel may include hydrogels incorporating homopolymers; copolymers (e.g., tetrapolymers of hydroxymethylmethacrylate, ethylene glycol, dimethylmethacrylate, and methacrylic acid), copolymers of trimethylene carbonate and polyglycolic acid, polyglactin 910, glyconate, poly-p-dioxanone, polyglycolic acid, polyglycolic acid felt, poly-4-hydroxybutyrate, a combination of poly(L-lactide) and poly(L-lactide-co-glycolide), glycol methacrylate, poly-DL-lactide, or Primacryl); composites of oxidized regenerated cellulose, polypropylene, and polydioxanone or a composite of polypropylene and polygelcaprone; etc. In some variations, the formulations do not include one or more of polyvinyl alcohol, hydroxypropyl methylcellulose, polyethylene glycol 400 castor oil emulsion, carboxymethylcellulose sodium, propylene glycol, hydroxypropyl guar, carboxymethylcellulose sodium, white petrolatum, mineral oil, dextran 70, glycerin, hypromellose, flaxseed oil, fish oils, omega 3 and omega 6 fatty acids, lutein, or primrose oil. In some variations, the formulations do not include one or more of the carriers described in U.S. Pat. No. 4,888,354, e.g., such as one or more of oleic acid, ethanol, isopropanol, glycerol monooleate, glycerol dioleate, methyl laurate, propylene glycol, propanol or dimethyl sulfoxide. In some



variations, the formulations are substantially free of glycerol dioleate and isopropanol.

**[0373]** In particular embodiments of the present disclosure, the gel-forming polymer may be, for example, a polysaccharide. In certain embodiments of the present disclosure, the polysaccharide is gellan gum. Gellan gum refers to a heteropolysaccharide elaborated by the bacterium *Pseudomonas elodea*, though the name "gellan gum" is more commonly used in the field. Gellan gum, in particular the formulation GELRITE® is described in detail in U.S. Pat. No. 4,861,760, in particular in its use in formulation of timolol. GELRITE®, a low acetyl clarified grade of gellan gum, is commercially available from Merck & Co (Rahway, N.J.) and gellan gum can be commercially obtained from, among others CPKelco (Atlanta, Ga.). The preparation of polysaccharides such as gellan gum is described in, for example, U.S. Pat. Nos. 4,326,053 and 4,326,052.

**[0374]** In certain embodiments of the present disclosure, the gel-forming polymer is present at a concentration of from about 0.03% to about 2% (w/v). In some embodiments of the present disclosure, the gel-forming polymer is present at a concentration from about 0.03% to about 1.75%; from about 0.03% to about 1.5%, from about 0.03% to about 1.25%, from about 0.03% to about 1%, from about 0.03% to about 0.9%, from about 0.03% to about 0.8%, from about 0.03% to about 0.7%, from about 0.03% to about 0.6%, from about 0.03% to about 0.5%, from about 0.05% to about 2%, from about 0.05% to about 1.75%; from about 0.05% to about 1.5%, from about 0.05% to about 1.25%, from about 0.05% to about 1%, from about 0.05% to about 0.9%, from about 0.05% to about 0.8%, from about 0.05% to about 0.7%, from about 0.05% to about 0.6%, from about 0.05% to about 0.5%, from about 0.1% to about 2%, from about 0.1% to about 1.75%; from about 0.1% to about 1.5%, from about 0.1% to about 1.25%, from about 0.1% to about 1%, from about 0.1% to about 0.9%, from about 0.1% to about 0.8%, from about 0.1% to about 0.7%, from about 0.1% to about 0.6%, from about 0.1% to about 0.5%, from about 0.2% to about 2%, from about 0.2% to about 1.75%; from about 0.2% to about 1.5%, from about 0.2% to about 1.25%, from about 0.2% to about 1%, from about 0.2% to about 0.9%, from about 0.2% to about 0.8%, from about 0.2% to about 0.7%, from about 0.2% to, about 0.6%, from about 0.2% to about 0.5%, or from about 0.5% to about 1.5%. In some embodiments of the present disclosure, the concentration of gel-forming polymer is about 0.1%, about 0.2%, about 0.4%, about 0.6%, about 0.8%, about 1%.

**[0375]** In particular embodiments of the present disclosure, the gel-forming polymer is gellan gum at a concentration of from about 0.05% to about 2% (w/v), from about 0.1% to about 2% (w/v), from about 0.1% to about 1% (w/v), from about 0.05% to about 1% (w/v) or from about 0.1% to about 0.6% (w/v). In some embodiments of the present disclosure, the concentration of gellan gum is about 0.1%, about 0.2%, about 0.4%, about 0.6%, about 0.8%, about 1%.

**[0376]** In some embodiments of the present disclosure of the formulations, the formulation may include additional components such as one or more preservatives, one or more surfactants, or one or more pharmaceutical agents. In particular embodiments of the present disclosure, the formulation may include additional components such as one or more preservatives, one or more surfactants, one or more tonicity agents, one or more buffering agents, one or more chelating agents, one or more viscosity-increasing agents, one or more salts, or one or more pharmaceutical agents. In certain of these embodiments of the present disclosure, the formulation may include (in addition to a HRS-Fc conjugate (or a pharmaceutically acceptable salt thereof) and carrier): one or more preservatives, one or more buffering agents (e.g., one, two, three, etc.), one or more chelating agents, and one or more salts. In some embodiments of the present disclosure, the formulation may include (in addition to a HRS-Fc conjugate (or a pharmaceutically acceptable salt thereof) and carrier): one or more preservatives, one or more tonicity agents, one or more buffering agents, one or more chelating agents, and one or more viscosity-increasing agents.

**[0377]** In some embodiments of the present disclosure, the viscosity of the formulation is about the same as the viscosity of a saline solution containing the same concentration of a HRS-Fc conjugate (or a pharmaceutically acceptable salt thereof). In some embodiments of the present disclosure, the formulation is



substantially free of gel-forming polymers. In certain embodiments of the present disclosure, where the carrier is water, the formulation may additionally include one or more chelating agents (e.g., EDTA disodium (EDTA), one or more preservatives (e.g., benzalkonium chloride, benzethonium chloride, chlorhexidine, chlorobutanol, methylparaben, phenylethyl alcohol, propylparaben, thimerosal, phenylmercuric nitrate, phenylmercuric borate, phenylmercuric acetate, or combinations of two or more of the foregoing), salt (e.g., NaCl) and one or more buffering agents (e.g., one or more phosphate buffers (e.g., dibasic sodium phosphate, monobasic sodium phosphate, combinations thereof, etc.), citrate buffers, maleate buffers, borate buffers, and combination of two or more of the foregoing.).

**[0378]** In particular embodiments of the present disclosure, the chelating agent is EDTA disodium, the preservative is benzalkonium chloride, the salt is NaCl, and the buffering agents are dibasic sodium phosphate and monobasic sodium phosphate. In certain of these embodiments of the present disclosure, the formulation is substantially free of polymer. In some embodiments of the present disclosure, the formulation is substantially free of substantially viscosity-increasing agent(s) (e.g., carboxymethylcellulose, polyanionic polymers, etc.). In some embodiments of the present disclosure, the viscosity of the formulation is about the same as the viscosity of a saline solution containing the same concentration of a HRS-Fc conjugate (or a pharmaceutically acceptable salt thereof). In some of these embodiments of the present disclosure, the concentration of a HRS-Fc conjugate (or a pharmaceutically acceptable salt thereof) is from about 0.02% to about 3%, from about 0.02% to about 2%, from about 0.02% to about 1% (w/v). In certain embodiments of the present disclosure, the concentration of a HRS-Fc conjugate (or a pharmaceutically acceptable salt thereof), is about 0.01%, about 0.02%, about 0.03%, about 0.05%, about 0.07%, about 0.1%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.8% or about 1% (w/v).

**[0379]** In certain embodiments of the present disclosure, where the carrier includes water, a viscosity-increasing agent may also be included in the formulation. The skilled artisan will be familiar with viscosity-increasing agents that are suitable (e.g., water-soluble cellulose derivatives (e.g., hypromellose (also known as HPMC, hydroxypropylmethyl cellulose, and hydroxypropylcellulose), hydroxyethylcellulose, carboxmethylcellulose, etc.), polyvinyl alcohol, polyvinyl pyrrolidone, chondroitin sulfate, hyaluronic acid, and soluble starches. It is intended that when viscosity-increasing agents are used, they are not included in high enough concentrations such that the formulation would form a gel prior to or after administration (e.g., wherein the concentration of the viscosity-increasing agent is not sufficient to induce gel formation).

**[0380]** While exact concentrations of viscosity-increasing agents will depend upon the selection and concentration of other components in the formulation as well as the particular viscosity-increasing agent(s) selected, in general, viscosity-increasing agents may be present in a concentration such that the viscosity of the resulting solution is less than about 1000 centipoise. In certain embodiments of the present disclosure, the viscosity of the formulation is less than about 900, less than about 800, less than about 700, less than about 600, less than about 500, less than about 400, less than about 300, less than about 200, less than about 150, less than about 100, less than about 50 centipoise. In some embodiments of the present disclosure, the viscosity of the formulation is about 200, about 150, about 100, about 50 centipoise. In particular embodiments of the present disclosure, the viscosity is less than about 200 centipoise. In others, less than about 120 centipoise or less than about 100 centipoise. In some embodiments of the present disclosure, the viscosity is about 100 centipoise. In others about 50 centipoise. In still other embodiments of the present disclosure the viscosity is about 200 centipoise. Methods for measuring viscosity are well known to the skilled artisan. For example, as described in United States Pharmacopoeia 29 (Chapter 911) Viscosity, page 2785. As is well known to the skilled artisan, formulations commonly considered "gels" will have viscosity significantly greater than 1000 centipoise, for example, greater than about 2000 centipoise, greater than about 5000 centipoise.

**[0381]** In some embodiments of the present disclosure, including (but not limited to) where the use of salts is contraindicated as described above, the formulation may further include one or more tonicity agents. As used



herein, the term "tonicity agent" and its cognates refers to agents that adjust the tonicity of the formulation, but are not salts (e.g., not NaCl), which, as will be appreciated by the skill artisan in view of the teaching provided herein, are contraindicated for some formulations due to the presence of certain of the gel-forming polymers or viscosity-increasing agents. These agents may be used to prepare formulations that are isotonic or near isotonic (e.g., somewhat hyper- or hypo-isotonic; e.g., within about  $\pm 20\%$ , about  $\pm 15\%$ , about  $\pm 10\%$ , about  $\pm 5\%$  of being isotonic). Tonicity agent(s) may also be used in formulations where the use of salts is not contraindicated.

**[0382]** Tonicity agents that may be used to adjust the tonicity of formulation the formulations described herein and are known to the skilled artisan and can be selected based on the teaching provided herein. For example, tonicity agents include polyols (e.g., sugar alcohols (e.g., mannitol, etc.), trihydroxy alcohols (e.g., glycerin, etc.), propylene glycol or polyethylene glycol, etc.), or combinations of two or more polyols. Likewise, the concentration of the tonicity agent(s) will depend upon the identity and concentrations of the other components in the formulation and can be readily determined by the skilled artisan in view of the teaching provided herein.

**[0383]** In certain embodiments of the present disclosure, the tonicity agent is glycerin or mannitol. In some embodiments of the present disclosure, the tonicity agent is glycerin. In other embodiments of the present disclosure it is, mannitol. In still others a combination of mannitol and glycerin may be used. Exemplary concentrations of tonicity agents include, for example from about 0.001 to about 3%. In some embodiments of the present disclosure, the concentration of the tonicity agent (e.g., mannitol or glycerin) is, for example, about 0.001% to about 2.7%, about 0.001% to about 2.5%, about 0.001% to about 2%, about 0.001% to about 1.5%, about 0.001% to about 1%, about 0.01% to about 3%, about 0.01% to about 2.7%, about 0.01% to about 2.5%, about 0.01% to about 2%, about 0.01% to about 1.5%, about 0.01% to about 1%, about 0.1% to about 3%, about 0.1% to about 2.7%, about 0.1% to about 2.5%, about 0.1% to about 2%, about 0.1% to about 1.5%, about 0.1% to about 1%, about 0.01% about 1% to about 3%; about 1% to about 2.5%; about 1% to about 2%; about 1% to about 1.8%; about 1% to about 1.5%; or about 0.001%, about 0.01%, about 0.05%, about 0.08%, about 0.1%, about 0.2%, about 0.5%, about 0.8%, about 1%, about 1.5%, about 1.8%, about 2%, about 2.2%, about 2.5%, about 2.8%, or about 3% (w/v). In certain embodiments of the present disclosure, the tonicity agent is mannitol. In some of these embodiments of the present disclosure, the carrier includes a gel-forming agent (e.g., gellan gum).

**[0384]** In some embodiments of the present disclosure, the tonicity agent is mannitol. In certain of these embodiments of the present disclosure, the carrier includes a viscosity-increasing agent (e.g., water soluble cellulose derivatives (e.g., hypromellose), polyvinyl alcohol, polyvinyl pyrrolidone, chondroitin sulfate, hyaluronic acid, or soluble starches).

**[0385]** In some embodiments of the present disclosure, the formulation may additionally include a preservative (e.g., benzalkonium chloride, benzethonium chloride, chlorhexidine, chlorobutanol, methylparaben, Phenylethyl alcohol, propylparaben, thimerosal, phenylmercuric nitrate, phenylmercuric borate, or phenylmercuric acetate, peroxides), or a combination of two or more of the foregoing preservatives. In certain embodiments of the present disclosure, the preservative is benzalkonium chloride.

**[0386]** As will be appreciated by the skilled artisan, preservatives may be present in concentrations of from about 0.001% to about 0.7% (w/v). In particular embodiments of the present disclosure, the preservative(s) may be present in a concentration of from about 0.001% to about 0.5% (w/v); from about 0.001% to about 0.05% (w/v), from about 0.001% to about 0.02% (w/v), from about 0.001% to about 0.015% (w/v), from about 0.001% to about 0.005% (w/v), from about 0.01% to about 0.02%, from about 0.002% to about 0.01%, from about 0.015% to about 0.05%, less than about  $<0.5\%$ , from about 0.005% to about 0.01%, from about 0.001% to about 0.15%, from about 0.002% to about 0.004%, from about 0.001% to about 0.002%. In some embodiments of the present disclosure the concentration of the preservative may be, for example, about



0.001%, about 0.005%, about 0.01%, about 0.02%, about 0.03%, about 0.05%, about 0.1%, about 0.2%, about 0.5%, or about 0.7% (w/v). Typical concentrations (w/v) for various commonly used preservatives are listed in **Table C** below.

| TABLE C                |                                       |
|------------------------|---------------------------------------|
| Preservative           | Approximate Concentration Range (w/v) |
| Benzalkonium chloride  | 0.01-0.02%                            |
| Benzethonium chloride  | 0.01-0.02%                            |
| Chlorhexidine          | 0.002-0.01%                           |
| Chlorobutanol          | <0.5%                                 |
| Methylparaben          | 0.015-0.05%                           |
| Phenylethyl alcohol    | <0.5%                                 |
| Propylparaben          | 0.005-0.01%                           |
| Thimerosal             | 0.001-0.15%                           |
| Phenylmercuric nitrate | 0.002-0.004%                          |
| Phenylmercuric borate  | 0.002-0.004                           |
| Phenylmercuric acetate | 0.001-0.002                           |

[0387] In certain embodiments of the present disclosure, the formulation may additionally include a surfactant, or combinations of two or more surfactants. In particular embodiments of the present disclosure, the formulation is substantially free of surfactant. As used herein, the term "substantially free" is intended to refer to levels of a particular component that are undetectable using routine detection methods and protocols known to the skilled artisan. For example, HPLC (including chiral HPLC, chiral HPLC/MS, LC/MS/MS etc.), thin layer chromatography, mass spectrometry, polarimetry measurements, gas-chromatography-mass spectrometry, or others.

[0388] In particular embodiments of the present disclosure, the formulation may further include a chelating agent (e.g., EDTA disodium (EDTA) (e.g., EDTA disodium (dihydrate), etc.) citrates, etc.). In some embodiments of the present disclosure, a combination of chelating agents may be present. As will be appreciated by those of skill in the field, chelating agents can be used to hinder degradation of the formulation components and thereby increase the shelf life of formulations. As will be appreciated by the skilled artisan, use of EDTA in combination with gellan gum formulation may be contraindicated as the EDTA can cause gel formation prior to administration of the gellan gum formulation.

[0389] Typical concentrations for chelating agents are from about 0.005% to 0.1% (w/v). For example, from about 0.005% to about 0.09%, from about 0.005% to about 0.08%, from about 0.005% to about 0.07%, from about 0.005%, to about 0.06%, from about 0.005% to about 0.05%, from about 0.005 to about 0.04%, from about 0.005% to about 0.03%, from about 0.01% to about 0.1%, from about 0.01% to about 0.09%, from about 0.01% to about 0.08%, from about 0.01% to about 0.07%, from about 0.01% to about 0.06%, from about 0.01% to about 0.05%, from about 0.01% to about 0.04%, etc. In certain embodiments of the present disclosure, the concentration of chelating agent(s) is about 0.005%, about 0.01%, about 0.02%, about 0.03%, about 0.05%, about 0.06%, about 0.07%, about 0.08%, about 0.09%, or about 0.1%.

[0390] In particular embodiments of the present disclosure, the chelating agent is EDTA disodium. In certain embodiments of the present disclosure, the chelating agent is EDTA disodium (dihydrate). In some of these embodiments of the present disclosure, the EDTA disodium dihydrate is present at a concentration of about 0.01% (w/v).



**[0391]** In some embodiments of the present disclosure, the formulation may additionally include one or more buffering agents (e.g., phosphate buffer(s) (e.g., sodium phosphate buffers (e.g., dibasic sodium phosphate, monobasic sodium phosphate, etc.), citrate buffers, maleate buffers, borate buffers, etc.). As will be appreciated by the skilled artisan, the one or more buffering agent(s) should be selected in combination with the other components of a given formulation to achieve a pH suitable for use (e.g., pH of about 4.5 to about 8).

**[0392]** In certain embodiments of the present disclosure, the buffering agent is a phosphate buffer or combination of two or more phosphate buffers. In certain embodiments of the present disclosure, the buffering agents are dibasic sodium phosphate and monobasic sodium phosphate.

**[0393]** Typical concentrations for buffering agent(s) for example, phosphate buffering agent(s) may be from about 0.005 molar to 0.1 molar. In some embodiments of the present disclosure, the buffering agent(s) may be at a concentration of about 0.01 to about 0.1, from about 0.01 to about 0.08, from about 0.01 to about 0.05, from about 0.01 to about 0.04, from about 0.02 to about 0.1, from about 0.02 to about 0.08, from about 0.02 to about 0.06, from about 0.02 to about 0.05, from about 0.02 to about 0.04 molar, etc. In particular embodiments of the present disclosure, there are two buffering agents. Exemplary buffering agents include a combination of dibasic sodium phosphate (e.g., dibasic sodium phosphate.7H<sub>2</sub>O) and monobasic sodium phosphate (e.g., monobasic sodium phosphate anhydrous). In some embodiments of the present disclosure, the concentration of the buffering agent(s) is about 0.005 molar, about 0.01 molar, about 0.02 molar, about 0.03 molar, about 0.04 molar, about 0.05 molar, about 0.06 molar, about 0.07 molar, or about 0.1 molar.

**[0394]** An additional aspect of the present disclosure of the disclosure includes use of the formulations as described herein in the manufacture of a medicament. Particularly, the manufacture of a medicament for use in the treatment and/or prevention of conditions as described herein. Further, the formulations, variously described herein, are also intended for use in the manufacture of a medicament for use in treatment and/or prevention of the conditions and, in accordance with the methods, described herein, unless otherwise noted.

**[0395]** Methods of formulation are well known in the art and are disclosed, for example, in Remington: The Science and Practice of Pharmacy, Mack Publishing Company, Easton, Pa., 19th Edition (1995). The compositions and agents provided herein may be administered according to the methods of the present disclosure in any therapeutically effective dosing regimen. The dosage amount and frequency are selected to create an effective level of the agent without harmful effects. The effective amount of a compound of the present disclosure will depend on the route of administration, the type of warm-blooded animal being treated, and the physical characteristics of the specific warm-blooded animal under consideration. These factors and their relationship to determining this amount are well known to skilled practitioners in the medical arts. This amount and the method of administration can be tailored to achieve optimal efficacy but will depend on such factors as weight, diet, concurrent medication and other factors which those skilled in the medical arts will recognize.

**[0396]** In certain embodiments of the present disclosure, the pharmaceutical compositions may be delivered by intranasal sprays, inhalation, and/or other aerosol delivery vehicles. Methods for delivering genes, polynucleotides, and peptide compositions directly to the lungs via nasal aerosol sprays have been described e.g., in U.S. Pat. No. 5,756,353 and U.S. Pat. No. 5,804,212. Likewise, the delivery of drugs using intranasal microparticle resins (Takenaga *et al.*, 1998) and lysophosphatidyl-glycerol compounds (U.S. Pat. No. 5,725,871) are also well-known in the pharmaceutical arts. Likewise, transmucosal drug delivery in the form of a polytetrafluoroethylene support matrix is described in U.S. Pat. No. 5,780,045.

**[0397]** In certain embodiments of the present disclosure, the delivery may occur by use of liposomes, nanocapsules, microparticles, microspheres, lipid particles, vesicles, and the like, for the introduction of the compositions of the present disclosure into suitable host cells. In particular, the compositions of the present



disclosure may be formulated for delivery either encapsulated in a lipid particle, a liposome, a vesicle, a nanosphere, a nanoparticle or the like. The formulation and use of such delivery vehicles can be carried out using known and conventional techniques.

**[0398]** In certain embodiments of the present disclosure, the agents provided herein may be attached to a pharmaceutically acceptable solid substrate, including biocompatible and biodegradable substrates such as polymers and matrices. Examples of such solid substrates include, without limitation, polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and  $\gamma$ -ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as poly(lactic-co-glycolic acid) (PLGA) and the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), poly-D-(-)-3-hydroxybutyric acid, collagen, metal, hydroxyapatite, bioglass, aluminate, bioceramic materials, and purified proteins.

**[0399]** In one particular embodiment of the present disclosure, the solid substrate comprises Atrigel™ (QLT, Inc., Vancouver, B.C.). The Atrigel® drug delivery system consists of biodegradable polymers dissolved in biocompatible carriers. Pharmaceuticals may be blended into this liquid delivery system at the time of manufacturing or, depending upon the product, may be added later by the physician at the time of use. When the liquid product is injected into the subcutaneous space through a small gauge needle or placed into accessible tissue sites through a cannula, water in the tissue fluids causes the polymer to precipitate and trap the drug in a solid implant. The drug encapsulated within the implant is then released in a controlled manner as the polymer matrix biodegrades with time.

**[0400]** In particular embodiments of the present disclosure, the amount of a HRS-Fc conjugate composition administered will generally range from a dosage of from about 0.1 to about 100 mg/kg, and typically from about 0.1 to 10 or 20 mg/kg where administered orally, subcutaneously, or intravenously. In particular embodiments of the present disclosure, a dosage is about 1 mg/kg, about 3 mg/kg, about 5 mg/kg, about 7.5 mg/kg, or about 10 mg/kg. In certain embodiments of the present disclosure, a composition is administered in a single dosage of 0.1 to 10 mg/kg or 0.5 to 5 mg/kg. In some embodiments of the present disclosure, a composition is administered in a dosage of 0.1 to 50 mg/kg, 0.5 to 20 mg/kg, or 5 to 20 mg/kg.

**[0401]** For humans, the daily dosage used may range from, about 0.1 mg/kg to 0.5 mg/kg, about 1 mg/kg to 5 mg/kg, about 5 mg/kg to 10 mg/kg, about 10 mg/kg to 20 mg/kg, about 20 mg/kg to 30 mg/kg, about 30 mg/kg to 50 mg/kg, and about 50 mg/kg to 100 mg/kg / 24 hours.

**[0402]** For HRS-Fc conjugates with longer half lives, the human dosage used may range, for example, from about 0.1 mg/kg/week to 0.5 mg/kg/week, about 1 mg/kg/week to 5 mg/kg/week, about 5 mg/kg/week to 10 mg/kg/week, about 10 mg/kg/week to 20 mg/kg/week, about 20 mg/kg/week to 30 mg/kg/week, about 30 mg/kg/week to 50 mg/kg/week, or about 50 mg/kg/week to 100 mg/kg/week.

**[0403]** HRS-Fc conjugates with still longer half lives may be dosed in humans about 0.1 mg/kg/month to 0.5 mg/kg/month, about 1 mg/kg/month to 5 mg/kg/month, about 5 mg/kg/month to 10 mg/kg/month, about 10 mg/kg/month to 20 mg/kg/month, about 20 mg/kg/month to 30 mg/kg/month, about 30 mg/kg/month to 50 mg/kg/month, or about 50 mg/kg/month to 100 mg/kg/month.

**[0404]** In various embodiments of the present disclosure, the dosage is about 50-2500 mg per day, 100-2500 mg/day, 300-1800 mg/day, or 500-1800 mg/day, or 500-2500 mg per week, 1000-2500 mg/week, 300-1800 mg/week, or 500-1800 mg/week, or 500-2500 mg per month, 1000-2500 mg/month, 300-1800 mg/month, or 500-1800 mg/month. In some embodiments of the present disclosure, the dosage is between about 100 to 600 mg/day, 100 to 600 mg/week, or 100 to 600 mg/month. In some embodiments of the present disclosure, the dosage is between about 300 and 1200 mg/day, 300 and 1200 mg/ week, or 300 and 1200 mg/month. In



particular embodiments of the present disclosure, the composition or agent is administered at a dosage of 100 mg/week, 2.4 mg/week 300 mg/week, 600 mg/week, 1000 mg/week, 1200 mg/week or 1800 mg/week, in one or more doses per week or per month (i.e., where the combined doses achieve the desired weekly or monthly dosage). In some embodiments of the present disclosure, a dosage is 100 mg bid, 150 mg bid, 240 mg bid, 300 mg bid, 500 mg bid, or 600 mg bid. In various embodiments of the present disclosure, the composition or agent is administered in single or repeat dosing. The initial dosage and subsequent dosages may be the same or different.

**[0405]** In some embodiments of the present disclosure, total daily dose may be about 0.001 mg, about 0.005 mg, about 0.01 mg, about 0.05 mg, about 0.1 mg, 0.5 mg, 1 mg, about 2 mg, about 3 mg, about 4 mg, about 5 mg, about 6 mg, about 7 mg, about 8 mg, about 9 mg, about 10 mg, about 20 mg, about 30 mg, about 40 mg, about 50 mg, about 60 mg, about 70 mg, about 80 mg, about 90 mg or about 100 mg / 24 hours.

**[0406]** In some embodiments of the present disclosure, total weekly dose may be about 0.001 mg, about 0.005 mg, about 0.01 mg, about 0.05 mg, about 0.1 mg, 0.5 mg, 1 mg, about 2 mg, about 3 mg, about 4 mg, about 5 mg, about 6 mg, about 7 mg, about 8 mg, about 9 mg, about 10 mg, about 20 mg, about 30 mg, about 40 mg, about 50 mg, about 60 mg, about 70 mg, about 80 mg, about 90 mg or about 100 mg / week.

**[0407]** In some embodiments of the present disclosure, total monthly dose may be about 0.001 mg, about 0.005 mg, about 0.01 mg, about 0.05 mg, about 0.1 mg, 0.5 mg, 1 mg, about 2 mg, about 3 mg, about 4 mg, about 5 mg, about 6 mg, about 7 mg, about 8 mg, about 9 mg, about 10 mg, about 20 mg, about 30 mg, about 40 mg, about 50 mg, about 60 mg, about 70 mg, about 80 mg, about 90 mg or about 100 mg / month.

**[0408]** For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. The progress of these and other therapies (e.g., *ex vivo* therapies) can be readily monitored by conventional methods and assays and based on criteria known to the physician or other persons of skill in the art.

**[0409]** It will be further appreciated that for sustained delivery devices and compositions the total dose of HRS contained in such delivery system will be correspondingly larger depending upon the release profile of the sustained release system. Thus, a sustained release composition or device that is intended to deliver HRS-Fc conjugates over a period of 5 days will typically comprise at least about 5 to 10 times the daily dose of HRS-Fc conjugate; a sustained release composition or device that is intended to deliver a HRS-Fc conjugate over a period of 365 days will typically comprise at least about 400 to 800 times the daily dose of the HRS-Fc conjugate (depending upon the stability and bioavailability of the HRS-Fc conjugate when administered using the sustained release system).

**[0410]** In certain embodiments of the present disclosure, a composition or agent is administered intravenously, e.g., by infusion over a period of time of about, e.g., 10 minutes to 90 minutes. In other related embodiments of the present disclosure, a composition or agent is administered by continuous infusion, e.g., at a dosage of between about 0.1 to about 10 mg/kg/hr over a time period. While the time period can vary, in certain embodiments of the present disclosure the time period may be between about 10 minutes to about 24 hours or between about 10 minutes to about three days.

**[0411]** In particular embodiments of the present disclosure, an effective amount or therapeutically effective amount is an amount sufficient to maintain a concentration of the HRS-Fc conjugate in the blood plasma of a subject above about 300 pM, above about 1 nM, above about 10 nM, above about 100 nM, or above about 1000 nM.

**[0412]** In certain embodiments of the present disclosure, an IV or SC dosage is an amount sufficient to achieve a blood plasma concentration ( $C_{max}$ ) of between about 1,000 nM to about 5,000 nM or between



about 200 nM to about 1,000 nM, or about 20 nM to about 200 nM.

**[0413]** In particular embodiments of the present disclosure, a HRS-Fc conjugate is administered in an amount and frequency sufficient to achieve in the mammal a blood plasma concentration having a mean trough concentration of between about 300 pM and about 1 nM and/or a steady state concentration of between about 300 pM and about 1 nM. In some embodiments of the present disclosure, the  $C_{min}$  of the HRS-Fc conjugate in the blood plasma of the mammal is maintained above about 1 nM and/or a steady state concentration of between about 1 nM and about 10 nM. In certain embodiments of the present disclosure, the  $C_{min}$  of the HRS-Fc conjugate in the blood plasma of the mammal is maintained above about 10 nM and/or a steady state concentration of between about 10 nM and about 100 nM. In certain embodiments of the present disclosure, the  $C_{min}$  of the HRS-Fc conjugate in the blood plasma of the mammal is maintained above about 100 nM and/or a steady state concentration of between about 100 nM and about 1000 nM.

**[0414]** In particular embodiments of the present disclosure, an effective amount of the HRS-Fc conjugate, or the blood plasma concentration of the HRS-Fc conjugate, is achieved or maintained, with a single administration, *e.g.*, for at least 15 minutes, at least 30 minutes, at least 45 minutes, at least 60 minutes, at least 90 minutes, at least 2 hours, at least 3 hours, at least 4 hours, at least 8 hours, at least 12 hours, at least 24 hours, at least 48 hours, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least one week, at least 2 weeks, at least one month, at least 2 months, at least 3 months, at least 4 months, or at least 6 months.

**[0415]** In particular embodiments of the present disclosure, the effective dosage achieves the blood plasma levels or mean trough concentration of a composition or agent described herein. These may be readily determined using routine procedures.

**[0416]** Embodiments of the present disclosure, in other aspects of the present disclosure, provide kits comprising one or more containers filled with one or more of the HRS-Fc conjugates, polypeptides, polynucleotides, antibodies, multiunit complexes, compositions thereof, *etc.*, of the disclosure, as described herein. The kits can include written instructions on how to use such compositions (*e.g.*, to modulate cellular signaling, angiogenesis, cancer, inflammatory conditions, diagnosis *etc.*).

**[0417]** The kits herein may also include a one or more additional therapeutic agents or other components suitable or desired for the indication being treated, or for the desired diagnostic application. An additional therapeutic agent may be contained in a second container, if desired. Examples of additional therapeutic agents include, but are not limited to anti-neoplastic agents, anti-inflammatory agents, antibacterial agents, antiviral agents, angiogenic agents, *etc.*

**[0418]** The kits herein can also include one or more syringes or other components necessary or desired to facilitate an intended mode of delivery (*e.g.*, stents, implantable depots, *etc.*).

**[0419]** Certain embodiments of the present disclosure now will be illustrated by the following Examples. Examples not falling under the scope of the appended claims do not form part of the invention.

## EXAMPLES

### EXAMPLE 1

#### PRODUCTION OF HIS TAGGED RESOKINE (HRS COMPRISING AMINO ACIDS 1-60)



**[0420] Codon optimization and gene synthesis.** DNA encoding Resokine (HRS(1-60)) was codon-optimized for *E. coli* expression using the algorithm developed by DNA2.0 (Menlo Park, CA).

**[0421]** The codon-optimized DNA sequence is as follows:

ATGGCAGAACGTGCGGCATTGGAAGAATTGGTTAAACTGCAAGGTGAACGTGTTCGTGG  
TCTGAAGCAGCAGAAGGCTAGCGCGGAGCTGATCGAAGAAGAGGTGGCCAAACTGCTG  
AAGCTGAAGGCGCAGCTGGGCCCCGACGAGAGCAAACAAAAGTTCGTCCTGAAAACCCC  
GAAACACCACCATCACCATCAC (SEQ ID NO:261)

**[0422]** The translated protein sequence is as follows:

MAERAALVELVKLQGERVRGLKQKASAELEEEVAKLLKLKAQLGPDESKQKFVLKTPKH  
HHHHH (SEQ ID NO:262)

**[0423]** Additionally, engineered versions of this construct were prepared with cysteine residues inserted close to the N-terminus (comprising additional N-terminal Met and Cys residues), C-terminus (comprising an additional C-terminal cysteine at position 61), and in the linker domain joining the 2 alpha helical sections of the molecule (comprising the mutation Ala 26→Cys). The codon optimized DNA sequences, and corresponding amino acid sequences for these constructs are listed below.

H-N4:1-H (codon-HRS(1-60)-M1MC-6xHis):

ATGTGTGCAGAAAGAGCCGCCCTGGAAGAGTTAGTTAAGTTGCAAGGTGAACGTGTCCG  
TGGTCTGAAGCAGCAGAAGGCTAGCGCGGAGCTGATCGAAGAAGAGGTGGCCAAACTG  
CTGAAGCTGAAGGCGCAGCTGGGCCCCGACGAGAGCAAACAAAAGTTCGTCCTGAAAAC  
CCCGAAACACCACCATCACCATCAC (SEQ ID NO:263)

**[0424]** The translated protein sequence is as follows:

MCAERAALVELVKLQGERVRGLKQKASAELEEEVAKLLKLKAQLGPDESKQKFVLKTPK  
HHHHHH (SEQ ID NO:264)

H-N4:2-H (codon-HRS(1-60)-A26C-6xHis):

ATGGCAGAACGTGCGGCATTGGAAGAATTGGTTAAACTGCAAGGTGAACGTGTTCGTGG  
TCTGAAGCAGCAGAAGTGCAGCGCGGAGCTGATCGAAGAAGAGGTGGCCAAACTGCTG  
AAGCTGAAGGCGCAGCTGGGCCCCGACGAGAGCAAACAAAAGTTCGTCCTGAAAACCCC  
GAAACACCACCATCACCATCAC (SEQ ID NO:265)

**[0425]** The translated protein sequence is as follows:

MAERAALVELVKLQGERVRGLKQKCSAELEEEVAKLLKLKAQLGPDESKQKFVLKTPKH  
HHHHH (SEQ ID NO:266)

H-N4:3-H (codon-HRS(1-60)-C61-6xHis):

ATGGCAGAACGTGCGGCATTGGAAGAATTGGTTAAACTGCAAGGTGAACGTGTTCGTGG  
TCTGAAGCAGCAGAAGGCTAGCGCGGAGCTGATCGAAGAAGAGGTGGCCAAACTGCTG  
AAGCTGAAGGCGCAGCTGGGCCCCGACGAGAGCAAACAAAAGTTCGTCCTGAAAACCCC  
GAAATGCCACCACCATCACCATCAC (SEQ ID NO:267)



**[0426]** The translated protein sequence is as follows:

MAERAALVELVKLQGERVRGLKQKASAEIEEEVAKLLKLKAQLGPDESKQKFVLKTPKC

HHHHHH (SEQ ID NO:268)

**[0427]** The corresponding genes were synthesized (DNA 2.0) with a C-terminal 6xHis tag and subcloned into the pJexpress411 expression vector where the T7 promoter was used to drive the transcription and kanamycin resistance was used for antibiotic selection.

**[0428] Expression strain.** BL21(DE3) competent cells (Novagen, cat. no. 69450) were transformed with the relevant codon-optimized expression construct, as described. Briefly, the plasmid (1  $\mu$ L) was added into 50  $\mu$ L of the competent cells. The reaction was mixed and incubated on ice for 30 minutes. The reaction was heat-shocked for at 42°C for 30 seconds followed by a cold-shock on ice for 2 minutes. Then the SOC medium (500  $\mu$ L) was added and the tube was incubated at 37°C, 250 rpm for 1 hour. Finally, an aliquot of the culture (50  $\mu$ L) was spread on the Kanamycin plate (Teknova S9641) and incubated at 37°C overnight. A single colony was picked and used for expression scale-up.

**[0429] Medium.** M9YE medium was prepared by mixing 200 mL sterile M9 minimal salt 5X (BD248510), 778 mL of 30g/L yeast extract in sterile purified water (BD212750), 20 mL sterilized 20% glucose (Sigma G7021) and 2 mL sterile 1.0 M MgSO<sub>4</sub> (Sigma M7506). The feeding solution contains 5% yeast extract, 50% glucose, trace elements and 2 g/L magnesium sulfate. Kanamycin sulfate (Invitrogen 15160) was added to a final concentration of 100  $\mu$ g/mL in both M9YE and feeding solution.

**[0430] Fed-batch fermentation.** A 4 L fermentor (Sartorius Biostat B plus) with MFCS/DA software was used for the fed-batch fermentation. The agitation was set at 1000 rpm. The pH value was controlled at 7.0 automatically by the addition of 30% ammonium hydroxide (Sigma 221228) and 30% phosphoric acid (Sigma P5811). The air was provided at a flow rate of 4 L/min with an oil-free diaphragm air compressor (Cole-Parmer). The air was passed through a 0.2  $\mu$ m Midisart 2000 filter (Sartorius 17805). The pure oxygen (West Air) was supplied automatically to control the dissolved oxygen level at 70%. The temperature was controlled at 30°C with a Neslab RTE7 circulator (Thermo Scientific). The foaming was controlled by addition of the antifoam 204 (Sigma A8311). The initial volume of M9YE medium in the fermentor was 3L. The fermentor was inoculated with 150 mL of the seed culture grown overnight at 30°C and 250 rpm. When the glucose was depleted in the vessel, the concentrated feeding solution was introduced into the vessel by a peristaltic pump set at 0.9 mL/min. When the optical density of the cells at 600 nm reached about 30, the culture was induced with 0.5 mM IPTG (Fisher Scientific BP1755). The culture was run overnight (about 18-hour fed-batch phase) and harvested by centrifugation at 6,000 x g for 1 hour. The cell pellet was stored at -20°C until purification. The expression of Resokine was confirmed on the SDS-PAGE.

**[0431] Purification of Proteins.** Resokine and the Cys variants thereof were purified from *E. coli* cell paste through cell lysis and clarification; immobilized metal affinity chromatography, and cation exchange chromatography. Frozen cells weighing 10 g and containing Resokine or Cys variants were thawed and resuspended in IX NiNTA buffer (50 mM Tris, 0.3 M NaCl 25 mM imidazole pH 8) at a 4:1 ml/g paste ratio along with 5 mM beta-mercaptoethanol (Sigma Cat#M7154-25ML) and 1 protease inhibitor cocktail tablet (Roche Cat #05 056 489 001). After all cells were resuspended, cells were lysed on ice in a Microfluidizer M-110Y, 2 passes at 15000 psi on ice to release soluble Resokine or Cys variants. NiNTA buffer was added after the second pass to flush remaining lysate through the Microfluidizer (100-120 ml final volume after lysis). Lysate was centrifuged for 15000 x g @ 4 C for 30 min. Supernatant was filtered 0.45/0.2  $\mu$ m with an Acropak 200 (Pall Cat#12094). Filtered supernatant is clarified lysate.

**[0432] Immobilized metal affinity chromatography (IMAC) purification.** Clarified lysate from 10 g cell paste was loaded onto a gravity-flow column containing 3 ml NiNTA resin (Qiagen # 30210) and pre-



equilibrated in NiNTA buffer. Resin was washed with 50 column volumes (CV) of 0.1% Triton X-114 in IX NiNTA buffer to remove endotoxin, then 30 CV IX NiNTA buffer, followed by elution with 5 CV NiNTA elution buffer (50 mM Tris, 0.3 M NaCl, 0.3 M imidazole pH 8 @ 4C).

**[0433] Cation exchange (CEX) chromatography purification.** CEX load was prepared by diluting NiNTA eluent 1/20x in CEX A buffer (10 mM sodium phosphate pH 7.0, 2 mM DTT), then loaded onto SP Sepharose High Performance column equilibrated in CEX A. Protein was eluted with a linear gradient of 0-100%B over 20 CV, where A=10 mM sodium phosphate pH 7.0, 2 mM DTT and B=10 mM sodium phosphate, 1 M sodium chloride 2 mM DTT pH 7.0, monitoring absorbance at 214 nm. Fractions were pooled corresponding to the main peak in the elution gradient by absorbance at 214 nm. CEX pool was buffer exchanged into IX PBS pH 7.4 (Gibco #10010) using Amicon Ultra-15 3 kD MWCO ultracentrifugal devices.

## EXAMPLE 2

### PRODUCTION OF HIS TAGGED FULL-LENGTH HISTIDYL-TRNA SYNTHETASE (HRS)

**[0434] Codon optimization and gene synthesis.** The full length HisRS gene was codon-optimized for *E. coli* expression and subcloned into pET21a vector where the T7 promoter was used to drive the transcription. In addition, a 5-amino acid linker and 6xHis tag were attached to the C-terminus.

**[0435]** The DNA sequence is as follows:

```
ATGGCGGAACGTGCCGCACTGGAAGAATTGGTTAAATTACAGGGAGAACGCGTACGTGG
TCTTAAACAACAAAAAGCCTCTGCGGAATTGATTGAAGAAGAAGTTGCCAAATTACTGA
AACTGAAAGCTCAACTTGGACCCGATGAAAGTAAACAAAAATTTGTGTTGAAAACGCCC
AAAGGAACCCGTGATTATAGTCCACGTCAAATGGCCGTTTCGTGAAAAAGTGTTGACGTT

ATTATTCGCTGTTTTAAACGTCACGGTGCTGAAGTAATCGATACCCCGTATTTGAATTG
AAAGAGACTCTGATGGGCAAATATGGTGAAGATTCTAAACTGATTTATGATTTGAAAGA
CCAAGGAGGTGAACTGCTGAGCCTGCGCTACGACTTAACTGTGCCTTTTGCCCGTTACTT
AGCCATGAATAAaTTaACCAACATCAAACGTTACCATATTGCAAAAGTATATCGCCGCGA
CAACCCTGCAATGACTCGTGGACGCTATCGCGAATTCTATCAGTGTGATTTTGATATTGC
CGGAAATTTGACCCGATGATCCCGGATGCCGAGTGTTTGAAAATTATGTGTGAAATTCT
GAGTTCGTTGCAGATCGGAGACTTTCTTGTAAGTTAATGACCGCCGTATTCTGGATGG
TATGTTTGCTATTTGCGGTGTTTCTGATTCCAAATTCCGTACAATCTGCTCAAGCGTGGAC
AAATTGGATAAAGTGTCTTGGAAGAAGTAAAAAATGAAATGGTGGGAGAAAAAGGCC
TGGCTCCAGAAGTAGCAGACCGTATTGGTGACTATGTTCAACAACATGGCGGTGTGTCCT
TAGTCGAACAGTTATTACAGGATCCTAAACTGAGCCAAAATAAACAAGCACTTGAAGGA
CTGGGAGATCTGAAATTACTCTTTGAATATCTGACCTTATTTGGGATTGATGATAAAATT
AGCTTTGATCTGAGCTTGGCCCGCGGTCTTGATTATTATACCGGCGTGATTTACGAAGCT
GTTCTCTTGCAAACCCAGCCAGGCGGGCGAAGAGCCTTTGGGAGTCGGCAGTGTGGC
AGCCGGTGGTCGTTATGATGGTTTGGTAGGAATGTTTGACCCTAAAGGCCGTAAAGTACC
ATGTGTGGGGCTTTCTATCGGTGTGCAACGTATCTTTTCTATTGTTGAACAACGTCTTGAA
GCTTTGGAGGAAAAGATCCGTACCACGGAAaCCAAGTCTTAGTTGCaAGTGCCCAAAAA
AAACTGTTAGAAGAACGCCTGAAACTCGTATCAGAACTTTGGGACGCCGGCATCAAGGC
CGAACTGCTGTATAAAAAGAACCCGAAATTGTTAAACCAACTCCAGTATTGTGAAGAAG
CTGGGATCCCACTCGTAGCTATTATTGGTGAGCAAGAATTAAGATGGCGTGATTAAAC
TGCGTTCAGTAACAAGCCGTGAAGAGGTAGATGTACGTCGCGAAGACTTAGTGGAAGAA
ATTAAACGCCGCACCGGTCAACCGTTATGTATTTGCGCGGCCGCACTCGAGCACCACCAC
CACCACCACTCA (SEQ ID NO: 260)
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CACCAACAC TGA (SEQ ID NO:207)

**[0436]** The sequence of the translated protein is as follows:

MAERAALVELVQLQGERVRGLKQKASAELEEEVAKLLKLKAQLGPDESKQKFVLKTPKG  
TRDYSPRQMAVREKVFVDVIIRCFKRHGAVIDTPVFELKETLMGKYGEDSKLIYDLKDQGG  
LLSLRYDLTVPFARYLAMNKLNTIKRYHIAKVYRRDNPAMTRGRYREFYQCDFDIAGNFDP  
MIPDAECLKIMCEILSSLQIGDFLVKVNDRRILDGMFAICGVSDSKFRTICSSVDKLDKVSWE  
VKNEMVGEKGLAPEVADRIGDYVQQHGGVSLVEQLLQDPKLSQNKQALEGLGDLKLLFEY  
LTLFGIDDKISFDLSLARGLDYYTGVIYEAVLLQTPAQAGEEPLGVGSVAAGGRYDGLVGMF  
DPKGRKVPCVGLSIGVERIFIVEQRLEALEEKIRTTETQVLVASAQKKLEERLKLSELWD  
AGIKAELLYKKNPKLLNQLQYCEEAGIPLVAHGEQELKDGVIKLRSVTSREEVDVRREDLVE  
EIKRRTGQPLCICAAALEHHHHHH (SEQ ID NO:270)

**[0437] Expression strain.** The BL21(DE3) competent cells (Novagen, cat. no. 69450) were transformed with the codon-optimized expression construct. Briefly, the plasmid (1  $\mu$ L) was added into 50  $\mu$ L of the competent cells. The reaction was mixed and incubated on ice for 30 minutes. The reaction was heat-shocked for at 42°C for 30 seconds followed by a cold-shock on ice for 2 minutes. Then the SOC medium (500  $\mu$ L) was added and the tube was incubated at 37°C, 250 rpm for 1 hour. Finally, an aliquot of the culture (50  $\mu$ L) was spread on the Ampicillin plate (Teknova S9641) and incubated at 37°C overnight. Single colony was picked and used for expression scale-up.

**[0438] Medium.** The M9YE medium was prepared by mixing 200 mL sterile M9 minimal salt 5X (BD248510), 778 mL of 30g/L yeast extract in sterile purified water (BD212750), 20 mL sterilized 20% glucose (Sigma G7021) and 2 mL sterile 1.0 M MgSO<sub>4</sub> (Sigma M7506). The feeding solution contains 5% yeast extract, 50% glucose, trace elements and 2 g/L magnesium sulfate. Ampicillin was added to a final concentration of 100  $\mu$ g/mL in both M9YE and feeding solution.

**[0439] Fed-batch fermentation.** A 4 L fermentor (Sartorius Biostat B plus) with MFCS/DA software was used for the fed-batch fermentation. The agitation was set at 1000 rpm. The pH value was controlled at 7.0 automatically by the addition of 30% ammonium hydroxide (Sigma 221228) and 30% phosphoric acid (Sigma P5811). The air was provided at a flow rate of 4 L/min with an oil-free diaphragm air compressor (Cole-Parmer). The air was passed through a 0.2  $\mu$ m Midisart 2000 filter (Sartorius 17805). The pure oxygen (West Air) was supplied automatically to control the dissolved oxygen level at 70%. The temperature was controlled at 30°C with a Neslab RTE7 circulator (Thermo Scientific). The foaming was controlled by addition of the antifoam 204 (Sigma A8311). The initial volume of M9YE medium in the fermentor was 3L. The fermentor was inoculated with 150 mL of the seed culture grown overnight at 30°C and 250 rpm. When the glucose was depleted in the vessel, the concentrated feeding solution was introduced into the vessel by a peristaltic pump set at 0.9 mL/min. When the optical density of the cells at 600 nm reached about 30, the culture was induced with 0.5 mM IPTG (Fisher Scientific BP1755). The culture was run overnight (about 18-hour fed-batch phase) and harvested by centrifugation at 6,000 x g for 1 hour. The cell pellet was stored at -20°C until purification. The expression of HisRS was confirmed on the SDS-PAGE.

**[0440] Purification of HisRS.** Frozen cell paste (40 g) were resuspended in 160 mL (i.e. 4 mL/g cell paste) of Lysis Buffer (20 mM Tris, 400 mM NaCl, 20 mM Imidazole, 14 mM  $\beta$ -ME, pH 8.0 at 4°C). Complete EDTA-FREE protease inhibitor tablets (Roche) were added to the suspension at a ratio of 1 tablet/50 mL. The suspension was passed through a microfluidizer (Microfluidics) twice at 15,000 psi with cooling by ice. The lysate was centrifuged at 35,000 x g for 45 min at 4°C. The supernatant was filtered through 0.22  $\mu$ m Acropak 200 capsule filters (Pall).

**[0441]** The clarified lysate was bound to the Ni-NTA resin (Qiagen), pre-equilibrated with Ni-NTA Binding



Buffer (20 mM Tris, 400 mM NaCl, 20 mM Imidazole, 5 mM  $\beta$ -ME, pH 8.0 at 4°C). The column was washed with 500 column volumes of Ni-NTA Binding Buffer + 0.1% Triton X-114 followed by 50 column volumes of the Ni-NTA Binding Buffer. The bound protein, HisRS, was eluted with 5 column volumes of Ni-NTA Elution Buffer (20 mM Tris, 400 mM NaCl, 500 mM Imidazole, 5 mM  $\beta$ -ME, pH 8.0 at 4°C).

**[0442]** The Ni-NTA eluate was further purified by an anion exchange column. Specifically, the Ni-NTA eluate was dialyzed against Q Binding Buffer (20 mM Tris, 50 mM NaCl, 1 mM DTT, pH 7.4) and loaded onto a 5 mL Q-sepharose column, pre-equilibrated with the Q Binding Buffer. The desired product was eluted off the column with a linear gradient of 0-1 M NaCl in the Q Binding Buffer over 10 column volumes. The purified HisRS was concentrated and buffer exchanged into PBS (Invitrogen product # 10010) + 1 mM DTT, and filtered through a 0.22  $\mu$ m sterile filter.

### **EXAMPLE 3**

#### **ACTIVE SITE TITRATION OF THE CYSTEINE RESIDUES IN FULL LENGTH HARS**

**[0443]** To determine the location and identity of the surface exposed cysteine residues in full length HARS, purified recombinant protein was incubated with iodoacetamide under native and denatured conditions to alkylate any surface exposed cysteine residues. Samples were then analyzed by limiting proteolysis followed by LC-mass analysis to determine the location and identity of the modified cysteine residues.

**[0444]** To perform the alkylation studies, full length, polyhistidine tagged HARS (6.65 mg/ml in PBS, 10% glycerol, 2 mM DTT, pH7.4, (Example 2) was first fully reduced by incubation with 10 mM DTT for 45 minutes at room temperature. Incubations with iodoacetamide were conducted with an iodoacetamide concentration at either 30 mM ("Low") or a 100 mM ("High") for 30 minutes in the dark, and were conducted on native and denatured samples of HARS to confirm that the reaction was successful. Denatured HARS was prepared by pre-incubation of the protein with 4M guanidine for 45 min at 50 C. After incubation with iodoacetamide, samples were dialyzed in PBS pH 7.4 at 4 C using 10KDa molecular weight cutoff dialysis membrane, and with at least 3 buffer exchanges, and then used for mass spectroscopy analysis as described below.

**[0445]** In brief, samples were prepared by diluting the proteins into 0.1% formic acid to a final concentration of 1m/ml and 5 $\mu$ g samples of the proteins were injected and analyzed by reverse phase HPLC followed by mass spectrum analysis using an Agilent TOF mass spectrometer. Samples were first separated on a C3 HPLC column (Agilent ZORBAX 300SB-C3, 5 $\mu$ m, 2.1x150mm column) using a linear gradient of (mobile phase B of 2-60%) over 18min (mobile phase A: 0.1% formic acid; mobile phase B: 0.1% formic acid in acetonitrile). Mass spectrometry analysis of the samples was in profile mode. Data was acquired and analyzed by MassHunter (Agilent). Measured molecular weight was calculated by MassHunter Bioconfirm Agilent).

**[0446]** The results (data not shown) demonstrated that under native conditions only 3 or 4 cysteine residues are readily modified, whereas by comparison when the protein is first denatured to disrupt its native conformation all 10 cysteines were readily denatured.

**[0447]** To identify the identity of the modified cysteine residues, samples before and after incubation with iodoacetamide were subjected to denaturation in 4 M Guanidine HCl at 37°C for 30min followed by proteolytic cleavage with LysC using a by a 10:1 ratio (w/w) at room temperature for 20h. Protein digests were analyzed by LC/MS/MS using Dionex HPLC and Thermo LTQ XL mass spectrometer. Samples were first separated on C18 HPLC column (Agilent ZORBAX 300SB-C18, 5 $\mu$ m, 2.1x150mm) using a gradient of mobile phase B (mobile phase A: 0.1% formic acid; mobile phase B: 0.1% formic acid in acetonitrile). The gradient start off with



1-3% B in 10min and then to 40% B in 76min. Separated protein digests were analyzed either by full MS in profile mode or by a full MS scan were analyzed by tandem MS/MS scan on the top three identified ions. Data was acquired and analyzed by Xcalibur (Thermo). Peptide sequencing was based on the MS/MS spectra of each peptide, in which b- and y-ion peaks match their theoretical ions. Identification of the peptides and mapping of the modification sites are based on the molecular weight and confirmed by peptide sequencing using MS/MS spectra, and are listed in **Table E1**.

| Table E1                                                       |           |                                                                |          |         |
|----------------------------------------------------------------|-----------|----------------------------------------------------------------|----------|---------|
| LC-MS Peptide mapping results after limiting trypsin digestion |           |                                                                |          |         |
| Cys residue                                                    | From - To | Sequence                                                       | RT (min) | MH+     |
| Cys83                                                          | 76-85     | VFDVIIRCFK (SEQ ID NO:271)                                     | 56.24    | 1239.68 |
| Cys174;<br>Cys191                                              | 155-193   | VYRRDNPAMTRGRYREFYQCDFDIAGNFDPMIPDAECL<br>K<br>(SEQ ID NO:272) | 61.27    | 4673.14 |
| Cys196                                                         | 194-210   | IMCEILSSLQIGDFLVK (SEQ ID NO:273)                              | 73.14    | 1909.01 |
| Cys224                                                         | 211-230   | VNDRRILDGMFAICGVSDSK (SEQ ID NO:274)                           | 58.53    | 2196.08 |
| Cys235                                                         | 231-240   | FRTICSSVDK (SEQ ID NO:275)                                     | 22.8     | 1155.57 |
| Cys235                                                         | 231-243   | FRTICSSVDKLDK (SEQ ID NO:276)                                  | 28.77    | 1511.79 |
| Cys379                                                         | 377-403   | VPCVGLSIGVERIFSIVEQRLEALEEK (SEQ ID NO:277)                    | 81.00    | 3013.63 |
| Cys445                                                         | 448-472   | LLNQLQYCEEAGIPLVAIIGEQLK (SEQ ID NO:278)                       | 72.46    | 2784.48 |
| Cys505;<br>Cys509                                              | 500-509   | RRTGQPLCIC (SEQ ID NO:279)                                     | 27.17    | 1146.57 |

[0448] The results revealed (data not shown) that Cys235, Cys507 and Cys509 are readily modified by iodoacetamide treatment and are thus likely to be surface-exposed residues that are readily amenable to chemical modification.

EXAMPLE 4

CREATION OF MODIFIED HRS POLYPEPTIDES WITH ALTERED CYSTEINE CONTENT

[0449] To determine whether any of the 10 naturally-occurring cysteine residues in full length HRS could be mutated to alternative naturally occurring amino acid residues, or deleted, primers were designed to selectively mutate each cysteine residue. To accomplish this, primers based on the following may be used (see **Table E2**).

| Table E2 |                                                                             |            |
|----------|-----------------------------------------------------------------------------|------------|
| Mutation | Oligo sequence                                                              | SEQ ID NO: |
| C83      | 5'- GTTTGACGTAATCATCCGTTGCTTCAAGCGCCACGGTGCAG-3'<br>(Forward)               | 280        |
| C83      | 5'- CTG CAC CGT GGC GCT TGA AGC AAC GGA TGA TTA CGT CAA<br>AC -3' (Reverse) | 281        |



| Table E2             |                                                                               |            |
|----------------------|-------------------------------------------------------------------------------|------------|
| Mutation             | Oligo sequence                                                                | SEQ ID NO: |
| C174                 | 5'- GCCGATACCGGGAATTCTACCAGTGTGATTTTGACATTGCTGGG-3' (Forward)                 | 282        |
| C174                 | 5'- CCC AGC AAT GTC AAA ATC ACA CTG GTA GAA TTC CCG GTA TCG GC -3' (Reverse)  | 283        |
| C191                 | 5'- CCATGATCCCTGATGCAGAGTGCCTGAAGATCATGTGCGAG-3' (Forward)                    | 284        |
| C191                 | 5'- CTC GCA CAT GAT CTT CAG GCA CTC TGC ATC AGG GAT CAT GG -3' (Reverse)      | 285        |
| C196                 | 5'- GCAGAGTGCCTGAAGATCATGTGCGAGATCCTGAGTTCACTTC-3' (Forward)                  | 286        |
| C196                 | 5'- GAA GTG AAC TCA GGA TCT CGC ACA TGA TCT TCA GGC ACT CTG C -3' (Reverse)   | 287        |
| C224                 | 5'- CTAGATGGGATGTTTGCTATCTGTGGTGTCTTGACAGCAAGTTC-3' (Forward)                 | 288        |
| C224                 | 5'- GAA CTT GCT GTC AGA AAC ACC ACA GAT AGC AAA CAT CCC ATC TAG -3' (Reverse) | 289        |
| C235                 | 5'- CAGCAAGTTCCGTACCATCTGCTCCTCAGTAGACAAGCTGG-3' (Forward)                    | 290        |
| C235                 | 5'- CCA GCT TGT CTA CTG AGG AGC AGA TGG TAC GGA ACT TGC TG -3' (Reverse)      | 291        |
| C379                 | 5'- GGGCGCAAGGTGCCATGTGTGGGGCTCAGCATTGGGG-3' (Forward)                        | 292        |
| C379                 | 5'- CCC CAA TGC TGA GCC CCA CAC ATG GCA CCT TGC GCC C -3' (Reverse)           | 293        |
| C455                 | 5'- CTGAACCAGTTACAGTACTGTGAGGAGGCAGGCATCCC-3' (Forward)                       | 294        |
| C455                 | 5'- GGG ATG CCT GCC TCC TCA CAG TAC TGT AAC TGG TTC AG -3' (Reverse)          | 295        |
| C507                 | 5'- GAGAACAGGCCAGCCCCTCTGCATCTGCTAGAACCCAGC-3' (Forward)                      | 296        |
| C507                 | 5'- GCT GGG TTC TAG CAG ATG CAG AGG GGC TGG CCT GTT CTC -3' (Reverse)         | 297        |
| C509                 | 5'- CCAGCCCCTCTGCATCTGCTAGAACCCAGCTTTCTTG-3' (Forward)                        | 298        |
| C509                 | 5'- CAA GAA AGC TGG GTT CTA GCA GAT GCA GAG GGG CTG G -3' (Reverse)           | 299        |
| Last 3 codon removal | 5' GAACAGGCCAGCCCCTCTAGAACCCAGCTTTCTTG 3' (Forward)                           | 300        |
| Last 3 codon removal | 5'- CAA GAA AGC TGG GTT CTA GAG GGG CTG GCC TGT TC -3' (Reverse)              | 301        |

[0450] To confirm the active site titration data, the crystal structure of full length HRS was analyzed using the



program Getarea 1.1 to assess the relative location of the 10 cysteine residues. The results (data not shown) suggest that in addition to C235, C507 and C509, the cysteines at positions C174, C191 and C224 of SEQ ID NO:1, are at least partially exposed to the surface and could likely be modified via standard reagents. Additionally, analysis of the crystal structure of HRS suggests that C174 and C191 are capable of making an internal disulfide bond, while C507 and C509 are capable of making interchain disulfide bonds within the HRS dimer, both potentially contributing to microheterogeneity that could be beneficially eliminated.

**[0451]** To directly assess the significance of the two C-terminal cysteine residues in contributing to interchain disulfide bond formation, His-tagged versions of the full length and the C-terminally deleted versions of HRS (HRS(1-506)) were compared by SDS PAGE analysis before and after reduction. The results, shown in **Figure 3**, demonstrate that full length HRS is a ~50:50 mixture of non-covalent and SS- linked dimer, while HRS(1-506) dramatically reduces the SS-linked dimer. Comparison of the two proteins by competitive ELISA, as described below, revealed that both proteins had comparable IC50 values with respect to Jo-1 antibody binding (data not shown). The dramatically reduced interchain disulfide bond formation associated with HRS(1-506) suggests that this variant is a suitable starting point for the development of improved next generation product forms.

**[0452]** To determine whether any of the remaining four partially exposed cysteine residues in full length HRS could be mutated to alternative naturally occurring amino acid residues, primers were designed to selectively mutate C174, C191, C224 and C235 residues. To accomplish this, the following primers were used as listed in **Table E3**:

| <b>Table E3</b> |                                                                  |                   |
|-----------------|------------------------------------------------------------------|-------------------|
| <b>Mutation</b> | <b>Oligo sequence</b>                                            | <b>SEQ ID NO:</b> |
| C191A           | CCCGGATGCCGAGGCTTTGAAAATTATGTG (Forward)                         | 302               |
| C191A           | CAC ATA ATT TTC AAA GCC TCG GCA TCC GGG (Reverse)                | 303               |
| C191S           | GATCCCGGATGCCGAGAGTTTGAAAATTATGTGTG (Forward)                    | 304               |
| C191S           | CAC ACA TAA TTT TCA AAC TCT CGG CAT CCG GGA TC<br>(Reverse)      | 305               |
| C191V           | GA TCCCGGA TGCCGAGGTTTTGAAAA TT A TGTGTG (Forward)               | 306               |
| C191V           | CAC ACA TAA TTT TCA AAA CCT CGG CAT CCG GGA TC<br>(Reverse)      | 307               |
| C174A           | CGCGAATTCTATCAGGCTGATTTTGATATTGCCGG (Forward)                    | 308               |
| C174A           | CCG GCA ATA TCA AAA TCA GCC TGA TAG AAT TCG CG<br>(Reverse)      | 309               |
| C174V           | CGCGAATTCTATCAGGTTGATTTTGATATTGCCG (Forward)                     | 310               |
| C174V           | CGG CAA TAT CAA AAT CAA CCT GAT AGA ATT CGC G (Reverse)          | 311               |
| C224S           | GGT A TGTTTGCT A TTTCCGGTGTTTCTGA TTCC (Forward)                 | 312               |
| C224S           | GGA ATC AGA AAC ACC GGA AAT AGC AAA CAT ACC (Reverse)            | 313               |
| C235S           | CCAAATTCCGTACAATCTCCTCAAGCGTGGACAAATTGG (Forward)                | 314               |
| C235S           | CCA ATT TGT CCA CGC TTG AGG AGA TTG TAC GGA ATT TGG<br>(Reverse) | 315               |
| C191A           | CCCGGATGCCGAGGCTTTGAAAATTATGTG (Forward)                         | 316               |
| C191A           | CAC ATA ATT TTC AAA GCC TCG GCA TCC GGG (Reverse)                | 317               |

**[0453]** Mutations were introduced by mutagenesis using the QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent, cat. no. 210518) following the manufacturer's instructions. After mutagenesis, the sample were



treated with Dpn I enzyme at 37°C and transformed into XL10 gold competent cells using routine procedures. Multiple colonies are grown in terrific broth overnight at 37°C and the resulting plasmids are purified with QIAprep Spin Miniprep Kit (Qiagen cat. no.27106). The plasmids are sequenced to confirm the identity of the amino acid substitution of each clone. The representative clones were transformed into NovaBlue competent cells (Novagen cat. no. 70181) and grown in 250ml M9YE medium at 37 °C overnight. A maxiprep was performed using the HiSpeed Plasmid Maxi Kit (Qiagen cat. no. 12663) to create a plasmid stock of mutant for further analysis. The concentration and purity were determined by measuring A260, A280 and A230. The purified plasmids were stored at -20°C before transfection into E. coli or mammalian cells following standard protocols.

**[0454]** To assess the impact of the mutation of each residue, representative clones were transformed into E. coli, or mammalian cells, and the production yields, endotoxin contents, stability and relative activity in an ELISA assay to determine Jo-1 antibody binding as described below.

**[0455] Protein production.** BL21(DE3) competent cells (Novagen, cat. no. 69450) or W3110 cells (ATTC) were transformed with the codon-optimized expression constructs encoding the reduced cysteine constructs as described above. The expression system, fermentation media, fermentation conditions and purification steps used to produce recombinant protein were essentially the same as those described in Example 4 below, after adjusting for the production scale, and amount of cell paste used. **Table E4** below shows the purification yields, and endotoxin levels for the proteins made.

| <b>Table E4</b>                                                              |                                |                            |
|------------------------------------------------------------------------------|--------------------------------|----------------------------|
| <b>Purification yields and endotoxin levels of reduced cysteine variants</b> |                                |                            |
| <b>Name</b>                                                                  | <b>Yield (mg/g cell paste)</b> | <b>Endotoxin (EU / mg)</b> |
| HRS(1-506)                                                                   | +++                            | 0.32                       |
| HRS(1-506)C174V                                                              | ++                             | 0.71                       |
| HRS(1-506)C174A                                                              | ++                             | 0.30                       |
| HRS(1-506)C191A                                                              | ++                             | 0.46                       |
| HRS(1-506)C191V                                                              | +++                            | 0.33                       |
| HRS(1-506)C1191S                                                             | +++                            | 0.32                       |
| HRS(1-506)C224S                                                              | ++                             | 0.54                       |
| HRS(1-506)C235S                                                              | +++                            | 0.60                       |
| +++ greater than 7 mg protein / g cell paste;                                |                                |                            |
| ++ greater than 5 mg/g cell paste                                            |                                |                            |
| + less than 5 mg/g cell paste.                                               |                                |                            |

**[0456]** The results show that all of the reduced variants were relatively well expressed, and were successfully purified with low endotoxin levels. In particular the reduced cysteine variants based on the mutation of Cys191, and Cys235 displayed favorable expression levels; though all clones exhibited reasonable levels of expression, and low endotoxin levels.

**[0457]** To assess the impact of the cysteine mutations on the charge heterogeneity of the purified proteins, samples of each clone were analyzed by isoelectric focusing. Samples (10 µg) were loaded onto an isoelectric focusing gel (pH 3-10) using a Life Technologies Novex pH 3-10 IEF gel 1.0mm (Cat No. P/N EC6645BOX), Novex IEF Marker 3-10 (Cat No. P/N 391201), Novex pH 3-10IEF buffer kit (Cat. No. P/N LC5317), run with IX cathode buffer (upper chamber) and IX anode buffer (lower chamber) at 100V for 1 hour, 200V for 1 hour, and 500V for 30 minutes. Gels were fixed with 12% TCA with 3.5% sulfosalicylic acid for 30 minutes and stained with Expedeon InstantBlue (Cat No. P/N ISB1L). The data, (results not shown) demonstrate that the mutation



of the cysteine at position 174 significantly reduced isoelectric point heterogeneity, consistent with the possibility that this cysteine residue undergoes an intramolecular disulfide bond formation with cysteine 191.

**[0458]** To assess the impact of the cysteine modifications on the thermal stability, aggregation propensity, structure, and tRNA synthetase activity of the resultant proteins, the proteins were assessed by differential scanning fluorimetry, size exclusion HPLC (SE-HPLC), competitive ELISA and active site titration. The results are shown in **Table E5** below.

**[0459]** Differential scanning fluorimetry was performed on protein samples by monitoring fluorescence as a function of the fluorescence intensity of a lipophilic dye during thermal denaturation. Studies were carried on samples after they were diluted to 0.5 mg/mL into 100  $\mu$ L final volume of PBS pH 7.0 (150 mM NaCl, 20 mM phosphate) and mixed with a thermal shift dye solution, which was prepared by diluting the stock solution (Applied Biosystems/Life Technologies, P/N 4461146) 20-fold in ultrapure distilled water (Gibco, P/N 10977). Five  $\mu$ L of the diluted dye was added to 100  $\mu$ L of sample. The mixture was plated into a 384 well clear optical reaction plate (Applied Biosystems/ Life Technologies P/N 4309849) at 20  $\mu$ L each well and 4 well replicates per sample. The plate was read by the ViiA 7 Real Time PCR Instrument (Applied Biosystems/Life Technologies, P/N 4453552). The thermal denaturation protocol commenced with a rate of change of 1.6°C/s, until a temperature of 25°C was attained, at which point the instrument held this temperature for 2 minutes, before further increasing the temperature to 99 °C, at a rate of 0.5°C/s at which point this temperature was held for a further 2 minutes.

**[0460]** Size exclusion HPLC analysis was completed on the purified protein samples using a TSKgel Super SW3000, 4.6mm ID x 30 cm, 4  $\mu$ m particle size, 250 Å column (Tosoh, 18675) using a mobile phase of 200mM NaPhosphate, 150mM NaCl pH 7.0, at a flow rate of 0.3 ml/min, with an Agilent 1260 HPLC system equipped with a vacuum degasser, binary/quaternary pump, thermostatted autosampler, thermostatted column compartment, diode array detector (DAD), and Chemstation chromatography software). Un-diluted samples (40  $\mu$ g) of each protein were injected after brief centrifugation. System suitability sample (bovine serum albumin, BSA, Thermo Scientific, P/N: 23209) and internal control (wild type HRS) were used to bracket samples to ensure the validity of the test.

**[0461]** Competitive ELISAs were performed in 96-well plates (Immulon 4HBX) which had been coated with a 50  $\mu$ L solution of full length his-tagged HARS, adjusted to a concentration of 2  $\mu$ g/mL with PBS. Plates were sealed and incubated overnight at 4°C. Prior to use, plates were washed five times with PBST and subsequently blocked with 100  $\mu$ L 1%BSA in PBS for one hour at room temperature. While the ELISA plates were blocking, the reduced cysteine competition molecules (over a concentration range of  $1 \times 10^{-6}$ M to  $1 \times 10^{-13}$  M) were incubated with  $\alpha$ -Jo-1 antibodies (GenWay GWB-FB7A3D or Immunovision HJO-0100) at 1:10,000 dilution in 1%BSA PBS in a separate incubation plate (Costar 3357 96-well) for one hour at 4°C. After blocking was finished, the ELISA plates were washed three times with PBST and 50  $\mu$ L of solution containing antibody and competitor was added to the ELISA plate and the samples incubated at room temperature for 1.5 hours. Following initial binding incubation, plates were washed five times with PBST. Next, 50  $\mu$ L of detection antibody (AbD Serotec Goat Anti-human IgG F(ab')<sub>2</sub>:HRP 0500-0099) was added a 1:5,000 dilution and incubated for one hour at room temperature. Following secondary binding incubation, plates were washed with five times PBST and 50  $\mu$ L TMB substrate (Thermo Scientific Pierce TMB Substrate PI-34021) was added. Reactions proceeded for 8 minutes at which point 50  $\mu$ L of 2M sulfuric acid stop solution was added. Colorimetric quantification was performed using a SpectraMax plate reader at 450 nM.

**[0462]** To determine the number of catalytic active sites in each HARS506 cysteine variant the active site titration assay (as described in Fersht et al., (1975) Biochemistry) was employed. Briefly, assays were performed at room temperature with 5  $\mu$ M HARS, 10 mM MgCl<sub>2</sub>, 50  $\mu$ M ATP, 20 mM L-histidine, 2  $\mu$ g/mL inorganic pyrophosphatase, 1.65  $\mu$ M [ $\gamma$ -<sup>32</sup>P]ATP in standard buffer (100 mM HEPES pH 7.5, 20 mM KCl). Reactions were initiated with enzyme in low profile PCR plates and time points were quenched in 96-well



PVDF multiScreen filter plates Millipore containing HClO<sub>4</sub> /charcoal slurry (1:4 7% HClO<sub>4</sub>:10% charcoal slurry) at 30s, 1 min, 2 min, 4 min, 6 min and 10 min. After mixing up and down by pipetting and samples were spun into a collection plate with Supermix scintillant, and counted in a Microbetae plate reader.

| <b>Name</b>     | <b>Tm</b> | <b>% Low molecular weight aggregates</b> | <b>IC50 by ELISA Assay</b> | <b>Active site titration</b> |
|-----------------|-----------|------------------------------------------|----------------------------|------------------------------|
| HRS(1-506)      | 49.0      | 2.0                                      | 0.15                       | 63.3                         |
| HRS(1-506)C174V | 47.8      | 7.8                                      | 0.39                       | 55.5                         |
| HRS(1-506)C174A | 49.2      | 3.0                                      | 0.19                       | 59.8                         |
| HRS(1-506)C191A | 44.7      | 5.1                                      | 0.14                       | 66.2                         |
| HRS(1-506)C191V | 47.8      | 1.8                                      | 0.16                       | 60.8                         |
| HRS(1-506)C191S | 45.8      | 2.3                                      | 0.16                       | 63.2                         |
| HRS(1-506)C224S | 48.9      | 4.9                                      | 0.14                       | 60.5                         |
| HRS(1-506)C235S | 48,8      | 3.1                                      | 0.14                       | 64.6                         |

**[0463]** The results from these studies confirm that all of the cysteine mutants are active, with little or no loss in activity, stability, or conformation as measured by active site titration, ELISA binding and T<sub>m</sub> determinations for thermal denaturation. Active site titration of tRNA synthetase activity reveals that all of the reduced cysteine mutants are active, and thus suitable for use in any of the compositions, methods and kits of the disclosure. In general the Cys191 substitutions displayed overall lower thermostability, while the Cys174 mutants exhibited significantly less heterogeneity as determined by isoelectric focusing.

### EXAMPLE 5

**CREATION OF MODIFIED (TAG FREE) HRS POLYPEPTIDES WITH A C-TERMINAL TRUNCATION (HisRS<sup>N8</sup>) or (HRS(1-506))**

**[0464]** To delete the last three amino acids and the linker between wild type HisRS and the His-tag, primers were designed for use with QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent, cat no 210519). To accomplish this, the following primers are used as listed in **Table E6**.

| Table E6           |                                                 |            |
|--------------------|-------------------------------------------------|------------|
| Mutation           | Oligo sequence                                  | SEQ ID NO: |
| Delete<br>CICAAALE | 5'-CGCCGCACCGGTCAACCGTTACACCACCACCACCACCACTG-3' | 318        |
|                    | (Forward)                                       |            |
|                    |                                                 |            |



| Table E6         |                                                                   |            |
|------------------|-------------------------------------------------------------------|------------|
| Mutation         | Oligo sequence                                                    | SEQ ID NO: |
| Delete CICA AALE | 5'-CAG TGG TGG TGG TGG TGG TGT AAC GGT TGA CCG GTG CCG CG -<br>3' | 319        |
|                  | (Reverse)                                                         |            |

[0465] The deletion was made per the QuikChange Lightning Site-Directed Mutagenesis Kit manufacturer's instructions. After mutagenesis, the sample was treated with Dpn I enzyme at 37°C and transformed into XL10 gold competent cells using routine procedures. Multiple colonies were grown in luria-bertani broth overnight at 37°C and the resulting plasmids were purified with QIAprep Spin Miniprep Kit (Qiagen cat. no.27106). The plasmids were sequenced to confirm the identity of the amino acid substitution of each clone. To delete the His tag, primers were designed for use with QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent, cat no 210519). To accomplish this, the following primers were used as listed in Table E7.

| Table E7               |                                                                 |            |
|------------------------|-----------------------------------------------------------------|------------|
| Mutation               | Oligo sequence                                                  | SEQ ID NO: |
| Delete His-tag Forward | 5'- CGC CGC ACC GGT CAA CCG TTA TGA GAT CCG GCT GCT AAC -<br>3' | 320        |
| Delete His-tag Reverse | 5'- GTT AGC AGC CGG ATC TCA TAA CGG TTG ACC GGT GCG GCG -<br>3' | 321        |

[0466] The deletion was made per the QuikChange Lightning Site-Directed Mutagenesis Kit manufacturer's instructions, as described above.

[0467] **Protein production.** BL21(DE3) competent cells (Novagen, cat. no. 69450) or W3110 cells (ATTC) were transformed with the codon-optimized expression construct encoding HisRS<sup>N8</sup> (HRS(1-506)) as described in Example 2. The expression system, fermentation media, and fermentation conditions used to produce recombinant proteins were essentially the same as those described in Example 2.

[0468] **Purification of tag-free HisRS<sup>N8</sup> (HisRS(1-506)).** Frozen cell paste (400 g) was resuspended in 4-volumes (1600 mL) of Lysis Buffer (50mM Tris, 50mM NaCl, 5mM MgCl<sub>2</sub>, 2mM L-Cysteine, pH7.4). Complete EDTA-FREE protease inhibitor tablets (Roche, Cat # 05 056 489 001) were added to the suspension at a ratio of 1 tablet/50 mL. The suspension was passed through a microfluidizer (Microfluidics) twice at 18,000 psi with cooling by ice. The lysate was centrifuged at 15,000 x g for 45 min at 4°C. The supernatant was filtered through 2-3 AcroPak 1500 capsules (0.8/0.2 µm, Pall, PN12675).

[0469] The clarified lysate was loaded onto a 382 ml Q HP column (5x19.5 cm) pre-equilibrated with Q Buffer A (50 mM Tris, 50 mM NaCl, pH 7.4). The product was eluted with a linear gradient of 0-30% Q Buffer B (50 mM Tris, 1 M NaCl, pH 7.4) over 10 column volumes (CV). Fractions were collected at ½ CV/fraction and analyzed by SDS-PAGE. Pooling was based on gel analysis.

[0470] A 3.5 M ammonium sulfate solution was added to the Q HP pool above to a final concentration of 1.2 M. The mixture was filter through an AcroPak 200 (0.2 um) and loaded onto a 481 ml Phenyl HP column



(5x24.5 cm) pre-equilibrated with 20 mM Tris, 1.2 M ammonium sulfate, pH 7.0. The product was eluted with a linear gradient of 1.2 - 0 M ammonium sulfate in 20 mM Tris/pH 7.0 over 10 CV. Fractions (½ CV/fraction) containing the product based on SDS-PAGE analysis were pooled.

**[0471]** The Phenyl Pool from above was concentrated to 0.5 L via a TFF system, consisting of a Pellicon Mini cassette holder (Millipore Cat#XX42PMINI), a Masterflex I/P pump, and 2 x 0.1 m<sup>2</sup> cassette (30 kD MWCO, Novasep Cat#PP030M01L). The concentrated solution was then buffer exchanged with 6 diavolumes (3 L) of CHT Buffer A (10 mM sodium phosphate, 150 mM NaCl, pH 7.0). The retentate was filtered through a 0.2 µm Millex GP-50 filter (Millipore part # SLGP 05010) before proceeding to the next step.

**[0472]** The above solution was loaded onto a 380 ml ceramic hydroxyapatite (CHT) column (5x19.4 cm) pre-equilibrated with CHT Buffer A. The column was washed with Buffer A and followed by 6% Buffer B (500 mM sodium phosphate, 150 mM NaCl, pH 7.0). The product was eluted with a linear gradient of 6-56% Buffer B over 10 CV. Fractions (½ CV/fraction) containing the product based on SDS-PAGE analysis were pooled.

**[0473]** Using the same TFF system, the CHT Pool was concentrated to ~0.2 L, buffer exchanged with 6 diavolumes of the current formulation buffer (20 mM sodium phosphate, 150 mM NaCl, pH 7.0), and concentrated to a target concentration of ~10 mg/ml. The product solution was filtered through a 0.2 µm Millex GP-50 filter (Millipore part # SLGP 05010), and stored in -80°C freezer.

#### **EXAMPLE 6**

#### **EVALUATION OF HRS(1-60) (RESOKINE) AS AN ANTI-INFLAMMATORY AGENT**

**[0474]** To evaluate the potential anti-inflammatory property of HRS derived polypeptides, an N-terminal, naturally occurring splice variant comprising amino acids 1-60 of HRS ("Resokine") was tested in a TNBS induced model of colitis. Studies were performed in male BDF-1 mice, with 12 mice / group; Budesonide was added at 5 mg / kg orally.

**[0475]** In this study Resokine was administered daily by IV injection, starting 3 days prior to TNBS treatment, at a concentration of 1 or 5 mg/Kg. The data shown in **Figure 4** demonstrates that treatment with Resokine at either concentration resulted in a significant increase in survival. Accordingly Resokine appears to have potent anti-inflammatory effects, consistent with the hypothesis that HRS polypeptides are involved in the local control of inflammatory processes.

#### **EXAMPLE 7**

#### **EVALUATION OF HRS POLYPEPTIDES FOR THE TREATMENT OF STATIN-INDUCED MYOSITIS AND RHABDOMYOLYSIS**

**[0476]** Statins are HMG CoA Reductase inhibitors which inhibit the synthesis of mevalonate, the rate limiting step in cholesterol synthesis. Statin therapy has proved beneficial in lowering cholesterol levels in patients. However, side-effects and complications of statin therapy include muscle weakness, myositis and rhabdomyolysis. Muscle myopathy is a complication with several statins on the market and patients are often removed from their statin-therapy if they exhibit any of these symptoms. Like many other myopathies,



muscular dystrophies and inflammatory disorders of muscle, disease progression in statin induced myopathy appears to occur as the result of an initial chemical, genetic or physical injury, which becomes increasingly inflamed as a result of immune cell invasion into the damaged muscle cells.

**[0477]** Accordingly statin induced myopathy represents a broadly applicable model system to study drug induced myositis, which is directly applicable to other myopathies and muscular dystrophies, all of which all share a common inflammatory component which mediates disease progression by promoting immune cell invasion of the damaged muscle tissue.

**[0478]** The purpose of this study was to evaluate the efficacy of HRS(1-506) in reversing the effects of statin-induced muscular myositis, as indicated by altered circulating enzyme levels, and changes in gene expression of muscle function and inflammatory markers in response to treatment with HRS(1-506).

**[0479]** To achieve this, rats were dosed daily with 1mg/kg Cerivastatin and then switched to an every other day (qod) dosing with Cerivastatin. The goal of this dosing regimen was to maintain a sustained disease state in the animals, but not to have such severe disease that rat survival is greatly impacted. The efficacy of a dose range of HRS(1-506) was then evaluated in rats after statin-dosing had already initiated measureable changes in circulating markers of myositis.

**[0480] Protocol and Methods.** In this study, 10 week old female Sprague-Dawley rats were treated with 1mg/kg Cerivastatin ((Sigma, Cat No. SML0005) in 0.5% methylcellulose, starting on day 1 via oral gavage. After 7 days of daily administration, rats were switched to an every other day dosing strategy (qod) on days 9, 11 and 13. HRS(1-506) and vehicle administration were initiated on day 6 through intravenous injection and rats were dosed daily to day 14 (shown schematically in **Figure 5A**). All rats were taken down on day 15, 24 hours after the final test article dosing and 48 hours after the last statin administration. HRS(1-506) was administered at 3 doses (0.3, 1.0 and 3.0 mg/kg) in 20mM NaPO<sub>4</sub>, 0.15M NaCl, pH 7.0 daily.

**[0481]** To address the primary objective of this study, the following study measurements and endpoints were performed: rat survival, weight, circulating serum CK levels at days 12 and 15, H&E staining on day 15 hamstring samples, Troponin-I ELISA, CBC on day 15 blood, qPCR on hamstring samples and serum endogenous HARS levels.

**[0482] qPCR methods.** Mouse hamstring was excised from the animals and stored at -80 °C until analysis. Tissue was prepped in groups of 10 hamstrings using Qiagen's RNeasy Fibrous Tissue Midi Kit (Catalog #75742). Once RNA was eluted from the Qiagen column, it was run on an Agilent's Bioanalyzer 2100 to test RNA integrity and NanoDrop to determine RNA concentration and purity. RNA was then stored at -80 °C.

**[0483]** Reverse transcription (RT) of RNA to cDNA was performed in a 96 well PCR plate format in Eppendorfs Mastercycler PCR machine with the following program: 37°C for 60 minutes, 95 °C for 5 minutes. The edge wells of the 96 well plate were not used and filled with 50mL water to prevent evaporation of inside wells. 20mL RNA and 30mL of reverse transcription master mix (Ambion's TaqMan PreAmp Cells to CT Kit catalog #4387299) was used per sample RT. Once RT was completed, next step was to pre-amplify genes of interest in the sample cDNA. Primers of genes of interest (DELTAgene primers designed by Fluidigm) were combined to a final concentration of 200nM. Using these primers, genes of interest were pre-amplified in each sample. Pre-amplification was performed in 10mL reactions (2.5mL cDNA, 7.5mL Pre-Amp mastermix) in 384-well format using an Applied Biosystems ViiA7 PCR machine with the following program: 95°C for 10 minutes, 14 cycles of 95°C for 15 seconds and 60°C for 4 minutes. After pre-amplification step, exonuclease (New England BioLabs catalog #M0293L) was added to remove unincorporated primers from each sample. This exonuclease reaction was also completed in the ViiA7 PCR machine with the following program: 37 °C for 30 minutes, 80°C for 15 minutes. After exonuclease, the RT sample was further diluted 1:5 (7mL exonuclease sample + 18mL low EDTA buffer).



**[0484]** The chip used to run qPCR on Fluidigm's Biomark system was a 96.96 Dynamic Array IFC for Gene Expression. The chip was first primed with the IFC controller HX as per manufacturer's recommendations before sample and assays were loaded. To prepare assays to be loaded on a chip, 4.4mL assay master mix (Fluidigm's 2X Assay Loading Reagent catalog #8500736 and low EDTA TE) to 3.6mL 20mM forward and reverse primers for each gene of interest were prepared in a 96 well plate. To prepare samples, 4.5mL sample master mix (Ambion's 2X TaqMan Gene Expression Master Mix, Fluidigm's 20X DNA Binding Dye Sample Loading Reagent catalog number 100-0388, and Biotium's 20X EvaGreen catalog #31000) was added to 3mL diluted pre-amplified/exonuclease sample in a 96 well plate. Once the chip had been primed, 5mL sample or assay prepared above were loaded onto the chip. The chip was then returned to the IFC controller for the samples to be loaded into the chip. After the chip had finished loading, qPCR could then be run on the Biomark using preset program for 96.96 Dynamic Array for Gene Expression with a melt curve to determine primer specificity. Relative gene expression was determined by the delta delta Ct method.

**[0485] Quantification of extracellular HARS.** A 96 well based ELISA was developed in-house using 2 mouse anti-HARS monoclonal antibodies M03 (Sigma #SAB1403905, and Abnova #H00003035-M03) and M01 (Abgent #AT2317a) in a sandwich format to detect HARS in rat serum. Assays were run in 96 well Costar plates (Costar 96-well plate #3369) using a seven point standard curve which was generated ranging from 75 to 0.1 ng/ml using a stock solution of HRS(1-506); (7.5 mg/ml in 20 mM NaPO<sub>4</sub>, 0.15 M NaCl pH 7.0, using 1x PBST (0.05% Tween-20) as a diluent). The M01 mouse monoclonal, clone 1C8 (Abgent #AT2317a) was biotinylated in house and used as the detection antibody, and the M03 mouse monoclonal antibody (Sigma #SAB1403905, lot#11238, 0.5mg/mL and Abnova #H00003035-M03, lot#11238, 0.5mg/mL) was used as a capture antibody. Casein (Thermo Scientific #37528) was used as a blocking agent, and 1x PBST (0.05% Tween-20) was used as a wash buffer. Antibody binding was quantified using Streptavidin-HRP (Invitrogen cat#434323, Lot# 816755A) using TMB Substrate (Thermo #34021) and with 2M sulfuric acid as the stop solution.

**[0486]** ELISA assays were run by coating plates overnight with 0.6 to 2 µg/ml M03 antibody in IX PBS, which were then blocked by incubation with casein for one hour 1 hour, and washed 3x with PBST. Plates were then incubated with standards and samples for 1 hour, washed 3 x PBST, and then incubated with 500 ng/ml biotinylated-M01 diluted in PBST, 1 hour, washed 3 x PBST, incubated with 200 ng/ml streptavidin-HRP for 1 hour, washed 3x with PBST, and then the TMB substrate added for 4 minutes. Reactions were stopped with stop solution and absorbance read at 450 nm.

**[0487]** The results were quantified based on the standard curve based on the average raw absorbance values without background subtraction. Prism was used for standard curve fitting. Model: Log(agonist) vs. response fit [4-parameter logistic regression] Percent recovery was calculated for each individual concentration point (not averaged) by:

$$\frac{(\text{measured} - \text{actual}) \times 100\%}{(\text{actual})}$$

**[0488] Other Readouts.** Rats were weighed daily. Serum samples were taken on days 1, 8, 12 (via tail vein) and day 15 (terminal) to be used for circulating enzyme analysis (Idexx) and serum skeletal muscle Troponin-I measurements, were measured using a commercial ELISA kit. Urinalysis was performed on days 3, 5, 8, 10, 12 and 15 prior to dosing on that day. CBC analysis was run on blood isolated on day 15 prior to euthanizing rats. On day 15, the rats were euthanized and a portion of the hamstring muscle and lung (not inflated) was placed in 10% NBF for paraffin embedding and H&E staining of sections (Premier Laboratory). Another portion of hamstring muscle and lung was placed at -80C to use for RNA extraction and profiling. Liver, kidney and heart were also isolated on day 15 and placed in zinc-formalin for paraffin embedding (TSRI Histology) for long-term tissue storage.



**[0489] Results.** There was 100% survival in this study, and all rats survived to the scheduled takedown on day 15. Statin-dosed rats had lower average weights than control rats not dosed with statin. On day 15, the statin + vehicle group had the lowest average rat weight of all the groups, whereas the Statin + 3mg/kg HRS(1-506)-dosed group had the highest weight average of all the statin-treated animals (data not shown). CBC analysis showed overall similar patterns of changes between different animal treatment groups (data not shown).

**[0490]** A small increase in serum CK was observed in statin treated rats over untreated controls on days 12 and 15. On day 12, rats dosed with 1mg/kg and 3mg/kg HRS(1-506) had smaller, tighter CK averages compared to Statin + Vehicle treated animals (**Figures 6A-B**), consistent with a positive impact of HRS(1-506) treatment on statin induced myositis, also consistent with a positive effect of HRS(1-506) on muscle function, muscle troponin C levels were also reduced in HRS(1-506) treated animals (**Figure 5B**). Moreover endogenous serum HRS levels were elevated in statin-treated rats compared to rats not receiving statin (**Figure 7**), suggesting that the release of HRS may play a role as an endogenous regulator of muscle inflammation. H&E staining on hamstrings demonstrated reduced muscle degeneration/necrosis and inflammation scores in statin-treated rats dosed with 1mg/kg and 3mg/kg HRS(1-506) compared to vehicle-dosed and 0.3mg/kg HRS(1-506)-dosed rats (**Figure 8**).

**[0491]** To further investigate the mechanistic basis for the effects of HRS on statin induced myopathy, changes in gene expression in the hamstrings from treated animals was examined after the completion of the study. RNA profiling was performed on hamstring muscles isolated from the rats on day 15 as described above. The results from these studies demonstrated that all 13 genes that were elevated by more than 5 fold in response to statin treatment were reduced by treatment with HRS(1-506) (see **Table E8**; and **Figures 9-10**)

| Table E8                            |                                     |                                    |           |
|-------------------------------------|-------------------------------------|------------------------------------|-----------|
| Gene regulated by more than 25 fold | Gene regulated by more than 10 fold | Gene regulated by more than 4 fold | No Change |
| CD8a                                | MCP1                                | CD11a                              | HARS      |
| MMP9                                | CD8b                                | CD11b                              | HARS2     |
| IL6                                 | CCR5                                | CD45                               | DARS      |
| IL10                                | CD18                                | SDC1                               | GARS      |
|                                     |                                     | IFNg                               | QARS      |

**[0492]** Transcriptional profiling of statin treated rat hamstrings: revealed that 10 diabetes/metabolic syndrome related genes (**Figure 11**) and several housekeeping genes (data not shown) were not significantly impacted by HRS treatment. By contrast, transcriptional profiling of statin treated rat hamstrings of 26 immune cell marker genes revealed significant changes in a larger number of genes (see **Figures 12-14**), including the dose dependent inhibition of ITGAL(CD11a), CD11b, CD8a, CD8b, CD18, CCR5, PTPPC and (CD45R) expression. Additionally HRS(1-506) was effective in reducing the expression of a number of inflammatory marker genes including IL6, MCP1, IL10 and IFN gamma (see **Figures 15-16**). Transcriptional changes were also observed in 14 adhesion, development, and fibrosis related genes (see **Figures 17-18**), the muscle contractility gene Neb (data not shown), and in genes associated with muscular wasting, atrophy, and myogenesis (see **Figures 19-20**).

**[0493] Conclusions.** Decreased CK, serum Troponin-I and muscle cell degeneration/necrosis and muscle inflammation were all observed in animals receiving higher doses of HRS(1-506), either at 1.0mg/kg or 3.0mg/kg in contrast to animals receiving either Vehicle or low dose 0.3mg/kg HRS(1-506). RNA profiling data supported these results by demonstrating reduced CD8a, IL-6, MCP-1 and MMP-9 expression in hamstrings of statin-treated rats dosed with higher doses of HRS(1-506). Up-regulation of these genes is most likely due to increased immune cell infiltrate into damaged muscle tissue. Based on the identity of the expressed genes,



the infiltrating immune cells are likely to be made up of one of more of the following cell types, T cells, dendritic cell, NK cells, and macrophage/monocytes. All of these cell types have been associated with muscle inflammation, and the ability of the HRS polypeptides, including HRS(1-506) to mediate a dramatic inhibition of this immune cell influx suggests that HRS polypeptides such as HRS(1-506) represent potent immunoregulators, which are capable of acting as potent immunomodulators in a broad range of inflammatory and autoimmune diseases and disorders.

EXAMPLE 8

PREPARATION OF HRS-FC POLYPEPTIDES

[0494] N-terminal and C-terminal Fc-histidyl tRNA synthetase (HRS-Fc) fusion proteins were prepared, purified, and analyzed as follows.

[0495] **Plasmid construction.** The human IgG1 Fc domain was amplified by polymerase chain reaction (PCR) before inserting into the C-term or N-term of the HRS polypeptide HRS(1-60) via sequential PCR reactions using the primers below, and the resulting amplified DNA fragments inserted into C-term or N-term of HRS(1-60) located in the pET28 expression vector (Novagen 69864). It will be appreciated that the creation of the N-terminal Fc fusion protein results in the deletion/replacement of the N-terminal methionine in HRS(1-60) with the C-terminal amino acid of the Fc domain, and vice versa where appropriate.

[0496] The following primers were used to create the N-terminally fused HRS(1-60) Fc fusion protein (Fc-HRS(2-60)) (Table E9):

| Table E9         |                                                                  |            |
|------------------|------------------------------------------------------------------|------------|
| Primer Sequences |                                                                  |            |
| Primer Name      | Sequence                                                         | SEQ ID NO: |
| FcNSV9-F         | TAATTTGTTTAACTTTAAGAAGGAGATATACATATGTCTGACAAAACCTC<br>ACACATGCCC | 323        |
| FcNSV9tail       | AGCCTCTCCCTGTCTCCGGGTAAAGCAGAGCGTGCGGCGCTGG                      | 324        |
| FcNSV9-R         | CCAGCGCCGCACGCTCTGCTTTACCCGGAGACAGGGAGAGGCT                      | 325        |
| FcNSV9-F2        | TTTTGTTTAACTTTAAGAAGGAGATATACATATGTCTGACAAAACCTCACA<br>CATGCCC   | 326        |
| FcNSV9tail2      | CTCTCCCTGTCTCCGGGTAAAGCAGAGCGTGCGGCGC                            | 327        |
| FcNSV9-R2        | GCGCCGCACGCTCTGCTTTACCCGGAGACAGGGAGAG                            | 328        |

[0497] The following primers were used to create the C-terminally fused HRS(1-60) Fc fusion protein (HRS(1-60)-Fc) (Table E10).



| Table E10        |                                                                 |            |
|------------------|-----------------------------------------------------------------|------------|
| Primer Sequences |                                                                 |            |
| Primer Name      | Sequence                                                        | SEQ ID NO: |
| FcCSV9-F         | CAAACAGAAATTTGTGCTCAAAACCCCCAAGTCTGACAAAAC TCACAC<br>ATGCCCACCG | 329        |
| FcCSV9tail       | AGCCTCTCCCTGTCTCCGGGTAAATGAGATCCGGCTGCTAACAAAGCC<br>C           | 330        |
| FcCSV9-R         | GGGCTTTGTTAGCAGCCGGATCTCATTTACCCGGAGACAGGGAGAGGCT               | 331        |
| FcCSV9-F2        | CAGAAATTTGTGCTCAAAACCCCCAAGTCTGACAAAAC TCACACATGC<br>CC         | 332        |
| FcCSV9tail2      | CTCTCCCTGTCTCCGGGTAAATGAGATCCGGCTGCTAACAAAG                     | 333        |
| FcCSV9-R2        | CTTTGTTAGCAGCCGGATCTCATTTACCCGGAGACAGGGAGAG                     | 334        |

[0498] The PCR reactions were performed using recommended thermal cycling parameters, and the PCR-amplified fragments were verified on by gel electrophoresis. Sequences were confirmed by performing alignments with the theoretical sequences using EMBOSS Pairwise Alignment Algorithms. The cloned DNA and protein sequences of Fc-HRS(2-60) and HRS(1-60)-Fc are shown below.

DNA sequence of Fc-HRS(2-60) (N-terminal Fc fusion):  
ATGTCTGACAAAAC TCACACATGCCACCGTGCCAGCACCTGAACTCCTGGGGGGACC  
GTCAGTCTTCCTCTTCCCCC AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGA  
GGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGT  
ACGTGGACGGCGTGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAA  
CAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAA  
GGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCAGCCCCATCGAGAAAACCATCT  
CCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAG  
GAGATGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGA  
CATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAAC TACAAGACCACGCCTC  
CCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCA  
GGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCACGAGGCTCTGCACAACCACT  
ACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAAGCAGAGCGTGCGGCGCTGGAGGAG  
CTGGTGAAACTTCAGGGAGAGCGCGTGCGAGGCCTCAAGCAGCAGAAGGCCAGCGCCG  
AGCTGATCGAGGAGGAGGTGGCGAAACTCCTGAAACTGAAGGCACAGCTGGGTCTTGAT  
GAAAGCAAACAGAAATTTGTGCTCAAAACCCCCAAG TGA (SEQ ID NO:335)

DNA sequence of HRS(1-60)-Fc (C-terminal Fc fusion).  
ATGGCAGAGCGTGCGGCGCTGGAGGAGCTGGTGAAACTTCAGGGAGAGCGCGTGCGAG  
GCCTCAAGCAGCAGAAGGCCAGCGCCGAGCTGATCGAGGAGGAGGTGGCGAAACTCCT  
GAAACTGAAGGCACAGCTGGGTCTGATGAAGCAAACAGAAATTTGTGCTCAAAACCCC  
CAAGTCTGACAAAAC TCACACATGCCACCGTGCCAGCACCTGAACTCCTGGGGGGAC  
CGTCAGTCTTCCTCTTCCCCC AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTG  
AGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGG  
TACGTGGACGGCGTGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACA



ACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCA  
AGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATC  
TCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGA  
GGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCG  
ACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACCACGCC  
TCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAG  
CAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCACGAGGCTCTGCACAACC  
ACTACACGCAGAAGAGC CTC TCC CTG TCT CCG GGTAATGA (SEQ ID NO:336)

DNA sequence of Fc-HRS(2-60) (N-terminal Fc fusion)  
MSDKTHTCPPCAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD  
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG  
QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGSE  
  
FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKAERAALEELVKLQGERVR  
GLKQQKASAEIEEEVAKLLKLKAQLGPDESKQKFVLKTPK (SEQ ID NO:337)

Protein sequence of HRS(1-60)-Fc (C-terminal Fc fusion)  
MAERAALEELVKLQGERVRGLKQQKASAEIEEEVAKLLKLKAQLGPDESKQKFVLKTPKS  
DKTHTCPPCAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV  
EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP  
REPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGSFFL  
YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK  
(SEQ ID NO:338)

[0499] Additional N-terminal and C-terminal Fc-histidyl tRNA synthetase (HRS-Fc) DNA constructs were prepared as follows.

[0500] **Plasmid construction.** HRS (2-60) with an N-terminal Fc or HRS (1-60) with a C-terminal Fc (Example 8) were subcloned into a modified pET24b vector (EMD, Gibbstown, NJ) containing a TAC promoter instead of T7 ("pET24b\_TAC"). The Fc-HRS (2-60) and HRS (1-60)-Fc were amplified by polymerase chain reaction (PCR) using the primers below which contain a 5' NdeI site and a 3' XhoI site, and the resulting amplified DNA was subcloned into pET24b\_TAC using the NdeI and XhoI restriction sites.

[0501] The following primers were used to amplify the Fc-HRS (2-60) (N-terminal Fc fusion) (Table E11):

| Table E11        |                                   |            |
|------------------|-----------------------------------|------------|
| Primer Sequences |                                   |            |
| Primer Name      | Sequence                          | SEQ ID NO: |
| 1921-NdeI-FWD    | GATATACATATGTCTGACAAACTCACACATGCC | 343        |
| 1921-XhoI-REV    | GATCCTCGAGTCACTTGGGGGTTTTG        | 344        |

[0502] The following primers were used to amplify the HRS (1-60)-fc (C-terminal Fc fusion) (Table E12).

| Table E12        |                            |            |
|------------------|----------------------------|------------|
| Primer Sequences |                            |            |
| Primer Name      | Sequence                   | SEQ ID NO: |
| 1922-NdeI-FWD    | GATATACATATGGCAGAGCGTGCGG  | 345        |
| 1922-XhoI-REV    | GATCCTCGAGTCATTTACCCGGAGAC | 346        |



| Table E12        |          |            |
|------------------|----------|------------|
| Primer Sequences |          |            |
| Primer Name      | Sequence | SEQ ID NO: |

[0503] The PCR reactions were performed using recommended thermal cycling parameters, and the PCR-amplified fragments were verified by gel electrophoresis. Sequences were confirmed by performing alignments with the theoretical sequences using DNASTAR Lasergene SeqMan Pro. The cloned DNA and protein sequences of Fc-HRS(2-60) and HRS(1-60)-Fc are shown below.

DNA sequence of HRS(2-60)-Fc (C-terminal Fc fusion):

ATGTCTGACAAACTCACACATGCCACCGTGCCACGACCTGAACTCCTGGGGGGACC  
GTCAGTCTTCCTCTTCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGA  
GGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGT  
ACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAA  
CAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAA  
GGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCATCGAGAAAACCATCT  
CCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAG  
GAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGA  
CATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACCACGCCTC  
CCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCA  
GGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCACGAGGCTCTGCACAACCACT  
ACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAAGCAGAGCGTGCGGCGCTGGAGGAG  
CTGGTGAAACTTCAGGGAGAGCGCGTGCAGAGGCTCAAGCAGCAGAAGGCCAGCGCCG  
AGCTGATCGAGGAGGAGGTGGCGAAACTCCTGAAACTGAAGGCACAGCTGGGTCCTGAT  
GAAAGCAAACAGAAATTTGTGCTCAAAACCCCCAAG tga (SEQ ID NO:347)

DNA sequence of HRS(1-60)-Fc (C-terminal Fc fusion):

ATGGCAGAGCGTGCGGCGCTGGAGGAGCTGGTGAAACTTCAGGGAGAGCGCGTGCGAG  
GCCTCAAGCAGCAGAAGGCCAGCGCCGAGCTGATCGAGGAGGAGGTGGCGAAACTCCT  
GAAACTGAAGGCACAGCTGGGTCTGATGAAAGCAAACAGAAATTTGTGCTCAAAACCC  
CCAAGTCTGACAAAACTCACACATGCCACCGTGCCACGACCTGAACTCCTGGGGGGGA  
CCGTGAGTCTTCCTCTTCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCT  
GAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTG  
GTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTAC  
AACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGC  
AAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCATCGAGAAAACCAT  
CTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGG  
AGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGC  
GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACCACGC  
CTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGA  
  
GCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCACGAGGCTCTGCACAAC  
CACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA tga (SEQ ID NO:348)

Protein sequence of Fc-HRS(2-60) (N-terminal Fc fusion):

MSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD  
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG  
QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSF  
FLYSKLTVDKSRWQQGNVVFSCVMHEALHNHYTQKSLSLSPGKAERAALEELVKLQGERVR



GLKQQKASAELIEEEVAKLLKLKAQLGPDESKQKFVLKTPK. (SEQ ID NO:349)

Protein sequence of HRS(1-60)-Fc (C-terminal Fc fusion):  
MAERAAL EELVKLQGERVRGLKQQKASAELIEEEVAKLLKLKAQLGPDESKQKFVLKTPKS  
DKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV  
EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP  
REPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFL  
YSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:350)

[0504] Additional N-terminal and C-terminal Fc-histidyl tRNA synthetase (HRS-Fc) DNA constructs were prepared as follows.

[0505] **Plasmid construction.** HRS (2-40), (2-45), (2-50), (2-55), (2-66) with an N-terminal Fc or HRS (1-40), (1-45), (1-50), (1-55), (1-66) with a C-terminal Fc were generated using Quikchange Mutagenesis (Agilent, Santa Clara, CA). Previously generated pET24b\_TAC constructs containing Fc-HRS(2-60) and HRS(1-60)-Fc in combination with the primers listed below, were used in the Quikchange reaction to generate the HRS-Fc constructs.

[0506] The following primers were used to amplify the Fc-HRS (2-40), (2-45), (2-50), (2-55), (2-66) polypeptides (N-terminal Fc fusion) (Table E13):

| Table E13            |                                                                               |            |
|----------------------|-------------------------------------------------------------------------------|------------|
| Primer Sequences     |                                                                               |            |
| Primer Name          | Sequence                                                                      | SEQ ID NO: |
| Fc-H-aa2-40 FWD      | 5'- GGT GGC GAA ACT CCT GAA ATG ACT CGA GGA TCC GGC TGC -3'                   | 351        |
| Fc-H-aa2-40 REV      | 5'- GCA GCC GGA TCC TCG AGT CAT TTC AGG AGT TTC GCC ACC -3'                   | 352        |
| Fc-H-aa2-45 FWD      | 5'- CTG AAG GCA CAG CTG TGA CTC GAG GAT CCG GCT GC -3'                        | 353        |
| Fc-H-aa2-45 REV      | 5'- GCA GCC GGA TCC TCG AGT CAC AGC TGT GCC TTC AG -3'                        | 354        |
| Fc-H-aa2-50 FWD      | 5'- GGG TCC TGA TGA AAG CTG ACT CGA GGA TCC GGC TGC -3'                       | 355        |
| Fc-H-aa2-50 REV      | 5'- GCA GCC GGA TCC TCG AGT CAG CTT TCA TCA GGA CCC -3'                       | 356        |
| Fc-H-aa2-55 FWD      | 5'- GCA AAC AGA AAT TTG TGT GAC TCG AGG ATC CGG CTG C -3'                     | 357        |
| Fc-H-aa2-55 REV      | 5'- GCA GCC GGA TCC TCG AGT CAC ACA AAT TTC TGT TTG C -3'                     | 358        |
| Fc-H-add-aa61-66 FWD | 5'- GCT CAA AAC CCC CAA GGG AAC CCG TGA TTA TAG TTG ACT                       | 359        |
|                      | CGA GGA TCC GG -3'                                                            |            |
| Fc-H-add-aa61-66 REV | 5'- CCG GAT CCT CGA GTC AAC TAT AAT CAC GGG TTC CCT TGG<br>GGG TTT TGA GC -3' | 360        |



| Table E13        |                     |            |
|------------------|---------------------|------------|
| Primer Sequences |                     |            |
| Primer Name      | Sequence            | SEQ ID NO: |
|                  | GGG TTT TGA GGC -3' |            |

[0507] The following primers were used to amplify the HRS (1-40), (1-45), (1-50), (1-55), (1-66) -Fc (C-terminal Fc fusion): (Table E14).

| Table E14            |                                                                           |            |
|----------------------|---------------------------------------------------------------------------|------------|
| Primer Sequences     |                                                                           |            |
| Primer Name          | Sequence                                                                  | SEQ ID NO: |
| H-aa1-40-Fc FWD      | 5'- GGT GGC GAA ACT CCT GAA ATC TGA CAA AAC TCA CAC ATG C -3'             | 361        |
| H-aa1-40-Fc REV      | 5'- GCA TGT GTG AGT TTT GTC AGA TTT CAG GAG TTT CGC CAC C -3'             | 362        |
| H-aa1-45-Fc FWD      | 5'- CTG AAA CTG AAG GCA CAG CTG TCT GAC AAA ACT CAC ACA TGC -3'           | 363        |
| H-aa1-45-Fc REV      | 5'- GCA TGT GTG AGT TTT GTC AGA CAG CTG TGC CTT CAG TTT CAG -3'           | 364        |
| H-aa1-50-Fc FWD      | 5'- GCT GGG TCC TGA TGA AAG CTC TGA CAA AAC TCA CAC ATG C -3'             | 365        |
| H-aa1-50-Fc REV      | 5'- GCA TGT GTG AGT TTT GTC AGA GCT TTC ATC AGG ACC CAG C -3'             | 366        |
| H-aa1-55-Fc FWD      | 5'- GAA AGC AAA CAG AAA TTT GTG TCT GAC AAA ACT CAC ACA TGC -3'           | 367        |
| H-aa1-55-Fc REV      | 5'- GCA TGT GTG AGT TTT GTC AGA CAC AAA TTT CTG TTT GCT TTC -3'           | 368        |
| H-add-aa61-66-Fc FWD | 5'- GCT CAA AAC CCC CAA GGG AAC CCG TGA TTA TAG TTC TGA CAA AAC TCA C -3' | 369        |
| H-add-aa61-66-Fc REV | 5'- GTG AGT TTT GTC AGA ACT ATA ATC ACG GGT TCC CTT GGG GGT TTT GAG C -3' | 370        |



**[0508]** The PCR reactions were performed using manufacturer recommended thermal cycling parameters. Sequences were confirmed by performing alignments with the theoretical sequences using DNASTAR Lasergene SeqMan Pro. The cloned DNA and protein sequences of the HRS-Fc constructs are shown below.

DNA sequence of Fc-HRS (2-40) (N-terminal Fc fusion):

```

ATGTCTGACAAAACCTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGG
GACCGTCAGTCTTCCTCTTCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCC
CTGAGGTCACATGCGTGTTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAAC
TGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGT
ACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATG
GCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACC
ATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCCG
GGAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCA
GCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACCAC
GCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAA
GAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCACGAGGCTCTGCACA
ACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAAGCAGAGCGTGCGGCGCTG
GAGGAGCTGGTGAACTTCAGGGAGAGCGCGTGCGAGGCCTCAAGCAGCAGAAGGCCA
GCGCCGAGCTGATCGAGGAGGAGGTGGCGAAACTCCTGAAATGA (SEQ ID NO:371)

```

DNA sequence of Fc-HRS (2-45) (N-terminal Fc fusion):

```

ATGTCTGACAAAACCTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGG
GACCGTCAGTCTTCCTCTTCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCC
CTGAGGTCACATGCGTGTTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAAC
TGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGT
ACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATG
GCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACC
ATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCCG
GGAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCA
GCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACCAC
GCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAA
GAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCACGAGGCTCTGCACA
ACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAAGCAGAGCGTGCGGCGCTG
GAGGAGCTGGTGAACTTCAGGGAGAGCGCGTGCGAGGCCTCAAGCAGCAGAAGGCCA
GCGCCGAGCTGATCGAGGAGGAGGTGGCGAAACTCCTGAAACTGAAGGCACAGCTGTGA
(SEQ ID NO:372)

```

DNA sequence of Fc-HRS (2-50) (N-terminal Fc fusion):

```

ATGTCTGACAAAACCTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGG
GACCGTCAGTCTTCCTCTTCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCC
CTGAGGTCACATGCGTGTTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAAC
TGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGT
ACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATG
GCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACC
ATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCCG
GGAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCA
GCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACCAC
GCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAA
GAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCACGAGGCTCTGCACA
ACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAAGCAGAGCGTGCGGCGCTG
GAGGAGCTGGTGAACTTCAGGGAGAGCGCGTGCGAGGCCTCAAGCAGCAGAAGGCCA

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GCGCCGAGCTGATCGAGGAGGAGGTGGCGAAACTCCTGAAACTGAAGGCACAGCTGGGT  
CCTGATGAAAGCTGA (SEQ ID NO:373)

DNA sequence of Fc-HRS (2-55) (N-terminal Fc fusion):

ATGTCTGACAAAACCTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGG  
GACCGTCAGTCTTCCTCTTCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCC  
CTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAAC  
TGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGT  
ACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATG  
GCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACC  
ATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCCG  
GGAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCA  
GCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACCAC  
GCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAA  
GAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCACGAGGCTCTGCACA  
ACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAAGCAGAGCGTGCGGCGCTG  
GAGGAGCTGGTGAAACTTCAGGGAGAGCGCGTGCGAGGCCTCAAGCAGCAGAAGGCCA  
GCGCCGAGCTGATCGAGGAGGAGGTGGCGAAACTCCTGAAACTGAAGGCACAGCTGGGT  
CCTGATGAAAGCAAACAGAAATTTGTGTGA (SEQ ID NO:374)

DNA sequence of Fc-HRS(2-66) (N-terminal Fc fusion):

ATGTCTGACAAAACCTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGG  
GACCGTCAGTCTTCCTCTTCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCC  
CTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAAC  
TGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGT  
ACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATG  
GCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACC  
ATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCCG  
GGAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCA  
GCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACCAC  
GCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAA  
GAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCACGAGGCTCTGCACA  
ACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAAGCAGAGCGTGCGGCGCTG  
GAGGAGCTGGTGAAACTTCAGGGAGAGCGCGTGCGAGGCCTCAAGCAGCAGAAGGCCA  
GCGCCGAGCTGATCGAGGAGGAGGTGGCGAAACTCCTGAAACTGAAGGCACAGCTGGGT  
CCTGATGAAAGCAAACAGAAATTTGTGCTCAAAACCCCCAAGGGAACCCGTGATTATAG  
TTGA (SEQ ID NO:375)

DNA sequence of HRS(1-40)-Fc (C-terminal Fc fusion):

ATGGCAGAGCGTGCGGCGCTGGAGGAGCTGGTGAAACTTCAGGGAGAGCGCGTG  
CGAGGCCTCAAGCAGCAGAAGGCCAGCGCCGAGCTGATCGAGGAGGAGGTGGCGAAAC  
TCCTGAAATCTGACAAAACCTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGG  
GACCGTCAGTCTTCCTCTTCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCC  
CTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAAC  
TGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGT  
ACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATG  
GCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACC  
ATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCCG  
GGAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCA  
GCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACCAC



GCC TCCC GTG CTG GACT CCG ACG GCT CCT TCT TCCT CTAC AGCA AGCT CAC CGT GGA CAA  
GAG CAG GTGG CAG CAG GGG AAC GTCT TCT CAT GCT CCG TGAT GCAC GAG GCT CTG CACA  
ACCA CTAC ACG CAGA AGAG CCTCT CCGT GTCT CCG GGTAA ATGA (SEQ ID NO:376)

DNA sequence of HRS(1-45)-Fc (C-terminal Fc fusion):

ATGGCAGAGCGTGCGGGCGCTGGAGGAGCTGGTGAAACTTCAGGGAGAGCGCGTGCGAG  
GCCTCAAGCAGCAGAAGGCCAGCGCCGAGCTGATCGAGGAGGAGGTGGCGAAACTCCT  
GAAACTGAAGGCACAGCTGTCTGACAAAACCTCACACATGCCACCGTGCCAGCACCTG  
AACTCCTGGGGGGACCGTCAGTCTTCTCTTCCCCC AAAACCCAAGGACACCCTCATGA  
TCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAG  
GTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCG  
GGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGG  
ACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCC  
ATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCT  
GCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAG  
GCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAAC  
TACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTC  
ACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCACGA  
GGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ  
ID NO:377)

DNA sequence of HRS(1-50)-Fc (C-terminal Fc fusion):

ATGGCAGAGCGTGCGGCGCTGGAGGAGCTGGTGAAACTTCAGGGAGAGCGCGTGCGAG  
GCCTCAAGCAGCAGAAGGCCAGCGCCGAGCTGATCGAGGAGGAGGTGGCGAAACTCCT  
GAAACTGAAGGCACAGCTGGGTCTTGATGAAAGCTCTGACAAAACCTCACACATGCCCCAC  
CGTGCCCAGCACCTGAACTCCTGGGGGGGACCGTCAGTCTTCTCTTCCCCC AAAACCCA  
AGGACACCCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGC  
CACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGC  
CAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCA  
CCGTCTCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAA  
GCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACC  
ACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGA  
CCTGCCTGGTCAAAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGG  
CAGCCGGAGAACA ACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTC  
CTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATG  
CTCCGTGATGCACGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCC  
GGGTAAATGA (SEQ ID NO:378)

DNA sequence of HRS(1-55)-Fc (C-terminal Fc fusion):

ATGGCAGAGCGTGCGGCGCTGGAGGAGCTGGTGAACTTCAGGGAGAGCGCGTGCGAG  
GCCTCAAGCAGCAGAAGGCCAGCGCCGAGCTGATCGAGGAGGAGGTGGCGAACTCCT  
GAAACTGAAGGCACAGCTGGGTCTTGATGAAAGCAAACAGAAATTTGTGTCTGACAAAA  
CTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGGACCGTCAGTCTTCCTCT  
TCCCCC AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTACATGCGTG  
TGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTG  
GAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTG  
TGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGC  
AAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGG  
GCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGA  
ACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGT  
GGCAGACCAATCCCGAGCCCGGAGACAACTAAGACAGGACCGCTCCCGTCTGCACTCG



GGGAGAGCAA TGGGACGCGGAGAAACAAC TACAAGAACGCTCCCGTGGTGGACTCC  
GACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGG  
GAACGTCTTCTCATGCTCCGTGATGCACGAGGCTCTGCACAACCACTACACGCAGAAGA  
GCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO: 379)

DNA sequence of HRS(1-66)-Fc (C-terminal Fc fusion):

ATGGCAGAGCGTGCGGCGCTGGAGGAGCTGGTGAAACTTCAGGGAGAGCGCGTGCGAG  
GCCTCAAGCAGCAGAAGGCCAGCGCCGAGCTGATCGAGGAGGAGGTGGCGAAACTCCT  
GAAACTGAAGGCACAGCTGGGTCTGATGAAAGCAAACAGAAATTTGTGCTCAAAACCC  
CCAAGGGAACCCGTGATTATAGTTCTGACAAAACCTCACACATGCCACCGTGCCCAGCA  
CCTGAACTCCTGGGGGGACCGTCAGTCTTCTCTTCCCCCAAAACCCAAGGACACCCTC

ATGATCTCCCGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGACCC  
TGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGC  
CGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCAC  
CAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGC  
CCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACA  
CCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTC  
AAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAA  
CAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAA  
GCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGC  
ACGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA  
(SEQ ID NO:380)

Protein sequence of Fc-HRS (2-40) (N-terminal Fc fusion):

MSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD  
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG  
QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSF  
FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKAERAALEELVKLQGERVR  
GLKQQKASAELIEEEVAKLLK (SEQ ID NO:381)

Protein sequence of Fc-HRS (2-45) (N-terminal Fc fusion):

MSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD  
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG  
QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSF  
FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKAERAALEELVKLQGERVR  
GLKQQKASAELIEEEVAKLLKLKAQL (SEQ ID NO:382)

Protein sequence of Fc-HRS (2-50) (N-terminal Fc fusion):

MSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD  
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG  
QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSF  
FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKAERAALEELVKLQGERVR  
GLKQQKASAELIEEEVAKLLKLKAQLGPDES (SEQ ID NO:383)

Protein sequence of Fc-HRS (2-55) (N-terminal Fc fusion):

MSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD  
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG  
QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSI  
  
FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKAERAALEELVKLQGERVR  
GLKQQKASAELIEEEVAKLLKLKAQLGPDESKQKFV (SEQ ID NO:384)



Protein sequence of Fc-HRS (2-66) (N-terminal Fc fusion):

MSDKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD  
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG  
QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGGSF  
FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKAERAALAEELVKLQGERVR  
GLKQQKASAELIEEEVAKLLKLKAQLGPDESKQKFVLKTPKGTRDYS (SEQ ID NO:385)

Protein sequence of HRS(1-40)-Fc (C-terminal Fc fusion):

MAERAALAEELVKLQGERVRGLKQQKASAELIEEEVAKLLKSDKTHTCPPCPAPPELLGGPSVF  
LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV  
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS  
LTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGGSFFLYSKLTVDKSRWQQGNVFSCS  
VMHEALHNHYTQKSLSLSPGK (SEQ ID NO:386)

Protein sequence of HRS(1-45)-Fc (C-terminal Fc fusion):

MAERAALAEELVKLQGERVRGLKQQKASAELIEEEVAKLLKLKAQLSDKTHTCPPCPAPPELLG  
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS  
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK  
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGGSFFLYSKLTVDKSRWQQGN  
VFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:387)

Protein sequence of HRS(1-50)-Fc (C-terminal Fc fusion):

MAERAALAEELVKLQGERVRGLKQQKASAELIEEEVAKLLKLKAQLGPDESSDKTHTCPPCPA  
PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE  
EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR  
EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGGSFFLYSKLTVDKSRW  
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:388)

Protein sequence of HRS(1-55)-Fc (C-terminal Fc fusion):

MAERAALAEELVKLQGERVRGLKQQKASAELIEEEVAKLLKLKAQLGPDESKQKFVSDKTHT  
CPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA  
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV  
YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGGSFFLYSKLT  
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:389)

Protein sequence of HRS(1-66)-Fc (C-terminal Fc fusion):

MAERAALAEELVKLQGERVRGLKQQKASAELIEEEVAKLLKLKAQLGPDESKQKFVLKTPKG  
TRDYSSDKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW  
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK  
AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDS  
DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:390)

**[0509]** N-terminal Fc-histidyl tRNA synthetase (HRS-Fc) tandem fusion DNA construct was prepared as follows.

**[0510] Plasmid construction.** HRS (2-60)\_HRS (2-60) with an N-terminal Fc was generated using pET24b\_TAC\_Fc-HRS (2-60) construct. The HRS (2-60) gene was PCR amplified with the primers listed below, which contain a 5' and 3' XhoI site. The PCR reactions were performed using recommended thermal cycling parameters. pET24b\_TAC\_Fc-HRS (2-60) construct was digested with XhoI, dephosphorylated, and gel purified. The PCR generated fragment was also digested with XhoI and gel purified. The gel purified HRS (2-60) was subcloned into the XhoI site of pET24b\_TAC\_Fc-HRS (2-60). To generate the final construct,



QuikChange mutagenesis was used to remove the stop codon and XhoI site between the tandem HRS (2-60) fragments using the primers listed below. Sequences were confirmed by performing alignments with the theoretical sequences using DNASTAR Lasergene SeqMan Pro.

[0511] The following primers were used to amplify the Fc-HRS (2-60) (Table E15):

| Table E15                       |                                                           |            |
|---------------------------------|-----------------------------------------------------------|------------|
| Primer Sequences                |                                                           |            |
| Primer Name                     | Sequence                                                  | SEQ ID NO: |
| XhoI-H-aa2-60 FWD               | 5'-TAT TCT CGA GGC AGA GCG TGC GGC -3'                    | 391        |
| H-aa2-60-stop-XhoI REV          | 5' - GCGCCTCGAGTCACTTGGGGGTTTTG - 3'                      | 392        |
| delete stop-XhoI concatemer FWD | 5' - GTG CTC AAA ACC CCC AAG GCA GAG CGT GCG GCG CTG G -3 | 393        |
| delete stop-XhoI concatemer REV | 5'- CCA GCG CCG CAC GCT CTG CCT TGG GGG TTT TGA GCA C -3' | 394        |

DNA sequence of Fc-HRS(2-60) HRS(2-60) (N-terminal Fc fusion):  
ATGTCTGACAAACTCACACATGCCACCGTGCCACGACCTGAACTCCTGGGGGGACC  
GTCAGTCTTCCTCTTCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGA  
GGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGT  
  
ACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAA  
CAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAA  
GGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCAGCCCCATCGAGAAAACCATCT  
CCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAG  
GAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGA  
CATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGAGAACTACAAGACCACGCCTC  
CCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCA  
GGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCACGAGGCTCTGCACAACCACT  
ACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAAGCAGAGCGTGCGGCGCTGGAGGAG  
CTGGTGAAACTTCAGGGAGAGCGCGTGCGAGGCCTCAAGCAGCAGAAGGCCAGCGCCG  
AGCTGATCGAGGAGGAGGTGGCGAAACTCCTGAAACTGAAGGCACAGCTGGGTCCTGAT  
GAAAGCAAACAGAAATTTGTGCTCAAAACCCCCAAGGCAGAGCGTGCGGCGCTGGAGG  
AGCTGGTGAAACTTCAGGGAGAGCGCGTGCGAGGCCTCAAGCAGCAGAAGGCCAGCGC  
CGAGCTGATCGAGGAGGAGGTGGCGAAACTCCTGAAACTGAAGGCACAGCTGGGTCCTG  
ATGAAAGCAAACAGAAATTTGTGCTCAAAACCCCCAAGTGA (SEQ ID NO:395)

Protein sequence of Fc-HRS(2-60) HRS(2-60) (N-terminal Fc fusion):  
MSDKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD  
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG  
QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSF  
FLYSKLTVDKSRWQQGNVFSVSMHEALHNHYTQKSLSLSPGKAERAALEELVKLQGERVR  
GLKQQKASAELIEEEVAKLLKLKAQLGPDESKQKFVLKTPKAERAALEELVKLQGERVRGL  
KQQKASAELIEEEVAKLLKLKAQLGPDESKQKFVLKTPK (SEQ ID NO:396)

[0512] Preparation and purification of HRS(1-60)-Fc and Fc-HRS(2-60) fusion proteins. *E. coli* strain. The *E. coli* BL21-CodonPlus® (DE3) RIPL Competent Cells (Agilent 230280) transformed with the pET expression constructs described above were used for initial production of Fc fusion proteins.



**[0513] Media.** M9YE medium was prepared by mixing sterile 5X M9 minimal salt (BD 248510), yeast extract solution in sterile purified water (BD 212750), sterilized 20% glucose (Sigma G7021), and sterile 1.0 M MgSO<sub>4</sub> (Sigma M7506). For the feeding solution, the yeast extract solution (5%), glucose solution (50%), and 10 ml concentrated trace element solution (containing Fe<sup>3+</sup>, Mn<sup>2+</sup>, boric acid, Mo<sup>6+</sup>, Co<sup>2+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup> and EDTA), as well 10 ml magnesium sulfate solution, were autoclaved separately. The components were mixed just prior to the fed-batch phase. Kanamycin sulfate was added to a final concentration of 100 µg/ml in the culture medium.

**[0514] Fed-batch fermentation.** A 0.5L Multifors fermentors (HT-Infors) with Iris software was used for the fed-batch fermentation process. The agitation was set at 1000 rpm. The pH value was controlled at 7.0 automatically by the addition of 30% ammonium hydroxide (Sigma 221228) and 30% phosphoric acid (Sigma P5811). Air was provided at a flow rate of 0.5 L/min with an oil-free diaphragm air compressor (Cole-Parmer) and passed through a 0.2µm filter. The dissolved oxygen level was controlled at 70% by providing pure oxygen (West Air). The temperature was controlled at 30°C with a Neslab RTE7 circulator (Thermo Scientific). Foaming was controlled by addition of the antifoam 204 (Sigma A8311).

**[0515]** The initial volume of M9YE medium in the fermentor was 0.3 L. The fermentor was inoculated with 15 ml of the seed culture grown overnight at 30°C and 250 rpm. When the carbon source was depleted in the vessel, the concentrated feeding solution was introduced into the vessel by a peristaltic pump at 0.12ml/min. When the optical density of the cells at 600 nm reached exponential phase, the culture was induced with 0.5 mM IPTG (Fisher Scientific BP1755). The culture was grown overnight (about 17-hour induction) and the final OD<sub>600</sub> reached about 120. The cells were harvested by centrifugation at 8,000g for 30 min. The supernatant was decanted and the pellet was stored at - 20°C until purification.

**[0516]** Additional HRS-Fc fusion proteins were prepared using a modified pET24b vector (EMD, Gibbstown, NJ) containing a TAC promoter instead of T7 ("pET24b\_TAC") and transformed into UT5600 competent cells. UT5600 competent cells were prepared from bacterial stock obtained from the Coli Genetic Stock Center (CGSC, Yale). UT5600 is a K12 derivative strain of *E. coli* and is designated as genotype: F, *araC14*, *leuB6*(Am), *secA206*(aziR), *lacY1*, *proC14*, *tsx-67*,  $\Delta$ (*ompT-fepC*)266, *entA403*, *glnX44*(AS),  $\lambda^-$ , *trpE38*, *rjbC1*, *rpsL109*(strR), *xylA5*, *mtl-1*, *thiE1*.

**[0517]** Expression vectors comprising these constructs were transformed into UT5600 cells using standard procedures, and glycerol stocks prepared.

**[0518] Fermentation Medium.** UT5600\_M9\_YE medium was prepared by mixing, for the batch media: 16 grams/L Yeast Extract (Difco 212750), 8 g/L Glycerol (Sigma G2025), 11.28 g/L M9 Salts (Difco 248510) and 100µl/L Antifoam 204 (Sigma AG6426) to Deionized water and sterilized via autoclave. Post autoclave additions were 0.64 ml/L Trace Metals Solution, 2.3 ml/L 100x Magnesium Sulfate and 45.83 g/L L-Leucine. Feed Media was prepared by mixing 250 g/L Yeast Extract, 225 g/L Glycerol and 100µl/L Antifoam 204 to deionized water and sterilized via autoclave. Post-sterilization additions were 10ml/L Trace Metals Solution, 2.3 ml/L 100x Magnesium Sulfate, 45.83 ml/L L-Leucine.

**[0519] Fed-batch fermentation.** A 0.5 L fermentor (Infors) with MFCS/DA software was used for the fed-batch fermentation. The agitation was set to cascade at 500-1200 rpm. The pH value was controlled at 7.0±0.1 automatically by the addition of 30% ammonium hydroxide (Sigma 221228) and 30% phosphoric acid (Sigma P5811). The air was provided at a flow rate of 0.5 L/min with an oil-free diaphragm air compressor (Cole-Parmer). The air was passed through a 0.2 µm Midisart 2000 filter (Sartorius 17805). The pure oxygen (West Air) was supplied automatically to control the dissolved oxygen level at 30%. The temperature was controlled at 30°C with a Neslab RTE7 circulator (Thermo Scientific). The foaming was controlled by addition of the antifoam 204 (Sigma A8311) as needed. The initial volume of UT5600\_M9\_YE medium in the fermentor



was 0.24L. The fermentor was inoculated with  $\approx 10$  OD Units (approximately 1-2 ml of seed at OD 5-10) of the seed culture grown for 6 hours at 37°C and 250 rpm. When the batch glycerol was depleted in the vessel ( $\sim 4$  hours), the concentrated feeding solution was introduced into the vessel by a peristaltic pump set on an exponential feeding program. When the optical density of the cells at 600 nm reached  $\sim 150$ , the culture was induced with 0.5 mM IPTG (Fisher Scientific BP1755). The culture was incubated 4 hours post induction and harvested by centrifugation at 10,000 x g for 30 minutes. Approximate final cell pellet yield was 150-200 grams per liter wet cell weight (WCW). The cell pellet was stored at - 80°C until purification. The expression of target protein was confirmed via SDS-PAGE and western blot to Goat anti-Human IgG, HRP conjugated antibody (Thermo p/n 31413).

**[0520] Purification of FC fusion proteins.** Frozen cell pellets were resuspended in 4 volumes (*i.e.*, 4 mL/g cell pellet) of Lysis Buffer (50 mM Tris, 500 mM NaCl, 14 mM  $\beta$ -ME, pH 7.5). Complete EDTA-FREE protease inhibitor tablets (Roche) were added to the suspension at a ratio of 1 tablet/50 mL. The suspension was passed through a microfluidizer (Microfluidics) twice at 14,000 psi with cooling by ice. The lysate was centrifuged at  $\geq 10,000$  x g for 45 min at 4°C. The supernatant was filtered through 0.45+0.22  $\mu$ m Sartobran capsule filters (Sartorius).

**[0521]** The clarified lysate was bound to the MabSelect resin (GE Healthcare), pre-equilibrated with Binding Buffer (50 mM Tris, 500 mM NaCl, pH 7.5) at a ratio of 1 ml resin per 10 g cell paste. The column was washed with 500 column volumes of Binding Buffer + 0.1% Triton X-114 followed by 100 column volumes of the Binding Buffer. The bound protein, fusion proteins were eluted with 3.75 column volumes of Elution Buffer (0.1 M glycine, 0.5 M Arginine, pH 3.0) to a collection tube containing 1.25 column volumes of Neutralization Buffer (1 M Tris, pH 8.0).

**[0522]** Optionally, for further removal of high molecular weight species the material was concentrated in Amicon 30 kDa ultracentrifugal concentrating devices (Millipore) and loaded onto a HiLoad Superdex 200 pg 16/600 size-exclusion chromatography column (GE Healthcare). The material was eluted in 1.1 column volumes of 1X PBS pH 7.4 (Gibco #10010), and fractions corresponding to the main peak based on the process chromatogram absorbance at 280 nm were pooled.

**[0523]** If size-exclusion chromatography was not performed, the purified Fc fusion proteins were buffer exchanged into a buffer containing PBS, at pH 7.4. The dialyzed protein was passed through a Q membrane filter (Sartobind-Q from Sartorius or Mustang-Q from Pall) or a Q-Sepharose column (GE Healthcare) for further endotoxin removal, and filtered through a 0.22  $\mu$ m sterile filter.

**[0524] Scaleable purification process for FC fusion proteins.** The purification process using MabSelect followed by size-exclusion chromatography was used to purify multiple Fc fusion proteins using a robust process effective with minimal purification development. However, the use of detergent wash during Protein A (MabSelect) and a size-exclusion chromatography step limits the ability to scale up purification. A purification process for scale-up was also developed for Fc fusion proteins using lysate flocculation, Protein A chromatography, cation exchange (CEX), and ceramic hydroxyapatite (CHT) chromatography.

**[0525]** Resuspension, and lysis were performed as described above, with the omission of protease inhibitor tablets and  $\beta$ -ME from the Lysis Buffer. After lysis, the lysate was flocculated with addition of polyethyleneimine, Mw 1300 (Sigma Aldrich) to 0.04% (v/v) and incubated for 30 min @ 4 °C. Centrifugation and clarification were performed as described above. Protein A chromatography was performed on clarified lysate using MabSelect resin in a packed chromatography column at a load ratio of 1 ml resin per 4 g cell paste, with a wash step of 5 column volumes in 50 mM Tris, 500 mM NaCl pH 7.5, followed by elution in 3 column volumes of 0.1 M glycine pH 3.0 and neutralization in 0.3 column volumes of 1 M Tris pH 8.0. Following Protein A, CEX load was prepared by 5x dilution of post-Protein A eluent in CEX Equilibration buffer (20 mM sodium phosphate, pH 6.0), loaded onto a SP Sepharose High Performance column, washed with 5 column volumes of Equilibration



buffer, and eluted over a linear sodium chloride gradient from 0 to 300 mM NaCl over 10 column volumes. CEX fractions were pooled based on SDS-PAGE analysis of elution peak fractions. Ceramic hydroxyapatite was performed on CEX pool by loading onto a CHT Type I 40 µm column (Bio-Rad) equilibrated in CHT Equilibration Buffer (5 mM sodium phosphate, 150 mM sodium chloride, 1 µM calcium chloride pH 6.5), washed with 5 columns of equilibration buffer and eluted over a linear sodium chloride gradient from 150 mM to 1.5 M sodium chloride over 10 column volumes and a 1.5 M NaCl hold for up to 20 column volumes to complete the elution. Following CHT, protein was buffer exchanged into 1X PBS pH 7.4 using Amicon 30 kDa centrifugal concentrating devices (Millipore).

**[0526]** The fusion protein concentration was determined by Bradford protein assay (Thermo Scientific) or by UV absorbance at 280 nm. The fusion protein concentration was determined by Bradford protein assay (Thermo Scientific). The endotoxin level was below 4 EU/mg as determined by EndoSafe PTS LAL assay (Charles River).

**[0527] Analysis of HRS-Fc fusion proteins.** The purified HRS-Fc fusion proteins were analyzed by SDS-PAGE as shown in **Figure 21**. Samples of 10 µg protein load were run on NuPAGE 4-12% Bis-Tris gels, 150 V for 60 minutes in MOPS-SDS buffer, and stained with Instant Blue. Reduced samples had 25 mM DTT, and were heated at 95 °C for 10 minutes in 1X LDS buffer prior to loading.

**[0528]** The purified HRS-Fc fusion proteins were also analyzed by a size-exclusion chromatography (SEC) method. The samples were loaded to a TSK-Gel Super SW3000 column (TOSOH, 4.6mm ID x 30cm, 4µm) on an Agilent 1260 HPLC system. A 30 minute isocratic run was carried out at 0.3 ml/min with a mobile phase containing 0.1 M NaCl, 0.2M Na phosphate and 5% 2-propanol at pH7. UV detection was performed at 280nm. The chromatogram is shown in **Figure 22**.

**[0529]** Approximately 83% of the protein is in the desired dimer form. after Protein A and prior to size-exclusion chromatography purification, with the remaining quantity present as high molecular weight species. After size-exclusion chromatography, the proportion of dimer increases to 95 to 99%. Using the Protein A, cation exchange, and hydroxyapatite purification process the proportion of dimer is greater than 99%. Most of the dimer protein contains the inter-chain disulfide bond in the Fc hinge region, while some non-covalent dimer also exists.

**[0530]** Analysis of the intact mass spectral data obtained using LC/ESI-MS demonstrates that the molecular size of the FC fusion proteins under non-reducing conditions is consistent with the expected molecular mass of approximately 64,520 daltons (data not shown). The CD spectra of the Fc fusion proteins in the far and near UV regions reveals that the structure of the fusion proteins is consistent with the expected domain structures. Additionally the deconvoluted differential scanning calorimetry data obtained from the HRS-Fc fusion proteins demonstrates that the Fc fusion proteins are folded with two major thermal transitions characteristic of the CH1 and CH2 domains of the Fc component (data not shown), consistent with predicted structures.

**[0531]** To assess the pharmacokinetic characteristics of the HRS-Fc fusion protein constructs compared to the unmodified HRS proteins, proteins were administered to normal C57BL/6 mice via a single intravenous or subcutaneous bolus at a dose of 8 mg/kg. Blood was serially sampled with sampling times distributed across nine animals per product form. For each time point, sera from three independent mice were drawn. Test article concentrations were measured by ELISA and pharmacokinetic parameters were derived using non-compartmental analysis on Phoenix software.

**[0532]** The results, shown in **Figures 23A, 23B, and 23C** demonstrate that the creation of the Fc fusion proteins resulted in significantly enhanced half life, exposure and SC bioavailability



| Table E16                                                                                                         |       |                                        |                               |                   |                                     |                             |                                        |
|-------------------------------------------------------------------------------------------------------------------|-------|----------------------------------------|-------------------------------|-------------------|-------------------------------------|-----------------------------|----------------------------------------|
| Pharmacokinetic analysis of HRS-Fc fusion proteins                                                                |       |                                        |                               |                   |                                     |                             |                                        |
| Product Form                                                                                                      | Route | V <sub>d</sub> <sup>1</sup><br>(ml/kg) | CL <sup>2</sup><br>(ml/hr/kg) | Half-life<br>(hr) | Bioavailability <sup>3</sup><br>(%) | AUC <sup>4</sup><br>(hr*nM) | Fold increase in exposure <sup>5</sup> |
| HRS(1-60)                                                                                                         | IV    | 119                                    | 574                           | 0.5               | --                                  | 1,835                       | --                                     |
| Fc-HRS(2-60)                                                                                                      | IV    | 176                                    | 2.8                           | 72                | --                                  | 89,916                      | 209                                    |
| HRS(1-60)-Fc                                                                                                      | IV    | 127                                    | 5.1                           | 33                | --                                  | 49,364                      | 115                                    |
| HRS(1-60)                                                                                                         | SC    | 1,457                                  | 1,541                         | 0.7               | 37                                  | 683                         |                                        |
| Fc-HRS(2-60)                                                                                                      | SC    | 452                                    | 4.4                           | 71                | 63                                  | 56,307                      | 352                                    |
| HRS(1-60)-Fc                                                                                                      | SC    | 537                                    | 10.2                          | 37                | 50                                  | 24,623                      | 154                                    |
| <sup>1</sup> Volume of distribution at steady state for IV administration or terminal phase for SC administration |       |                                        |                               |                   |                                     |                             |                                        |
| <sup>2</sup> Clearance for IV administration or clearance as a function of bioavailability for SC administration  |       |                                        |                               |                   |                                     |                             |                                        |
| <sup>3</sup> Compared to same product form administered IV                                                        |       |                                        |                               |                   |                                     |                             |                                        |
| <sup>4</sup> Area under the curve from time of dose predicted to infinity                                         |       |                                        |                               |                   |                                     |                             |                                        |
| <sup>5</sup> Fold increase in molar exposure compared to unmodified protein delivered the same route              |       |                                        |                               |                   |                                     |                             |                                        |

**[0533]** The pharmacokinetic analysis shown in **Table E16** demonstrates that HRS-Fc fusion constructs exhibit significantly improved systemic exposure, clearance and half-life compared to the unmodified proteins. Creation of the Fc fusion proteins also improved the subcutaneous bioavailability of the proteins compared to the unmodified proteins. In particular Fc-HRS(1-60) increased exposure compared to the unmodified protein by 200 to 300 fold depending on the route of administration, additionally SC bioavailability and half life were both significantly enhanced.

#### EXAMPLE 9

#### TESTING OF FC FUSION PROTEINS IN TNBS INDUCED COLITIS

**[0534]** The large intestine is lined by an epithelial mucosa that is invaginated into flask-like structures, crypts. Unlike the small intestine there are no villi in this region, with the top of the crypts opening onto a flat table region. The cells of the crypt are generated by stem cells located at the crypt base, whose daughter cells divide rapidly and differentiate into the predominant colonocytes and mucin producing goblet cells (a smaller number of endocrine cells and M cells are also produced). The size of the crypt and the number of goblet cells per crypt increase along the large intestine from caecum to rectum, presumably to aid the passage of faeces and provide sufficient mucosal and stem cell protection as water is absorbed from the faeces.



**[0535]** Normally, the rate of cell production in the intestinal crypt is precisely matched to the rate of cell loss - a very sensitive homeostatic mechanism operates. Disruption of the mucosal barrier allows bacterial entry into the body, with resultant disease implications. Conversely, hyperplasia can generate polyps and ultimately tumours. Disruption of the intestinal barrier can be caused by exposure to non-cell type specific (often proliferation specific) cytotoxic agents - typically anti-cancer therapies. However, perturbation of epithelial cell turnover is also a common feature of inflammatory diseases.

**[0536]** Current rodent models of inflammatory bowel disease (IBD) include for example models generated by triggering an autoimmune disease by manipulation of the T-cell population, irritating the mucosal lining of the intestine by the accumulation of particulate material in the large bowel (such as with DSS, dextran sulphate sodium), or chemical disruption of the epithelium (such as with Trinitrobenzene sulfonate, TNBS).

**[0537]** In any of these models disease severity may be assessed by a variety of subjective assessments of the observed pathological grades, as well as more objective and quantitative measures of the damage, to provide more meaningful insights into the underlying biology. It is possible to broaden the analysis by using computer assisted length / area measurements to map the changes in the mucosa / submucosa and obtain more quantitative measures of the damage, and hence therapeutic efficacy. While each of these models has different pros and cons, the TNBS colitis model is an established model of various aspects of the present disclosure of inflammatory bowel disease in humans, which has been successfully used to validate and optimize the efficacy of human therapeutics.

**[0538] TNBS Mouse Model.** In this model of colitis, colonic irritation is induced by intracolonic administration of TNBS in ethanol. This provokes an acute colitis that has a TH1-type cytokine profile, which is characterised by the expression of genes coding for TNF- $\alpha$ , IFN- $\gamma$  and IL-12 amongst others (Fichtner-Feigl et al. J. Clin. Invest. 2005. 115: 3057-3071). The colitis can be severe and localised to the area of the colon into which the TNBS is introduced. The inflammatory response results in localised swelling, inflammatory cell infiltration, and epithelial loss.

**[0539]** In this study, the efficacy of the unmodified HRS polypeptide, (HRS(1-60)), was compared to the Fc fusion proteins Fc-HRS(2-60) and HRS(1-60)-Fc to assess their efficacy in ameliorating TNBS-induced acute colitis in mice. Three different dosing regimens, employing either i.v. or s.c. administration of test item, were evaluated. Budesonide (p.o.) was used as a reference item for the study.

**[0540] Animals and caging:** A total of 100 BDF-1 (H.Pylori-free, murine norovirus-free) male mice (Harlan Laboratories, UK) were used in the study. Animals were 8-10 weeks old on supply and used at 10-12 weeks of age. All mice were held in individually ventilated cages (IVCs) in an SPF (Specific Pathogen Free) barrier unit. The animals were identified by numbered cages and by ear punches.

**[0541] Diet and animal welfare:** The animals were fed Rat and Mouse Expanded diet from B & K. Water was supplied in HYDROPAC™ water pouches (filtered RO water; Hydropac/lab products, Delaware, USA). Both feed and water were available ad libitum. There was a constant room temperature of  $21 \pm 2^{\circ}\text{C}$  and a mean relative humidity of  $55 \pm 10\%$ . The day-night cycle was constant, with light and dark phases of 12 hours each (07:00hr/19:00hr switch). Animal health was monitored daily and cages were cleaned at regular intervals. All procedures were certified according to the UK Animal (Scientific Procedures) Act 1986.

**[0542] Groups, dosages, administration and formulations:** A total of 100 mice were randomised into ten study groups (Table E17). All the mice in any one cage received the same treatment and were ear punched for identification purposes. Daily body weight measurements were used to calculate the volume of test item or vehicle administered to the applicable groups.

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| Table E17           |                   |                                    |                     |                    |             |
|---------------------|-------------------|------------------------------------|---------------------|--------------------|-------------|
| Study groups        |                   |                                    |                     |                    |             |
| Treatment           | Dose of test item | Volume and route of administration | Frequency of dosing | Duration of dosing | Mouse codes |
| Vehicle (PBS) only  | -                 | 5ml/kg, i.v.                       | <i>q.d.</i>         | day 0 to day 3     | 1-6         |
| TNB S/vehicle (PBS) | -                 | 5ml/kg, i.v.                       | <i>q.d.</i>         | day 0 to day 3     | 7-18        |
| TNBS + budesonide   | -                 | 5ml/kg, i.v.                       | <i>q.d.</i>         | day 0 to day 3     | 19-28       |
| TNBS + HRS(1-60)    | 1mg/kg            | 5ml/kg, i.v.                       | <i>q.d.</i>         | day 0 to day 3     | 29-40       |
| TNBS + Fc-HRS(2-60) | 5mg/kg            | 5ml/kg, i.v.                       | <i>q.d.</i>         | day 0 to day 3     | 41-50       |
| TNBS + Fc-HRS(2-60) | 5mg/kg            | 5ml/kg, i.v.                       | once only           | day 0              | 51-60       |
| TNBS + Fc-HRS(2-60) | 15mg/kg           | 5ml/kg, s.c.                       | <i>q.d.</i>         | day 0 to day 3     | 61-70       |
| TNBS + HRS(1-60)-Fc | 5mg/kg            | 5ml/kg, i.v.                       | <i>q.d.</i>         | day 0 to day 3     | 71-80       |
| TNBS + HRS(1-60)-Fc | 5mg/kg            | 5ml/kg, i.v.                       | once only           | day 0              | 81-90       |
| TNBS + HRS(1-60)-Fc | 15mg/kg           | 5ml/kg, s.c                        | <i>q.d.</i>         | day 0 to day 3     | 91-100      |

**[0543] Preparation and administration of TNBS and test items.** **TNBS:** TNBS (Sigma; lot # SLBD6811V) was prepared as a 15mg/ml solution in saline/50% ethanol. A single dose of 200µl (3mg TNBS) was instilled into the colon, using a plastic catheter, placed 4cm proximal to the anal verge, at 11:00hr on study day 0. Animals were maintained in an inverted position for 1 minute after introduction of TNBS into the colon, in order to minimise leakage of the compound.

**[0544] HRS(1-60):** The test item was received as four vials of frozen stock solution (0.033ml volume in each) at 17.1mg/ml, which was stored at -80°C until use. On each day of test item administration, a single aliquot was taken and thawed on wet ice. After thawing, the contents of the vial were mixed by pipetting them up and down ten times. Subsequently, the test item was diluted with cold vehicle (sterile, x1 PBS, pH 7.4) to give a solution at 0.2mg/ml; the solution was mixed by pipetting up and down ten times. This solution was administered daily (from study day 0 until study day 3) by intravenous injection, at 5ml/kg, in order to give a dose of 1mg/kg.

**[0545] Fc-HRS(2-60):** The test item was received as four vials of frozen stock solution (one vial of 2.51ml and three vials of 2.01ml) at 4.7mg/ml, which was stored at -80°C until use. On each day of test item administration, a single aliquot was taken and thawed on wet ice. After thawing, the contents of the vial were mixed by pipetting them up and down ten times. Subsequently, the test item was diluted with cold vehicle (sterile, x1 PBS, pH 7.4) to give solutions at 3mg/ml and 1mg/ml; the solutions were mixed by pipetting up and down ten times. Fc-HRS(2-60) was administered according to 3 different regimens: i) the 1mg/ml solution was administered daily (from study day 0 until study day 3) by intravenous injection, at 5ml/kg, in order to give a dose of 5mg/kg; ii) the 1mg/ml solution was administered daily, once only on study day 0, by intravenous injection, at 5ml/kg, in order to give a dose of 5mg/kg; iii) the 3mg/ml solution was administered daily (from



study day 0 until study day 3) by subcutaneous injection, at 5ml/kg, in order to give a dose of 15mg/kg.

**[0546] HRS(1-60)-Fc:** The test item was received as four vials of frozen stock solution (one vial of 2.37ml and three vials of 1.89ml) at 4.99mg/ml, which was stored at -80°C until use. On each day of test item administration, a single aliquot was taken and thawed on wet ice. After thawing, the contents of the vial were mixed by pipetting them up and down ten times. Subsequently, the test item was diluted with cold vehicle (sterile, x1 PBS, pH 7.4) to give solutions at 3mg/ml and 1mg/ml; the solutions were mixed by pipetting up and down ten times. HRS(1-60)-Fc was administered according to 3 different regimens: i) the 1mg/ml solution was administered daily (from study day 0 until study day 3) by intravenous injection, at 5ml/kg, in order to give a dose of 5mg/kg; ii) the 1mg/ml solution was administered daily, once only on study day 0, by intravenous injection, at 5ml/kg, in order to give a dose of 5mg/kg; iii) the 3mg/ml solution was administered daily (from study day 0 until study day 3) by subcutaneous injection, at 5ml/kg, in order to give a dose of 15mg/kg.

**[0547] Budesonide:** Budesonide was obtained from Tocris (Tocris 1101, lot # 1A/128902) and was stored in the dark at ambient temperature until use. On each day of administration, budesonide was formulated as a 1mg/ml solution in peanut oil (Sigma). Budesonide was administered daily (from study day 0 until study day 3) by oral gavage, at 5ml/kg, in order to give a dose of 5mg/kg.

**[0548] Clinical examinations and analgesia.** Any animal demonstrating more than 15% weight loss was considered unwell and treatment may have been withheld. Any animal was culled if the weight loss was greater than 20%. Animal well-being was monitored daily. Once daily from day -1 until the end of the study, all mice were weighed and assessed for stool consistency, and the presence of overt blood in the stool or around the anus according to the criteria in Table E18.

| Table E18                                        |                              |                                                                               |                                    |
|--------------------------------------------------|------------------------------|-------------------------------------------------------------------------------|------------------------------------|
| Scoring criteria for in-life disease parameters. |                              |                                                                               |                                    |
| Score                                            | Weight Loss (% day 0 weight) | Stool observation                                                             | Overt blood (in stool/around anus) |
| 0                                                | <1%                          | Normal                                                                        | None                               |
| 1                                                | ≥1% <5%                      | Soft; empty colon/rectum at necropsy;<br>No observation in a 30 minute period | Slight                             |
| 2                                                | ≥5% <10%                     | Unformed                                                                      | Moderate                           |
| 3                                                | ≥10% <15%                    | Watery / gel-like                                                             | Severe                             |
| 4                                                | ≥15%                         |                                                                               |                                    |

**[0549] Use of analgesia:** Analgesia was not used in this study, at the direction of the Sponsor, as it may have interfered with test item action.

**[0550] Harvesting and preparation of tissue for histological examination:** Upon sacrifice, mice were anaesthetised with 4% isoflurane, with 2L/min O<sub>2</sub> and 2L/min N<sub>2</sub>O. When fully anaesthetised, blood was withdrawn by direct cardiac puncture and death confirmed by cervical dislocation.

**[0551] Preparation of whole blood and plasma samples.** Blood was collected by cardiac puncture from all mice into 1.5ml microcentrifuge tubes. Blood samples were immediately placed on ice and left to clot for 60 minutes. Samples were then centrifuged at 3000g for 7 minutes, at 4°C. Immediately after centrifugation, the serum was transferred by sterile pipette into pre-labelled vials and immediately frozen on dry ice.

**[0552] Preparation of intestinal samples.** The large intestine was removed and flushed with PBS and its length and wet weight were recorded, prior to cutting into proximal, mid and distal regions and fixation in



Carnoy's solution. In addition, a small sample of colon was snap-frozen in liquid nitrogen. Fixed tissue was dehydrated through a series of alcohols and xylene and embedded in paraffin, using a Leica TP1020 tissue processor and an EG1140H work station. Sections (5µm thick) were cut using a Leica RM2125RTF microtome, and air-dried on to microscope slides, overnight at 37°C. Subsequently, slides were dewaxed in xylene and rehydrated through graded alcohols to PBS. All sections were then stained with haematoxylin and eosin (H&E), and mounted.

**[0553] Histological analysis:** Histological sections were assessed in a blinded fashion. Sections were observed microscopically, and assigned a subjective severity score ranging between 0 and 5, according to the criteria outlined in **Table E19**. Up to twelve transverse cross-sections from the mid and distal large bowel were assessed.

| Table E19                                   |                                                                  |
|---------------------------------------------|------------------------------------------------------------------|
| Epistem's standard severity scoring system. |                                                                  |
| Severity Score                              | Description                                                      |
| 0                                           | Crypts appeared normal.                                          |
| 1                                           | Crypts present but damaged (abnormal pathology). No ulceration.  |
| 2                                           | Some crypts depleted and some ulceration/inflammation.           |
| 3                                           | 20-70% of crypts depleted and increased ulceration/inflammation. |
| 4                                           | >70% crypts depleted with substantial ulceration/inflammation.   |
| 5                                           | No crypts remaining. Totally ulcerated /inflamed.                |

**[0554] Statistical analysis:** Where mentioned, statistical comparisons of group data were performed using ANOVA, in combination with *post hoc* tests, using Graph Pad Prism.

**[0555] qPCR Analysis:** Mouse colon was excised from the animals and stored at -80 °C until analysis. RNA was prepped from colons using Qiagen's RNeasy Mini Kit (Catalog #74106). Once RNA was eluted from the Qiagen column, it was run on an Agilent Bioanalyzer 2100 to test RNA integrity and NanoDrop to determine RNA concentration and purity. RNA was then stored at -80 °C.

**[0556]** Reverse transcription (RT) of RNA to cDNA was performed in a 96 well PCR plate format in Eppendorf's Mastercycler PCR machine with the following program: 37 °C for 60 minutes, 95 °C for 5 minutes. The edge wells of the 96 well plate were not used and filled with 50mL water to prevent evaporation of inside wells. 100ng of RNA in 25mL of reverse transcription master mix (Life Technologies #4387406) was used per sample RT. Once RT was completed, the next step was to pre-amplify genes of interest in the sample cDNA. Primers of genes of interest (DELTAgene primers designed by Fluidigm) were combined to a final concentration of 200nM. Using these primers, genes of interest were pre-amplified in each sample. Pre-amplification was performed in 10mL reactions (2.5mL cDNA, 7.5mL Pre-Amp mastermix) in 384-well format using an Applied Biosystems ViiA7 PCR machine with the following program: 95 °C for 10 minutes, 14 cycles of 95 °C for 15 seconds and 60 °C for 4 minutes. After pre-amplification step, exonuclease (New England BioLabs catalog #M0293L) was added to remove unincorporated primers from each sample. This exonuclease reaction was also completed in the ViiA7 PCR machine with the following program: 37 °C for 30 minutes, 80 °C for 15 minutes. After exonuclease, the RT sample was further diluted 1:5 (7mL exonuclease sample + 18mL low EDTA buffer).

**[0557]** The chip used to run qPCR on Fluidigm's Biomark system was a 96.96 Dynamic Array IFC for Gene Expression. The chip was first primed with the IFC controller HX as per manufacturer's recommendations before sample and assays were loaded. To prepare assays to be loaded on a chip, 4.4mL assay master mix (Fluidigm's 2X Assay Loading Reagent catalog #8500736 and low EDTA TE) to 3.6mL 20mM forward and



reverse primers for each gene of interest were prepared in a 96 well plate. To prepare samples, 4.5mL sample master mix (Ambion's 2X TaqMan Gene Expression Master Mix, Fluidigm's 20X DNA Binding Dye Sample Loading Reagent catalog number 100-0388, and Biotium's 20X EvaGreen catalog #31000) was added to 3mL diluted pre-amplified/exonuclease sample in a 96 well plate. Once the chip had been primed, 5mL sample or assay prepared above were loaded onto the chip. The chip was then returned to the IFC controller for the samples to be loaded into the chip. After the chip had finished loading, qPCR could then be run on the Biomark using preset program for 96.96 Dynamic Array for Gene Expression with a melt curve to determine primer specificity. Relative gene expression was determined by the delta delta Ct method using multiple housekeeping genes.

## Results:

**[0558] In-life parameters** - All TNBS-recipient groups demonstrated an acute decrease in mean body weight, of between 8-10% of starting body weight over the first 24 hours following administration of the colitic agent; subsequently, there was a slower decrease in mean body weight, with all groups showing a significant decline in mean body weight to between 80-90% of starting weight by the end of the study ( $p \leq 0.0158$ ). The body weight of vehicle-only treated mice showed a minimal change over the period of the study, with a final mean body weight of  $99.6 \pm 1.7\%$  of starting body weight, by study day 4 (mean  $\pm$  standard deviation). The TNBS/vehicle group had the lowest mean final body weight, at  $79.8 \pm 1.8\%$ . Final mean body weights for some groups (i.e. TNBS/ Fc-HRS(2-60)-s.c. group and TNBS/budesonide group) were artificially high, due to the higher incidence of early morbidity in these groups. Of the groups that demonstrated the least early morbidity, the TNBS/ Fc-HRS(2-60)-QD -i.v. group had the highest mean final body weight of  $85.6 \pm 7.1\%$ , although this was not significantly different from the TNBS/vehicle group ( $p > 0.05$ ) (data not shown).

**[0559]** The majority of TNBS-recipient mice demonstrated diarrhoea at some stage during the study, although observations of mucosal bleeding were less common. There were reductions in the incidence of diarrhoea in response to some test item treatments. The TNBS/vehicle group had a cumulative (normalised) diarrhoea score of 31 by the end of the study on day 4; with the TNBS/ Fc-HRS(2-60)-QD-i.v. and TNBS/ HRS(1-60)-Fc-QD-i.v. groups having the lowest scores of 26 (data not shown).

**[0560]** The clinical condition of the mice can be best obtained by combining information on bleeding and diarrhoea together with a score for weight loss, in order to give a disease activity index (DAI) score. This can be most accurately determined on the day that a mouse is euthanised, as missing stool consistency observation data can be supplemented with observations of stool in the rectum. For mice surviving on day 4, there was a significant increase in the mean DAI scores for all the TNBS-recipient groups, in comparison to the untreated control group (at  $p \leq 0.0062$ ), except for the TNBS/ Fc-HRS(2-60)-s.c. group, although in this group only three mice remained in the study at the scheduled end point. The mean DAI for surviving mice on day 4 in the TNBS/vehicle group was  $7.13 \pm 0.64$ ; in comparison, the TNBS/budesonide and TNBS/ HRS(1-60)-Fc-once-i.v. groups had significantly lower mean DAIs, at  $4.33 \pm 1.97$  and  $4.20 \pm 3.03$ , respectively ( $p \leq 0.0304$ ). Inclusion of all mice, surviving to at least 07:00hr on day 1, in the calculation of DAI gave similar results, although the effect observed in the TNBS/budesonide and TNBS/ HRS(1-60)-Fc -once-i.v. groups was no longer significant ( $p > 0.05$ ) (see **Figure 24A**).

**[0561] Post mortem observations** - For the vehicle-only mice, mean large bowel weight and length were  $200.4 \pm 21.7\text{mg}$  and  $108.3 \pm 7.8\text{mm}$ , respectively; the mean large bowel weight:length ratio was  $1.85 \pm 0.12$  (mg/mm). All TNBS-treated groups demonstrated a significant increase in large bowel weight relative to the untreated control mice ( $p \leq 0.0215$ ), with the TNBS/vehicle group having a mean large bowel weight of  $368.4 \pm 70.1\text{mg}$ . The TNBS/budesonide group had the lowest mean large bowel weight of all the TNBS-recipient groups, at  $298.0 \pm 62.0\text{mg}$ . TNBS administration was associated also with a significant reduction in large bowel length in all TNBS-recipient groups ( $p \leq 0.0285$ ), with the TNBS/vehicle group having a mean large bowel



length of  $75.6 \pm 9.3\text{mm}$ ; in comparison, the TNBS/ HRS(1-60)-Fc -QD-i.v. group demonstrated a significant amelioration of large bowel shortening, with a mean large bowel length of  $94.9 \pm 11.8\text{mm}$  ( $p=0.0019$ ). The changes in large bowel weight and length resulted in a significant increase in large bowel weight:length ratio for all TNBS-recipient groups ( $p \leq 0.0116$ ). The TNBS/vehicle group had the highest mean large bowel weight:length ratio of all groups, at  $4.92 \pm 0.99$  (mg/mm). The TNBS/budesonide group, TNBS/ Fc-HRS(2-60)-s.c. group, TNBS/ HRS(1-60)-Fc -QD-i.v. group and the TNBS/ HRS(1-60)-Fc -once-i.v. group all demonstrated a significant reduction in large bowel weight:length ratio, relative to the TNBS/vehicle control group, although the mean ratio for the TNBS/ HRS(1-60)-Fc -QD-i.v. group was group was skewed by the number of mice that were euthanised early in the study. The mean large bowel weight:length ratios for the TNBS/budesonide and TNBS/ HRS(1-60)-Fc -QD-i.v. groups were  $3.57 \pm 1.01$  and  $3.56 \pm 0.80$ , respectively ( $p=0.0143$  and  $p=0.0456$ ) (see **Figure 24B**).

**[0562] Histopathology** - Histopathological changes associated with TNBS-induced colitis were assessed according to Epistem's standard histological scoring procedure. All TNBS-recipient groups demonstrated a significant increase in histopathology score ( $p \leq 0.0036$ ). The T cell/vehicle group had a mean histopathology score of  $2.32 \pm 0.65$ , with the TNBS/ HRS(1-60)-Fc -s.c. group having the highest mean histopathology score at  $2.41 \pm 0.68$ . Other treatment groups had lower mean histopathology scores than the TNBS/vehicle group, with the TNBS/budesonide and TNBS/ Fc-HRS(2-60)-QD-i.v. groups having the lowest scores of  $1.66 \pm 1.08$  and  $1.68 \pm 1.03$ , respectively, although these reductions were not statistically significant. On a regional basis, the effect of these treatments was more apparent in the distal third of the large bowel, although still not of statistical significance.

**[0563] Morbidity** - Only one group, TNBS/ HRS(1-60)-Fc -QD-i.v. demonstrated survival of all (eligible) mice (mouse 75 excluded due to large bowel perforation during TNBS administration and mouse 78 excluded due to lack of disease induction). Survival in other groups ranged from 80% (TNBS/ Fc-HRS(2-60)-QD-i.v. group) to 33.3% (TNBS/ Fc-HRS(2-60)-s.c. group); survival in the TNBS/budesonide group was 60%. Mice were euthanised when they presented with poor condition (withdrawn/failure to demonstrate normal behaviour); despite six-hourly checks, two mice (40 and 50) were found dead at 19:00hr on study day 3 (data not shown).

**[0564] qPCR Results.** To further investigate the mechanistic basis for the effects of HRS(1-60) and Fc-HRS(2-60) on TNBS induced colitis, changes in gene expression in the colons from animals was examined after the completion of the study. RNA profiling was performed on colons isolated from the mice on day as described above. The results from these studies demonstrated that seven genes were elevated by more than 10 fold in response to TNBS treatment were significantly reduced by treatment with Fc-HRS(2-60) (see **Table E20**; and **Figure 25**).

| Table E20                            |
|--------------------------------------|
| Genes regulated by more than 10 fold |
| IL6                                  |
| IL1b                                 |
| MCP1                                 |
| MMP3                                 |
| MMP9                                 |
| CD11B                                |
| IL10                                 |

**[0565]** Transcriptional profiling of TNBS treated mouse colons revealed many genes, including several housekeeping genes (data not shown) were not significantly impacted by Fc-HRS(2-60) treatment. By contrast, transcriptional profiling of TNBS treated mouse colons revealed significant reduction of TNBS



regulated immune and inflammatory related genes by Fc-HRS(2-60). This result was fortified by the finding that HRS(1-60) also significantly reduces TNBS induced levels of MCP1, MMP3, CD11b, and IL10 (see **Figures 26A-26H**).

**[0566] Conclusion:** Intracolonic administration of TNBS to BDF-1 mice resulted in the development of colitis characterised by acute weight loss, with a moderate increase in the incidence of diarrhoea and mucosal bleeding. *Post mortem* examination demonstrated significant change in the large bowel weight: length ratio and ulcerative lesions in the large bowel, with stenosis and accompanying stool accumulation within the bowel. Treatment with budesonide was associated with improvements in both in-life and post mortem disease parameters, and although having the lowest mean histopathology score of all TNBS-recipient groups, the reduction in this disease parameter did not achieve significance. Treatment with Fc-HRS(2-60)-QD-i.v. had a similar effect to budesonide in reducing histopathology score and was associated with superior survival. The TNBS/ HRS(1-60)-Fc -QD-i.v. group showed the highest level of survival and significant improvement in large bowel weight:length ratio, although it was less effective than either budesonide or Fc-HRS(2-60) in reducing the histopathology score. With regard to histopathology score, all three of these test items appeared to have their greatest effect on the distal third of the large bowel. Improvements in disease parameters were observed for the TNBS/ Fc-HRS(2-60)-s.c. group, and the TNBS/ HRS(1-60)-Fc -once-i.v. group (e.g. body weight and large bowel weight:length ratio). Additionally, transcriptional profiling of TNBS treated mouse colons revealed significant reduction of TNBS regulated immune and inflammatory related genes by Fc-HRS(2-60). This result was fortified by the finding that HRS(1-60) also significantly reduces TNBS induced levels of MCP1, MMP3, CD11b, and IL10, demonstrating the the HRS-Fc fusion proteins were active in immunomodulating gene expression in this system.

**[0567]** Overall, the data suggest that Fc-HRS(2-60) and HRS(1-60)-Fc have significant potential in the treatment of intestinal inflammation, and other inflammatory conditions.

## EXAMPLE 10

### IMPACT OF HRS-FC FUSION PROTEINS ON T CELL POPULATIONS

**[0568]** To evaluate the potential impact of HRS-Fc conjugates on T cell populations *in vivo*, Fc-HRS(2-60) was tested in a TNBS induced model of colitis, similar to that described above. Studies were performed in male BDF-1 mice. Briefly, male BDF-1 mice (Jackson Laboratories), were acclimated for a minimum of 1 week prior to experimentation, were fasted beginning 16 hours prior to TNBS administration and 13 hours prior to dosing. Fc-HRS(2-60) was administered at a single dose, 0.5 mg/Kg, IV at the same time as commencing TNBS treatment. Splenocytes from three animals per group were analyzed for immune populations. To choose the animals for analysis, the mice that had the most severe and the least severe disease scores were excluded (based on clinical observations, stool consistency and body weight). From the remaining animals, three animals from each treatment group were picked based on representing the mean of their respective group.

**[0569] Procedures.** Spleens were harvested from BDF-1 mice and placed directly into 10ml cold Cell Staining Buffer (Biolegend, catalog #420201, lot #B166478) on ice. Spleens were mechanically disassociated between two frosted autoclaved slides in a tissue culture dish. The cell suspension was filtered through a 70µm filter and cells were pelleted by centrifugation at 300g for 5 minutes, 4°C. Cells were washed once in 10ml Cell Staining Buffer and counted using the Nexcelom Cellometer Auto 2000 Cell Viability Counter. Splenocytes were reconstituted at  $5 \times 10^5$  cells/ml in Cell Staining Buffer and were immediately stained with antibodies for flow cytometry.



**[0570]** A total of  $1 \times 10^6$  cells were stained for T regulatory markers (CD4, CD25 and FOXP3) using the One Step Staining Mouse Treg Flow Kit (Biolegend catalog #13680, lot numbers #B177852, B177853, B174309, B176365) according to manufacturer's instructions.

**[0571]** For phenotyping of immune cells, a total of  $1 \times 10^6$  cells were stained with fluorescently-labeled antibodies against CD3 $\epsilon$  (catalog #100334, lot #162956), CD4 (catalog #100529, lot #B152907) and CD8 (catalog #100714, lot #B165226) (antibodies were purchased from Biolegend). Cells were stained with antibodies diluted at manufacturer's recommended concentrations in Cell Staining Buffer and incubated for 30 minutes at 4°C. Cells were thoroughly washed with Cell Staining Buffer and fixed using Stabilizing Fixative Buffer (BD Biosciences catalog #338036, lot #2291614).

**[0572]** Flow cytometry was performed at the VA Flow Cytometry Research Core facility (VA San Diego, La Jolla, CA). The samples were analyzed on a 3 laser BD Canto instrument (488 Argon, 633 HeNe, 405 Violet). Raw FACS files were analyzed using FlowJo software (TreeStar).

**[0573] Results.** Extracellularly-stained splenocytes were gated on a live lymphocyte population and CD3 $^+$  cells to determine a total T cell percentage. To generate population percentages of CD4 $^+$  and CD8 $^+$  cells, cells were gated into CD3 $^+$ CD4 $^+$  T cells and CD3 $^+$ CD8 $^+$  T double-positive cells from the live lymphocyte gate. T regulatory cells (Treg) were gated on a live lymphocyte gate and then on CD4 $^+$  cells. Treg cells were determined based on expression of CD25 and FoxP3. In these data (see Figures 27A-27D), each dot represents one animal, the line represents the mean.

**[0574]** In TNBS-treated mice, there was an increase in CD3 $^+$  T cells (**Figure 27A**), compared to naïve animals. By contrast, two out of the three Fc-HRS(2-60) treated mice showed a reduction in CD3 $^+$  T cells compared to the TNBS treated animals. To determine which CD3 $^+$  populations were accounting for this change, CD8 $^+$ , CD4 $^+$  and Treg cells populations were further investigated. **Figures 27B** and **Figure 27C** show that CD8 $^+$  T and CD4 $^+$  cells were elevated in TNBS-treated animals and treatment with Fc-HRS(2-60) reduced levels in both cases. Furthermore, Treg cells were depleted in TNBS-treated animals, compared to naïve animals, but were elevated compared to the TNBS treated animals in the Fc-HRS(2-60) treated group (**Figure 27D**). Moreover, one mouse treated with Fc-HRS(2-60) showed Treg levels similar to naïve animals. Together, these results suggest that TNBS alters T cell populations in the spleen to a more inflammatory phenotype through increased CD8 $^+$  T cells and decreased Treg cells and that treatment with Fc-HRS(2-60) restores these populations towards homeostatic levels.

**[0575]** These and other changes can be made to the embodiments of the present disclosure in light of the above-detailed description. In general, in the following claims, the terms used should not be construed to limit the claims to the specific embodiments of the present disclosure disclosed in the specification and the claims, but should be construed to include all possible embodiments of the present disclosure along with the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

## SEQUENCE LISTING

**[0576]**

<110> Ying BUECHLER et al.

<120> HISTIDYL-TRNA SYNTHETASE-FC CONJUGATES



&lt;130&gt; ATYR-116/01WO

&lt;150&gt; US 61/789,011

&lt;151&gt; 2013-03-15

&lt;160&gt; 396

&lt;170&gt; PatentIn version 3.5

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Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp  
50 55 60

Tyr Ser Pro Arg Gln Met Ala Val Arg Glu Lys Val Phe Asp Val Ile  
65 70 75 80

Ile Arg Cys Phe Lys Arg His Gly Ala Glu Val Ile Asp Thr Pro Val  
85 90 95

Phe Glu Leu Lys Glu Thr Leu Met Gly Lys Tyr Gly Glu Asp Ser Lys  
100 105 110

Leu

<210> 6

<211> 60

<212> PRT

<213> Homo sapiens

<400> 6

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys  
50 55 60

<210> 7

<211> 270

<212> PRT

<213> Homo sapiens

<400> 7

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu



```

1           5           10           15
Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu
20           25           30
Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp
35           40           45
Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp
50           55           60
Tyr Ser Pro Arg Gln Met Ala Val Arg Glu Lys Val Phe Asp Val Ile
65           70           75           80
Ile Arg Cys Phe Lys Arg His Gly Ala Glu Val Ile Asp Thr Pro Val
85           90           95
Phe Glu Leu Lys Glu Thr Leu Met Gly Lys Tyr Gly Glu Asp Ser Lys
100          105          110
Leu Ile Tyr Asp Leu Lys Asp Gln Gly Gly Glu Leu Leu Ser Leu Arg
115          120          125
Tyr Asp Leu Thr Val Pro Phe Ala Arg Tyr Leu Ala Met Asn Lys Leu
130          135          140
Thr Asn Ile Lys Arg Tyr His Ile Ala Lys Val Tyr Arg Arg Asp Asn
145          150          155          160
Pro Ala Met Thr Arg Gly Arg Tyr Arg Glu Phe Tyr Gln Cys Asp Phe
165          170          175
Asp Ile Ala Gly Asn Phe Asp Pro Met Ile Pro Asp Ala Glu Cys Leu
180          185          190
Lys Ile Met Cys Glu Ile Leu Ser Ser Leu Gln Ile Gly Asp Phe Leu
195          200          205
Val Lys Val Asn Asp Arg Arg Ile Leu Asp Gly Met Phe Ala Ile Cys
210          215          220
Gly Val Ser Asp Ser Lys Phe Arg Thr Ile Cys Ser Ser Val Asp Lys
225          230          235          240
Leu Asp Lys Val Gly Tyr Pro Trp Trp Asn Ser Cys Ser Arg Ile Leu
245          250          255
Asn Tyr Pro Lys Thr Ser Arg Pro Trp Arg Ala Trp Glu Thr
260          265          270
<210> 8
<211> 105
<212> PRT
<213> Homo sapiens
<400> 8
Arg Thr Thr Glu Thr Gln Val Leu Val Ala Ser Ala Gln Lys Lys Leu
1           5           10           15
Leu Glu Glu Arg Leu Lys Leu Val Ser Glu Leu Trp Asp Ala Gly Ile
20           25           30
Lys Ala Glu Leu Leu Tyr Lys Lys Asn Pro Lys Leu Leu Asn Gln Leu
35           40           45

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Gln Tyr Cys Glu Glu Ala Gly Ile Pro Leu Val Ala Ile Ile Gly Glu  
50 55 60

Gln Glu Leu Lys Asp Gly Val Ile Lys Leu Arg Ser Val Thr Ser Arg  
65 70 75 80

Glu Glu Val Asp Val Arg Arg Glu Asp Leu Val Glu Glu Ile Lys Arg  
85 90 95

Arg Thr Gly Gln Pro Leu Cys Ile Cys  
100 105

<210> 9

<211> 395

<212> PRT

<213> Homo sapiens

<400> 9

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Asp Phe Asp Ile  
50 55 60

Ala Gly Asn Phe Asp Pro Met Ile Pro Asp Ala Glu Cys Leu Lys Ile  
65 70 75 80

Met Cys Glu Ile Leu Ser Ser Leu Gln Ile Gly Asp Phe Leu Val Lys  
85 90 95

Val Asn Asp Arg Arg Ile Leu Asp Gly Met Phe Ala Ile Cys Gly Val  
100 105 110

Ser Asp Ser Lys Phe Arg Thr Ile Cys Ser Ser Val Asp Lys Leu Asp  
115 120 125

Lys Val Ser Trp Glu Glu Val Lys Asn Glu Met Val Gly Glu Lys Gly  
130 135 140

Leu Ala Pro Glu Val Ala Asp Arg Ile Gly Asp Tyr Val Gln Gln His  
145 150 155 160

Gly Gly Val Ser Leu Val Glu Gln Leu Leu Gln Asp Pro Lys Leu Ser  
165 170 175

Gln Asn Lys Gln Ala Leu Glu Gly Leu Gly Asp Leu Lys Leu Leu Phe  
180 185 190

Glu Tyr Leu Thr Leu Phe Gly Ile Asp Asp Lys Ile Ser Phe Asp Leu  
195 200 205

Ser Leu Ala Arg Gly Leu Asp Tyr Tyr Thr Gly Val Ile Tyr Glu Ala  
210 215 220

Val Leu Leu Gln Thr Pro Ala Gln Ala Gly Glu Glu Pro Leu Gly Val  
225 230 235 240

Gly Ser Val Ala Ala Gly Gly Arg Tyr Asp Gly Leu Val Gly Met Phe



|                                                                 |     |         |
|-----------------------------------------------------------------|-----|---------|
| 245                                                             | 250 | 255     |
| Asp Pro Lys Gly Arg Lys Val Pro Cys Val Gly Leu Ser Ile Gly Val |     |         |
| 260                                                             | 265 | 270     |
| Glu Arg Ile Phe Ser Ile Val Glu Gln Arg Leu Glu Ala Leu Glu Glu |     |         |
| 275                                                             | 280 | 285     |
| Lys Ile Arg Thr Thr Glu Thr Gln Val Leu Val Ala Ser Ala Gln Lys |     |         |
| 290                                                             | 295 | 300     |
| Lys Leu Leu Glu Glu Arg Leu Lys Leu Val Ser Glu Leu Trp Asp Ala |     |         |
| 305                                                             | 310 | 315 320 |
| Gly Ile Lys Ala Glu Leu Leu Tyr Lys Lys Asn Pro Lys Leu Leu Asn |     |         |
| 325                                                             | 330 | 335     |
| Gln Leu Gln Tyr Cys Glu Glu Ala Gly Ile Pro Leu Val Ala Ile Ile |     |         |
| 340                                                             | 345 | 350     |
| Gly Glu Gln Glu Leu Lys Asp Gly Val Ile Lys Leu Arg Ser Val Thr |     |         |
| 355                                                             | 360 | 365     |
| Ser Arg Glu Glu Val Asp Val Arg Arg Glu Asp Leu Val Glu Glu Ile |     |         |
| 370                                                             | 375 | 380     |
| Lys Arg Arg Thr Gly Gln Pro Leu Cys Ile Cys                     |     |         |
| 385                                                             | 390 | 395     |

<210> 10

<211> 359

<212> PRT

<213> Homo sapiens

<400> 10

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



Leu Phe Gly Ile Asp Asp Lys Ile Ser Phe Asp Leu Ser Leu Ala Arg  
165 170 175

Gly Leu Asp Tyr Tyr Thr Gly Val Ile Tyr Glu Ala Val Leu Leu Gln  
180 185 190

Thr Pro Ala Gln Ala Gly Glu Glu Pro Leu Gly Val Gly Ser Val Ala  
195 200 205

Ala Gly Gly Arg Tyr Asp Gly Leu Val Gly Met Phe Asp Pro Lys Gly  
210 215 220

Arg Lys Val Pro Cys Val Gly Leu Ser Ile Gly Val Glu Arg Ile Phe  
225 230 235 240

Ser Ile Val Glu Gln Arg Leu Glu Ala Leu Glu Glu Lys Ile Arg Thr  
245 250 255

Thr Glu Thr Gln Val Leu Val Ala Ser Ala Gln Lys Lys Leu Leu Glu  
260 265 270

Glu Arg Leu Lys Leu Val Ser Glu Leu Trp Asp Ala Gly Ile Lys Ala  
275 280 285

Glu Leu Leu Tyr Lys Lys Asn Pro Lys Leu Leu Asn Gln Leu Gln Tyr  
290 295 300

Cys Glu Glu Ala Gly Ile Pro Leu Val Ala Ile Ile Gly Glu Gln Glu  
305 310 315 320

Leu Lys Asp Gly Val Ile Lys Leu Arg Ser Val Thr Ser Arg Glu Glu  
325 330 335

Val Asp Val Arg Arg Glu Asp Leu Val Glu Glu Ile Lys Arg Arg Thr  
340 345 350

Gly Gln Pro Leu Cys Ile Cys  
355

<210> 11

<211> 399

<212> PRT

<213> Homo sapiens

<400> 11

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp  
50 55 60

Tyr Ser Pro Arg Gln Met Ala Val Arg Glu Lys Val Phe Asp Val Ile  
65 70 75 80

Ile Arg Cys Phe Lys Arg His Gly Ala Glu Val Ile Asp Thr Pro Val  
85 90 95

Phe Glu Leu Lys Val Asn Asp Arg Arg Ile Leu Asp Glu Met Phe Ala



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-----
      100      105      110
-----
Ile Cys Gly Val Ser Asp Ser Lys Phe Arg Thr Ile Cys Ser Ser Val
  115      120      125

Asp Lys Leu Asp Lys Val Ser Trp Glu Glu Val Lys Asn Glu Met Val
  130      135      140

Gly Glu Lys Gly Leu Ala Pro Glu Val Ala Asp Arg Ile Gly Asp Tyr
  145      150      155      160

Val Gln Gln His Gly Gly Val Ser Leu Val Glu Gln Leu Leu Gln Asp
      165      170      175

Pro Lys Leu Ser Gln Asn Lys Gln Ala Leu Glu Gly Leu Gly Asp Leu
      180      185      190

Lys Leu Leu Phe Glu Tyr Leu Thr Leu Phe Gly Ile Asp Asp Lys Ile
      195      200      205

Ser Phe Asp Leu Ser Leu Ala Arg Gly Leu Asp Tyr Tyr Thr Gly Val
      210      215      220

Ile Tyr Glu Ala Val Leu Leu Gln Thr Pro Ala Gln Ala Gly Glu Glu
  225      230      235      240

Pro Leu Gly Val Gly Ser Val Ala Ala Gly Gly Arg Tyr Asp Gly Leu
      245      250      255

Val Gly Met Phe Asp Pro Lys Gly Arg Lys Val Pro Cys Val Gly Leu
      260      265      270

Ser Ile Gly Val Glu Arg Ile Phe Ser Ile Val Glu Gln Arg Leu Glu
      275      280      285

Ala Leu Glu Glu Lys Ile Arg Thr Thr Glu Thr Gln Val Leu Val Ala
      290      295      300

Ser Ala Gln Lys Lys Leu Leu Glu Glu Arg Leu Lys Leu Val Ser Glu
  305      310      315      320

Leu Trp Asp Ala Gly Ile Lys Ala Glu Leu Leu Tyr Lys Lys Asn Pro
      325      330      335

Lys Leu Leu Asn Gln Leu Gln Tyr Cys Glu Glu Ala Gly Ile Pro Leu
      340      345      350

Val Ala Ile Ile Gly Glu Gln Glu Leu Lys Asp Gly Val Ile Lys Leu
      355      360      365

Arg Ser Val Thr Ser Arg Glu Glu Val Asp Val Arg Arg Glu Asp Leu
      370      375      380

Val Glu Glu Ile Lys Arg Arg Thr Gly Gln Pro Leu Cys Ile Cys
  385      390      395

<210> 12
<211> 473
<212> PRT
<213> Homo sapiens

<400> 12
Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu
  1      5      10      15

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Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp  
50 55 60

Tyr Ser Pro Arg Gln Met Ala Val Arg Glu Lys Val Phe Asp Val Ile  
65 70 75 80

Ile Arg Cys Phe Lys Arg His Gly Ala Glu Val Ile Asp Thr Pro Val  
85 90 95

Phe Glu Leu Lys Glu Thr Leu Met Gly Lys Tyr Gly Glu Asp Ser Lys  
100 105 110

Leu Ile Tyr Asp Leu Lys Asp Gln Gly Gly Glu Leu Leu Ser Leu Arg  
115 120 125

Tyr Asp Leu Thr Val Pro Phe Ala Arg Tyr Leu Ala Met Asn Lys Leu  
130 135 140

Thr Asn Ile Lys Arg Tyr His Ile Ala Lys Val Tyr Arg Arg Asp Asn  
145 150 155 160

Pro Ala Met Thr Arg Gly Arg Tyr Arg Glu Phe Tyr Gln Cys Val Asn  
165 170 175

Asp Arg Arg Ile Leu Asp Gly Met Phe Ala Ile Cys Gly Val Ser Asp  
180 185 190

Ser Lys Phe Arg Thr Ile Cys Ser Ser Val Asp Lys Leu Asp Lys Val  
195 200 205

Ser Trp Glu Glu Val Lys Asn Glu Met Val Gly Glu Lys Gly Leu Ala  
210 215 220

Pro Glu Val Ala Asp Arg Ile Gly Asp Tyr Val Gln Gln His Gly Gly  
225 230 235 240

Val Ser Leu Val Glu Gln Leu Leu Gln Asp Pro Lys Leu Ser Gln Asn  
245 250 255

Lys Gln Ala Leu Glu Gly Leu Gly Asp Leu Lys Leu Leu Phe Glu Tyr  
260 265 270

Leu Thr Leu Phe Gly Ile Asp Asp Lys Ile Ser Phe Asp Leu Ser Leu  
275 280 285

Ala Arg Gly Leu Asp Tyr Tyr Thr Gly Val Ile Tyr Glu Ala Val Leu  
290 295 300

Leu Gln Thr Pro Ala Gln Ala Gly Glu Glu Pro Leu Gly Val Gly Ser

305 310 315 320

Val Ala Ala Gly Gly Arg Tyr Asp Gly Leu Val Gly Met Phe Asp Pro  
325 330 335

Lys Gly Arg Lys Val Pro Cys Val Gly Leu Ser Ile Gly Val Glu Arg  
340 345 350



Ile Phe Ser Ile Val Glu Gln Arg Leu Glu Ala Leu Glu Glu Lys Ile  
355 360 365

Arg Thr Thr Glu Thr Gln Val Leu Val Ala Ser Ala Gln Lys Lys Leu  
370 375 380

Leu Glu Glu Arg Leu Lys Leu Val Ser Glu Leu Trp Asp Ala Gly Ile  
385 390 395 400

Lys Ala Glu Leu Leu Tyr Lys Lys Asn Pro Lys Leu Leu Asn Gln Leu  
405 410 415

Gln Tyr Cys Glu Glu Ala Gly Ile Pro Leu Val Ala Ile Ile Gly Glu  
420 425 430

Gln Glu Leu Lys Asp Gly Val Ile Lys Leu Arg Ser Val Thr Ser Arg  
435 440 445

Glu Glu Val Asp Val Arg Arg Glu Asp Leu Val Glu Glu Ile Lys Arg  
450 455 460

Arg Thr Gly Gln Pro Leu Cys Ile Cys  
465 470

<210> 13

<211> 469

<212> PRT

<213> Homo sapiens

<400> 13

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Glu Thr Leu Met  
50 55 60

Gly Lys Tyr Gly Glu Asp Ser Lys Leu Ile Tyr Asp Leu Lys Asp Gln  
65 70 75 80

Gly Gly Glu Leu Leu Ser Leu Arg Tyr Asp Leu Thr Val Pro Phe Ala  
85 90 95

Arg Tyr Leu Ala Met Asn Lys Leu Thr Asn Ile Lys Arg Tyr His Ile  
100 105 110

Ala Lys Val Tyr Arg Arg Asp Asn Pro Ala Met Thr Arg Gly Arg Tyr  
115 120 125

Arg Glu Phe Tyr Gln Cys Asp Phe Asp Ile Ala Gly Asn Phe Asp Pro  
130 135 140

Met Ile Pro Asp Ala Glu Cys Leu Lys Ile Met Cys Glu Ile Leu Ser  
145 150 155 160

Ser Leu Gln Ile Gly Asp Phe Leu Val Lys Val Asn Asp Arg Arg Ile  
165 170 175



Leu Asp Gly Met Phe Ala Ile Cys Gly Val Ser Asp Ser Lys Phe Arg  
180 185 190

Thr Ile Cys Ser Ser Val Asp Lys Leu Asp Lys Val Ser Trp Glu Glu  
195 200 205

Val Lys Asn Glu Met Val Gly Glu Lys Gly Leu Ala Pro Glu Val Ala  
210 215 220

Asp Arg Ile Gly Asp Tyr Val Gln Gln His Gly Gly Val Ser Leu Val  
225 230 235 240

Glu Gln Leu Leu Gln Asp Pro Lys Leu Ser Gln Asn Lys Gln Ala Leu  
245 250 255

Glu Gly Leu Gly Asp Leu Lys Leu Leu Phe Glu Tyr Leu Thr Leu Phe  
260 265 270

Gly Ile Asp Asp Lys Ile Ser Phe Asp Leu Ser Leu Ala Arg Gly Leu  
275 280 285

Asp Tyr Tyr Thr Gly Val Ile Tyr Glu Ala Val Leu Leu Gln Thr Pro  
290 295 300

Ala Gln Ala Gly Glu Glu Pro Leu Gly Val Gly Ser Val Ala Ala Gly  
305 310 315 320

Gly Arg Tyr Asp Gly Leu Val Gly Met Phe Asp Pro Lys Gly Arg Lys  
325 330 335

Val Pro Cys Val Gly Leu Ser Ile Gly Val Glu Arg Ile Phe Ser Ile  
340 345 350

Val Glu Gln Arg Leu Glu Ala Leu Glu Glu Lys Ile Arg Thr Thr Glu  
355 360 365

Thr Gln Val Leu Val Ala Ser Ala Gln Lys Lys Leu Leu Glu Glu Arg  
370 375 380

Leu Lys Leu Val Ser Glu Leu Trp Asp Ala Gly Ile Lys Ala Glu Leu  
385 390 395 400

Leu Tyr Lys Lys Asn Pro Lys Leu Leu Asn Gln Leu Gln Tyr Cys Glu  
405 410 415

Glu Ala Gly Ile Pro Leu Val Ala Ile Ile Gly Glu Gln Glu Leu Lys  
420 425 430

Asp Gly Val Ile Lys Leu Arg Ser Val Thr Ser Arg Glu Glu Val Asp  
435 440 445

Val Arg Arg Glu Asp Leu Val Glu Glu Ile Lys Arg Arg Thr Gly Gln  
450 455 460

Pro Leu Cys Ile Cys  
465

<210> 14

<211> 435

<212> PRT

<213> Homo sapiens

<400> 14

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
- - - - -



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



333                      360                      387  
 Lys Lys Asn Pro Lys Leu Leu Asn Gln Leu Gln Tyr Cys Glu Glu Ala  
     370                      375                      380  
 Gly Ile Pro Leu Val Ala Ile Ile Gly Glu Gln Glu Leu Lys Asp Gly  
     385                      390                      395                      400  
 Val Ile Lys Leu Arg Ser Val Thr Ser Arg Glu Glu Val Asp Val Arg  
                     405                      410                      415  
 Arg Glu Asp Leu Val Glu Glu Ile Lys Arg Arg Thr Gly Gln Pro Leu  
                     420                      425                      430  
 Cys Ile Cys  
                     435

&lt;210&gt; 15

&lt;211&gt; 171

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 15

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

&lt;210&gt; 16

&lt;211&gt; 211

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 16

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
     1                      5                      10                      15



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

&lt;210&gt; 17

&lt;211&gt; 141

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 17

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



Ile Ile Gly Glu Gln Glu Leu Lys Asp Gly Val Ile Lys Leu Arg Ser  
100 105 110

Val Thr Ser Arg Glu Glu Val Asp Val Arg Arg Glu Asp Leu Val Glu  
115 120 125

Glu Ile Lys Arg Arg Thr Gly Gln Pro Leu Cys Ile Cys  
130 135 140

<210> 18

<211> 143

<212> PRT

<213> Homo sapiens

<400> 18

Cys Leu Lys Ile Met Cys Glu Ile Leu Ser Ser Leu Gln Ile Gly Asp  
1 5 10 15

Phe Leu Val Lys Val Asn Asp Arg Arg Ile Leu Asp Gly Met Phe Ala  
20 25 30

Ile Cys Gly Val Ser Asp Ser Lys Phe Arg Thr Ile Cys Ser Ser Val  
35 40 45

Asp Lys Leu Asp Lys Val Ser Trp Glu Glu Val Lys Asn Glu Met Val  
50 55 60

Gly Glu Lys Gly Leu Ala Pro Glu Val Ala Asp Arg Ile Gly Asp Tyr  
65 70 75 80

Val Gln Gln His Gly Gly Val Ser Leu Val Glu Gln Leu Leu Gln Asp  
85 90 95

Pro Lys Leu Ser Gln Asn Lys Gln Ala Leu Glu Gly Leu Gly Asp Leu  
100 105 110

Lys Leu Leu Phe Glu Tyr Leu Thr Leu Phe Gly Ile Asp Asp Lys Ile  
115 120 125

Ser Phe Asp Leu Ser Leu Ala Arg Gly Leu Asp Tyr Tyr Thr Gly  
130 135 140

<210> 19

<211> 509

<212> PRT

<213> Mus musculus

<400> 19

Met Ala Asp Arg Ala Ala Leu Glu Glu Leu Val Arg Leu Gln Gly Ala  
1 5 10 15

His Val Arg Gly Leu Lys Glu Gln Lys Ala Ser Ala Glu Gln Ile Glu  
20 25 30

Glu Glu Val Thr Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Gln Asp  
35 40 45

Glu Gly Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp  
50 55 60

Tyr Ser Pro Arg Gln Met Ala Val Arg Glu Lys Val Phe Asp Val Ile  
65 70 75 80

Ile Arg Cys Phe Lys Arg His Gly Ala Glu Val Ile Asp Thr Pro Val  
85 90 95



[illegible]



435                      440                      445  
 Leu Asn Gln Leu Gln Tyr Trp Glu Glu Ala Gly Ile Pro Leu Val Ala  
 450                      455                      460  
 Ile Ile Gly Glu Gln Glu Leu Arg Asp Gly Val Ile Lys Leu Arg Ser  
 465                      470                      475                      480  
 Val Ala Ser Arg Glu Glu Val Asp Val Arg Arg Glu Asp Leu Val Glu  
 485                      490                      495  
 Glu Ile Arg Arg Arg Thr Asn Gln Pro Leu Ser Thr Cys  
 500                      505

&lt;210&gt; 20

&lt;211&gt; 509

&lt;212&gt; PRT

&lt;213&gt; Canis lupus familiaris

&lt;400&gt; 20

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



Leu Asp Lys Val Ser Trp Glu Glu Val Lys Asn Glu Met Val Gly Glu  
245 250 255

Lys Gly Leu Ala Pro Glu Val Ala Asp His Ile Gly Asp Tyr Val Gln  
260 265 270

Gln His Gly Gly Ile Ser Leu Val Glu Gln Leu Leu Gln Asp Pro Glu  
275 280 285

Leu Ser Gln Asn Lys Gln Ala Leu Glu Gly Leu Gly Asp Leu Lys Leu  
290 295 300

Leu Phe Glu Tyr Leu Thr Leu Phe Gly Ile Ala Asp Lys Ile Ser Phe  
305 310 315 320

Asp Leu Ser Leu Ala Arg Gly Leu Asp Tyr Tyr Thr Gly Val Ile Tyr  
325 330 335

Glu Ala Val Leu Leu Gln Thr Pro Val Gln Ala Gly Glu Glu Pro Leu  
340 345 350

Gly Val Gly Ser Val Ala Ala Gly Gly Arg Tyr Asp Gly Leu Val Gly  
355 360 365

Met Phe Asp Pro Lys Gly Arg Lys Val Pro Cys Val Gly Leu Ser Ile  
370 375 380

Gly Val Glu Arg Ile Phe Ser Ile Val Glu Gln Arg Leu Glu Ala Thr  
385 390 395 400

Glu Glu Lys Val Arg Thr Thr Glu Thr Gln Val Leu Val Ala Ser Ala  
405 410 415

Gln Lys Lys Leu Leu Glu Glu Arg Leu Lys Leu Val Ser Glu Leu Trp  
420 425 430

Asn Ala Gly Ile Lys Ala Glu Leu Leu Tyr Lys Lys Asn Pro Lys Leu  
435 440 445

Leu Asn Gln Leu Gln Tyr Cys Glu Glu Ala Gly Ile Pro Leu Val Ala  
450 455 460

Ile Ile Gly Glu Gln Glu Leu Lys Asp Gly Val Ile Lys Leu Arg Ser  
465 470 475 480

Val Ala Ser Arg Glu Glu Val Asp Val Pro Arg Glu Asp Leu Val Glu  
485 490 495

Glu Ile Lys Arg Arg Thr Ser Gln Pro Phe Cys Ile Cys

500

505

<210> 21

<211> 509

<212> PRT

<213> Bos taurus

<400> 21

Met Ala Asp Arg Ala Ala Leu Glu Asp Leu Val Arg Val Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Gln Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp



|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |  |  |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|--|--|--|
| 35  | 40  | 45  |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |  |  |
| Glu | Gly | Lys | Pro | Lys | Phe | Val | Leu | Lys | Thr | Pro | Lys | Gly | Thr | Arg | Asp |  |  |  |  |
| 50  |     |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |  |  |  |  |
| Tyr | Ser | Pro | Arg | Gln | Met | Ala | Val | Arg | Glu | Lys | Val | Phe | Asp | Val | Ile |  |  |  |  |
| 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |  |  |  |  |
| Ile | Ser | Cys | Phe | Lys | Arg | His | Gly | Ala | Glu | Val | Ile | Asp | Thr | Pro | Val |  |  |  |  |
|     |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |  |  |  |  |
| Phe | Glu | Leu | Lys | Glu | Thr | Leu | Thr | Gly | Lys | Tyr | Gly | Glu | Asp | Ser | Lys |  |  |  |  |
|     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |  |  |  |  |
| Leu | Ile | Tyr | Asp | Leu | Lys | Asp | Gln | Gly | Gly | Glu | Leu | Leu | Ser | Leu | Arg |  |  |  |  |
|     |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |  |  |  |  |
| Tyr | Asp | Leu | Thr | Val | Pro | Phe | Ala | Arg | Tyr | Leu | Ala | Met | Asn | Lys | Leu |  |  |  |  |
| 130 |     |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |  |  |  |  |
| Thr | Asn | Ile | Lys | Arg | Tyr | His | Ile | Ala | Lys | Val | Tyr | Arg | Arg | Asp | Asn |  |  |  |  |
| 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |  |  |  |  |
| Pro | Ala | Met | Thr | Arg | Gly | Arg | Tyr | Arg | Glu | Phe | Tyr | Gln | Cys | Asp | Phe |  |  |  |  |
|     |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |  |  |  |  |
| Asp | Ile | Ala | Gly | Gln | Phe | Asp | Pro | Met | Leu | Pro | Asp | Ala | Glu | Cys | Leu |  |  |  |  |
|     |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |  |  |  |  |
| Lys | Ile | Met | Cys | Glu | Ile | Leu | Ser | Ser | Leu | Gln | Ile | Gly | Asp | Phe | Leu |  |  |  |  |
|     |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |  |  |  |  |
| Val | Lys | Val | Asn | Asp | Arg | Arg | Ile | Leu | Asp | Gly | Met | Phe | Ala | Ile | Cys |  |  |  |  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |  |  |
| 210 |     |     |     |     |     | 215 |     |     |     |     |     | 220 |     |     |     |  |  |  |  |
| Gly | Val | Pro | Asp | Ser | Lys | Phe | Arg | Thr | Ile | Cys | Ser | Ser | Val | Asp | Lys |  |  |  |  |
| 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |  |  |  |  |
| Leu | Asp | Lys | Val | Ser | Trp | Glu | Glu | Val | Lys | Asn | Glu | Met | Val | Gly | Glu |  |  |  |  |
|     |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |     |  |  |  |  |
| Lys | Gly | Leu | Ala | Pro | Glu | Val | Ala | Asp | Arg | Ile | Gly | Asp | Tyr | Val | Gln |  |  |  |  |
|     |     |     | 260 |     |     |     |     | 265 |     |     |     |     | 270 |     |     |  |  |  |  |
| Gln | His | Gly | Gly | Val | Ser | Leu | Val | Glu | Gln | Leu | Leu | Gln | Asp | Pro | Lys |  |  |  |  |
|     |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |  |  |  |  |
| Leu | Ser | Gln | Asn | Lys | Gln | Ala | Leu | Glu | Gly | Leu | Gly | Asp | Leu | Lys | Leu |  |  |  |  |
|     |     | 290 |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |  |  |  |  |
| Leu | Phe | Glu | Tyr | Leu | Thr | Leu | Phe | Gly | Ile | Ala | Asp | Lys | Ile | Ser | Phe |  |  |  |  |
| 305 |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |  |  |  |  |
| Asp | Leu | Ser | Leu | Ala | Arg | Gly | Leu | Asp | Tyr | Tyr | Thr | Gly | Val | Ile | Tyr |  |  |  |  |
|     |     |     |     | 325 |     |     |     |     | 330 |     |     |     |     | 335 |     |  |  |  |  |
| Glu | Ala | Val | Leu | Leu | Gln | Pro | Pro | Ala | Arg | Ala | Gly | Glu | Glu | Pro | Leu |  |  |  |  |
|     |     |     | 340 |     |     |     |     | 345 |     |     |     |     | 350 |     |     |  |  |  |  |
| Gly | Val | Gly | Ser | Val | Ala | Ala | Gly | Gly | Arg | Tyr | Asp | Gly | Leu | Val | Gly |  |  |  |  |
|     |     | 355 |     |     |     |     | 360 |     |     |     |     | 365 |     |     |     |  |  |  |  |
| Met | Phe | Asp | Pro | Lys | Gly | Arg | Lys | Val | Pro | Cys | Val | Gly | Leu | Ser | Ile |  |  |  |  |
|     |     | 370 |     |     |     | 375 |     |     |     |     | 380 |     |     |     |     |  |  |  |  |



Gly Val Glu Arg Ile Phe Ser Ile Val Glu Gln Arg Leu Glu Ala Leu  
385 390 395 400

Glu Glu Lys Val Arg Thr Thr Glu Thr Gln Val Leu Val Ala Ser Ala  
405 410 415

Gln Lys Lys Leu Leu Glu Glu Arg Leu Lys Leu Ile Ser Glu Leu Trp  
420 425 430

Asp Ala Gly Ile Lys Ala Glu Leu Leu Tyr Lys Lys Asn Pro Lys Leu  
435 440 445

Leu Asn Gln Leu Gln Tyr Cys Glu Glu Thr Gly Ile Pro Leu Val Ala  
450 455 460

Ile Ile Gly Glu Gln Glu Leu Lys Asp Gly Val Ile Lys Leu Arg Ser  
465 470 475 480

Val Ala Ser Arg Glu Glu Val Asp Val Arg Arg Glu Asp Leu Val Glu  
485 490 495

Glu Ile Lys Arg Arg Thr Ser Gln Pro Leu Cys Ile Cys  
500 505

<210> 22

<211> 508

<212> PRT

<213> Rattus norvegicus

<400> 22

Met Ala Asp Arg Ala Ala Leu Glu Glu Leu Val Arg Leu Gln Gly Ala  
1 5 10 15

His Val Arg Gly Leu Lys Glu Gln Lys Ala Ser Ala Glu Gln Ile Glu  
20 25 30

Glu Glu Val Thr Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly His Asp  
35 40 45

Glu Gly Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp  
50 55 60

Tyr Ser Pro Arg Gln Met Ala Val Arg Glu Lys Val Phe Asp Val Ile  
65 70 75 80

Ile Arg Cys Phe Lys Arg His Gly Ala Glu Val Ile Asp Thr Pro Val  
85 90 95

Phe Glu Leu Lys Glu Thr Leu Thr Gly Lys Tyr Gly Glu Asp Ser Lys  
100 105 110

Leu Ile Tyr Asp Leu Lys Asp Gln Gly Gly Glu Leu Leu Ser Leu Arg  
115 120 125

Tyr Asp Leu Thr Val Pro Phe Ala Arg Tyr Leu Ala Met Asn Lys Leu  
130 135 140

Thr Asn Ile Lys Arg Tyr His Ile Ala Lys Val Tyr Arg Arg Asp Asn  
145 150 155 160

Pro Ala Met Thr Arg Gly Arg Tyr Arg Glu Phe Tyr Gln Cys Asp Phe  
165 170 175



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Asp Ile Ala Gly Gln Phe Asp Pro Met Ile Pro Asp Ala Glu Cys Leu
      180                      185                      190

Lys Ile Met Cys Glu Ile Leu Ser Ser Leu Gln Ile Gly Asn Phe Gln
      195                      200                      205

Val Lys Val Asn Asp Arg Arg Ile Leu Asp Gly Met Phe Ala Val Cys
      210                      215                      220

Gly Val Pro Asp Ser Lys Phe Arg Thr Ile Cys Ser Ser Val Asp Lys
      225                      230                      235                      240

Leu Asp Lys Val Ser Trp Glu Glu Val Lys Asn Glu Met Val Gly Glu
      245                      250                      255

Lys Gly Leu Ala Pro Glu Val Ala Asp Arg Ile Gly Asp Tyr Val Gln
      260                      265                      270

Gln His Gly Gly Val Ser Leu Val Glu Gln Leu Leu Gln Asp Pro Lys
      275                      280                      285

Leu Ser Gln Asn Lys Gln Ala Val Glu Gly Leu Gly Asp Leu Lys Leu
      290                      295                      300

Leu Phe Glu Tyr Leu Thr Leu Phe Gly Ile Asp Asp Lys Ile Ser Phe
      305                      310                      315                      320

Asp Leu Ser Leu Ala Arg Gly Leu Asp Tyr Tyr Thr Gly Val Ile Tyr
      325                      330                      335

Glu Ala Val Leu Leu Gln Met Pro Thr Gln Ala Gly Glu Glu Pro Leu
      340                      345                      350

Gly Val Gly Ser Ile Ala Ala Gly Gly Arg Tyr Asp Gly Leu Val Gly
      355                      360                      365

Met Phe Asp Pro Lys Gly Arg Lys Val Pro Cys Val Gly Leu Ser Ile
      370                      375                      380

Gly Val Glu Arg Ile Phe Ser Ile Val Glu Gln Lys Leu Glu Ala Ser
      385                      390                      395                      400

Glu Glu Lys Val Arg Thr Thr Glu Thr Gln Val Leu Val Ala Ser Ala
      405                      410                      415

Gln Lys Lys Leu Leu Glu Glu Arg Leu Lys Leu Ile Ser Glu Leu Trp
      420                      425                      430

Asp Ala Gly Ile Lys Ala Glu Leu Leu Tyr Lys Lys Asn Pro Lys Leu
      435                      440                      445

Leu Asn Gln Leu Gln Tyr Cys Glu Glu Ala Gly Ile Pro Leu Val Ala
      450                      455                      460

Ile Ile Gly Glu Gln Glu Leu Lys Asp Gly Val Ile Lys Leu Arg Ser
      465                      470                      475                      480

Val Thr Ser Arg Glu Glu Val Asp Val Arg Arg Glu Asp Leu Val Glu
      485                      490                      495

Glu Ile Arg Arg Arg Thr Ser Gln Pro Leu Ser Met
      500                      505

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&lt;210&gt; 23

&lt;211&gt; 500



&lt;212&gt; PRT

&lt;213&gt; Gallus gallus

&lt;400&gt; 23

Met Ala Asp Glu Ala Ala Val Arg Gln Gln Ala Glu Val Val Arg Arg  
 1 5 10 15

Leu Lys Gln Asp Lys Ala Glu Pro Asp Glu Ile Ala Lys Glu Val Ala  
 20 25 30

Lys Leu Leu Glu Met Lys Ala His Leu Gly Gly Asp Glu Gly Lys His  
 35 40 45

Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp Tyr Gly Pro Lys  
 50 55 60

Gln Met Ala Ile Arg Glu Arg Val Phe Ser Ala Ile Ile Ala Cys Phe  
 65 70 75 80

Lys Arg His Gly Ala Glu Val Ile Asp Thr Pro Val Phe Glu Leu Lys  
 85 90 95

Glu Thr Leu Thr Gly Lys Tyr Gly Glu Asp Ser Lys Leu Ile Tyr Asp  
 100 105 110

Leu Lys Asp Gln Gly Gly Glu Leu Leu Ser Leu Arg Tyr Asp Leu Thr  
 115 120 125

Val Pro Phe Ala Arg Tyr Leu Ala Met Asn Lys Ile Thr Asn Ile Lys  
 130 135 140

Arg Tyr His Ile Ala Lys Val Tyr Arg Arg Asp Asn Pro Ala Met Thr  
 145 150 155 160

Arg Gly Arg Tyr Arg Glu Phe Tyr Gln Cys Asp Phe Asp Ile Ala Gly  
 165 170 175

Gln Phe Asp Pro Met Ile Pro Asp Ala Glu Cys Leu Lys Ile Val Gln  
 180 185 190

Glu Ile Leu Ser Asp Leu Gln Leu Gly Asp Phe Leu Ile Lys Val Asn  
 195 200 205

Asp Arg Arg Ile Leu Asp Gly Met Phe Ala Val Cys Gly Val Pro Asp  
 210 215 220

Ser Lys Phe Arg Thr Ile Cys Ser Ser Val Asp Lys Leu Asp Lys Met  
 225 230 235 240

Pro Trp Glu Glu Val Arg Asn Glu Met Val Gly Glu Lys Gly Leu Ser  
 245 250 255

Pro Glu Ala Ala Asp Arg Ile Gly Glu Tyr Val Gln Leu His Gly Gly  
 260 265 270

Met Asp Leu Ile Glu Gln Leu Leu Gln Asp Pro Lys Leu Ser Gln Asn  
 275 280 285

Lys Leu Val Lys Glu Gly Leu Gly Asp Met Lys Leu Leu Phe Glu Tyr  
 290 295 300

Leu Thr Leu Phe Gly Ile Thr Gly Lys Ile Ser Phe Asp Leu Ser Leu  
 305 310 315 320

Ala Arg Gly Leu Asp Tyr Tyr Thr Gly Val Ile Tyr Glu Ala Val Leu  
 325 330 335



325 330 335

Leu Gln Gln Asn Asp His Gly Glu Glu Ser Val Ser Val Gly Ser Val  
340 345 350

Ala Gly Gly Gly Arg Tyr Asp Gly Leu Val Gly Met Phe Asp Pro Lys  
355 360 365

Gly Arg Lys Val Pro Cys Val Gly Ile Ser Ile Gly Ile Glu Arg Ile  
370 375 380

Phe Ser Ile Leu Glu Gln Arg Val Glu Ala Ser Glu Glu Lys Ile Arg  
385 390 395 400

Thr Thr Glu Thr Gln Val Leu Val Ala Ser Ala Gln Lys Lys Leu Leu  
405 410 415

Glu Glu Arg Leu Lys Leu Ile Ser Glu Leu Trp Asp Ala Gly Ile Lys  
420 425 430

Ala Glu Val Leu Tyr Lys Lys Asn Pro Lys Leu Leu Asn Gln Leu Gln  
435 440 445

Tyr Cys Glu Asp Thr Gly Ile Pro Leu Val Ala Ile Val Gly Glu Gln  
450 455 460

Glu Leu Lys Asp Gly Val Val Lys Leu Arg Val Val Ala Thr Gly Glu  
465 470 475 480

Glu Val Asn Ile Arg Arg Glu Ser Leu Val Glu Glu Ile Arg Arg Arg  
485 490 495

Thr Asn Gln Leu  
500

<210> 24  
<211> 437  
<212> PRT  
<213> Danio rerio

<400> 24

Met Ala Ala Leu Gly Leu Val Ser Met Arg Leu Cys Ala Gly Leu Met  
1 5 10 15

Gly Arg Arg Ser Ala Val Arg Leu His Ser Leu Arg Val Cys Ser Gly  
20 25 30

Met Thr Ile Ser Gln Ile Asp Glu Glu Val Ala Arg Leu Leu Gln Leu  
35 40 45

Lys Ala Gln Leu Gly Gly Asp Glu Gly Lys His Val Phe Val Leu Lys  
50 55 60

Thr Ala Lys Gly Thr Arg Asp Tyr Asn Pro Lys Gln Met Ala Ile Arg  
65 70 75 80

Glu Lys Val Phe Asn Ile Ile Ile Asn Cys Phe Lys Arg His Gly Ala  
85 90 95

Glu Thr Ile Asp Ser Pro Val Phe Glu Leu Lys Glu Thr Leu Thr Gly  
100 105 110

Lys Tyr Gly Glu Asp Ser Lys Leu Ile Tyr Asp Leu Lys Asp Gln Gly  
115 120 125



Gly Glu Leu Leu Ser Leu Arg Tyr Asp Leu Thr Val Pro Phe Ala Arg  
130 135 140

Tyr Leu Ala Met Asn Lys Ile Thr Asn Ile Lys Arg Tyr His Ile Ala  
145 150 155 160

Lys Val Tyr Arg Arg Asp Asn Pro Ala Met Thr Arg Gly Arg Tyr Arg  
165 170 175

Glu Phe Tyr Gln Cys Asp Phe Asp Ile Ala Gly Gln Tyr Asp Ala Met  
180 185 190

Ile Pro Asp Ala Glu Cys Leu Lys Leu Val Tyr Glu Ile Leu Ser Glu  
195 200 205

Leu Asp Leu Gly Asp Phe Arg Ile Lys Val Asn Asp Arg Arg Ile Leu  
210 215 220

Asp Gly Met Phe Ala Ile Cys Gly Val Pro Asp Glu Lys Phe Arg Thr  
225 230 235 240

Ile Cys Ser Thr Val Asp Lys Leu Asp Lys Leu Ala Trp Glu Glu Val  
245 250 255

Lys Lys Glu Met Val Asn Glu Lys Gly Leu Ser Glu Glu Val Ala Asp  
260 265 270

Arg Ile Arg Asp Tyr Val Ser Met Gln Gly Gly Lys Asp Leu Ala Glu  
275 280 285

Arg Leu Leu Gln Asp Pro Lys Leu Ser Gln Ser Lys Gln Ala Cys Ala  
290 295 300

Gly Ile Thr Asp Met Lys Leu Leu Phe Ser Tyr Leu Glu Leu Phe Gln  
305 310 315 320

Ile Thr Asp Lys Val Val Phe Asp Leu Ser Leu Ala Arg Gly Leu Asp  
325 330 335

Tyr Tyr Thr Gly Val Ile Tyr Glu Ala Ile Leu Thr Gln Ala Asn Pro  
340 345 350

Ala Pro Ala Ser Thr Pro Ala Glu Gln Asn Gly Ala Glu Asp Ala Gly  
355 360 365

Val Ser Val Gly Ser Val Ala Gly Gly Gly Arg Tyr Asp Gly Leu Val  
370 375 380

Gly Met Phe Asp Pro Lys Ala Gly Lys Cys Pro Val Trp Gly Ser Ala  
385 390 395 400

Leu Ala Leu Arg Gly Ser Ser Pro Ser Trp Ser Arg Arg Gln Ser Cys  
405 410 415

Leu Gln Arg Arg Cys Ala Pro Leu Lys Leu Lys Cys Leu Trp Leu Gln  
420 425 430

His Arg Arg Thr Phe  
435

<210> 25

<211> 506

<212> PRT



&lt;213&gt; Homo sapiens

&lt;400&gt; 25

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Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu
1          5          10          15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu
20          25          30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp
35          40          45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp
50          55          60

Tyr Ser Pro Arg Gln Met Ala Val Arg Glu Lys Val Phe Asp Val Ile
65          70          75          80

Ile Arg Cys Phe Lys Arg His Gly Ala Glu Val Ile Asp Thr Pro Val
85          90          95

Phe Glu Leu Lys Glu Thr Leu Met Gly Lys Tyr Gly Glu Asp Ser Lys
100         105         110

Leu Ile Tyr Asp Leu Lys Asp Gln Gly Gly Glu Leu Leu Ser Leu Arg
115         120         125

Tyr Asp Leu Thr Val Pro Phe Ala Arg Tyr Leu Ala Met Asn Lys Leu
130         135         140

Thr Asn Ile Lys Arg Tyr His Ile Ala Lys Val Tyr Arg Arg Asp Asn
145         150         155         160

Pro Ala Met Thr Arg Gly Arg Tyr Arg Glu Phe Tyr Gln Cys Asp Phe
165         170         175

Asp Ile Ala Gly Asn Phe Asp Pro Met Ile Pro Asp Ala Glu Cys Leu
180         185         190

Lys Ile Met Cys Glu Ile Leu Ser Ser Leu Gln Ile Gly Asp Phe Leu
195         200         205

Val Lys Val Asn Asp Arg Arg Ile Leu Asp Gly Met Phe Ala Ile Cys
210         215         220

Gly Val Ser Asp Ser Lys Phe Arg Thr Ile Cys Ser Ser Val Asp Lys
225         230         235         240

Leu Asp Lys Val Ser Trp Glu Glu Val Lys Asn Glu Met Val Gly Glu
245         250         255

Lys Gly Leu Ala Pro Glu Val Ala Asp Arg Ile Gly Asp Tyr Val Gln
260         265         270

Gln His Gly Gly Val Ser Leu Val Glu Gln Leu Leu Gln Asp Pro Lys
275         280         285

Leu Ser Gln Asn Lys Gln Ala Leu Glu Gly Leu Gly Asp Leu Lys Leu
290         295         300

Leu Phe Glu Tyr Leu Thr Leu Phe Gly Ile Asp Asp Lys Ile Ser Phe
305         310         315         320

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Asp Leu Ser Leu Ala Arg Gly Leu Asp Tyr Tyr Thr Gly Val Ile Tyr  
325 330 335

Glu Ala Val Leu Leu Gln Thr Pro Ala Gln Ala Gly Glu Glu Pro Leu  
340 345 350

Gly Val Gly Ser Val Ala Ala Gly Gly Arg Tyr Asp Gly Leu Val Gly  
355 360 365

Met Phe Asp Pro Lys Gly Arg Lys Val Pro Cys Val Gly Leu Ser Ile  
370 375 380

Gly Val Glu Arg Ile Phe Ser Ile Val Glu Gln Arg Leu Glu Ala Leu  
385 390 395 400

Glu Glu Lys Ile Arg Thr Thr Glu Thr Gln Val Leu Val Ala Ser Ala  
405 410 415

Gln Lys Lys Leu Leu Glu Glu Arg Leu Lys Leu Val Ser Glu Leu Trp  
420 425 430

Asp Ala Gly Ile Lys Ala Glu Leu Leu Tyr Lys Lys Asn Pro Lys Leu  
435 440 445

Leu Asn Gln Leu Gln Tyr Cys Glu Glu Ala Gly Ile Pro Leu Val Ala  
450 455 460

Ile Ile Gly Glu Gln Glu Leu Lys Asp Gly Val Ile Lys Leu Arg Ser  
465 470 475 480

Val Thr Ser Arg Glu Glu Val Asp Val Arg Arg Glu Asp Leu Val Glu  
485 490 495

Glu Ile Lys Arg Arg Thr Gly Gln Pro Leu  
500 505

<210> 26

<211> 48

<212> PRT

<213> Homo sapiens

<400> 26

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

<210> 27

<211> 80

<212> PRT

<213> Homo sapiens

<400> 27

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu

20

25

30



Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp  
50 55 60

Tyr Ser Pro Arg Gln Met Ala Val Arg Glu Lys Val Phe Asp Val Ile  
65 70 75 80

<210> 28

<211> 79

<212> PRT

<213> Homo sapiens

<400> 28

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp  
50 55 60

Tyr Ser Pro Arg Gln Met Ala Val Arg Glu Lys Val Phe Asp Val  
65 70 75

<210> 29

<211> 78

<212> PRT

<213> Homo sapiens

<400> 29

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp  
50 55 60

Tyr Ser Pro Arg Gln Met Ala Val Arg Glu Lys Val Phe Asp  
65 70 75

<210> 30

<211> 77

<212> PRT

<213> Homo sapiens

<400> 30

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30



Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp  
50 55 60

Tyr Ser Pro Arg Gln Met Ala Val Arg Glu Lys Val Phe  
65 70 75

<210> 31

<211> 76

<212> PRT

<213> Homo sapiens

<400> 31

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp  
50 55 60

Tyr Ser Pro Arg Gln Met Ala Val Arg Glu Lys Val  
65 70 75

<210> 32

<211> 75

<212> PRT

<213> Homo sapiens

<400> 32

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp  
50 55 60

Tyr Ser Pro Arg Gln Met Ala Val Arg Glu Lys  
65 70 75

<210> 33

<211> 74

<212> PRT

<213> Homo sapiens

<400> 33

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30



Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp  
50 55 60

Tyr Ser Pro Arg Gln Met Ala Val Arg Glu  
65 70

<210> 34

<211> 73

<212> PRT

<213> Homo sapiens

<400> 34

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp  
50 55 60

Tyr Ser Pro Arg Gln Met Ala Val Arg  
65 70

<210> 35

<211> 72

<212> PRT

<213> Homo sapiens

<400> 35

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp  
50 55 60

Tyr Ser Pro Arg Gln Met Ala Val  
65 70

<210> 36

<211> 71

<212> PRT

<213> Homo sapiens

<400> 36

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30



Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp  
50 55 60

Tyr Ser Pro Arg Gln Met Ala  
65 70

<210> 37

<211> 70

<212> PRT

<213> Homo sapiens

<400> 37

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp  
50 55 60

Tyr Ser Pro Arg Gln Met  
65 70

<210> 38

<211> 69

<212> PRT

<213> Homo sapiens

<400> 38

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp  
50 55 60

Tyr Ser Pro Arg Gln  
65

<210> 39

<211> 68

<212> PRT

<213> Homo sapiens

<400> 39

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30



Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp  
50 55 60

Tyr Ser Pro Arg  
65

<210> 40

<211> 67

<212> PRT

<213> Homo sapiens

<400> 40

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp  
50 55 60

Tyr Ser Pro  
65

<210> 41

<211> 66

<212> PRT

<213> Homo sapiens

<400> 41

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp  
50 55 60

Tyr Ser  
65

<210> 42

<211> 65

<212> PRT

<213> Homo sapiens

<400> 42

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15



Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp  
50 55 60

Tyr  
65

<210> 43

<211> 64

<212> PRT

<213> Homo sapiens

<400> 43

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp  
50 55 60

<210> 44

<211> 63

<212> PRT

<213> Homo sapiens

<400> 44

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg  
50 55 60

<210> 45

<211> 62

<212> PRT

<213> Homo sapiens

<400> 45

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45



Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr  
50 55 60

<210> 46

<211> 61

<212> PRT

<213> Homo sapiens

<400> 46

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly  
50 55 60

<210> 47

<211> 60

<212> PRT

<213> Homo sapiens

<400> 47

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys  
50 55 60

<210> 48

<211> 59

<212> PRT

<213> Homo sapiens

<400> 48

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro  
50 55

<210> 49

<211> 58

<212> PRT

<213> Homo sapiens

<400> 49



Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr  
50 55

<210> 50

<211> 57

<212> PRT

<213> Homo sapiens

<400> 50

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys  
50 55

<210> 51

<211> 56

<212> PRT

<213> Homo sapiens

<400> 51

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu  
50 55

<210> 52

<211> 55

<212> PRT

<213> Homo sapiens

<400> 52

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

-- -- -- -- --



Glu Ser Lys Gln Lys Phe Val  
50 55

<210> 53

<211> 54

<212> PRT

<213> Homo sapiens

<400> 53

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe  
50

<210> 54

<211> 53

<212> PRT

<213> Homo sapiens

<400> 54

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys

50

<210> 55

<211> 52

<212> PRT

<213> Homo sapiens

<400> 55

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln  
50

<210> 56

<211> 51

<212> PRT



<213> Homo sapiens

<400> 56

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15  
  
Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30  
  
Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45  
  
Glu Ser Lys  
50

<210> 57

<211> 50

<212> PRT

<213> Homo sapiens

<400> 57

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15  
  
Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30  
  
Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45  
  
Glu Ser  
50

<210> 58

<211> 49

<212> PRT

<213> Homo sapiens

<400> 58

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15  
  
Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30  
  
Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45  
  
Glu

<210> 59

<211> 48

<212> PRT

<213> Homo sapiens

<400> 59

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15  
  
Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30  
  
Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp



35 40 45

<210> 60  
<211> 47  
<212> PRT  
<213> Homo sapiens

<400> 60  
Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15  
Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30  
Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro  
35 40 45

<210> 61  
<211> 46  
<212> PRT  
<213> Homo sapiens

<400> 61  
Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15  
Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30  
Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly  
35 40 45

<210> 62  
<211> 45  
<212> PRT  
<213> Homo sapiens

<400> 62  
Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15  
Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30  
Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu  
35 40 45

<210> 63  
<211> 44  
<212> PRT  
<213> Homo sapiens

<400> 63  
Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15  
Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30  
Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln  
35 40



&lt;210&gt; 64

&lt;211&gt; 43

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 64

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Ala | Glu | Arg | Ala | Ala | Leu | Glu | Glu | Leu | Val | Lys | Leu | Gln | Gly | Glu |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     | 15  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Val | Arg | Gly | Leu | Lys | Gln | Gln | Lys | Ala | Ser | Ala | Glu | Leu | Ile | Glu |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Glu | Val | Ala | Lys | Leu | Leu | Lys | Leu | Lys | Ala |
|     |     | 35  |     |     |     |     | 40  |     |     |     |

&lt;210&gt; 65

&lt;211&gt; 42

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 65

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Ala | Glu | Arg | Ala | Ala | Leu | Glu | Glu | Leu | Val | Lys | Leu | Gln | Gly | Glu |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     | 15  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Val | Arg | Gly | Leu | Lys | Gln | Gln | Lys | Ala | Ser | Ala | Glu | Leu | Ile | Glu |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Glu | Val | Ala | Lys | Leu | Leu | Lys | Leu | Lys |
|     |     | 35  |     |     |     |     | 40  |     |     |

&lt;210&gt; 66

&lt;211&gt; 41

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 66

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Ala | Glu | Arg | Ala | Ala | Leu | Glu | Glu | Leu | Val | Lys | Leu | Gln | Gly | Glu |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     | 15  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Val | Arg | Gly | Leu | Lys | Gln | Gln | Lys | Ala | Ser | Ala | Glu | Leu | Ile | Glu |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Glu | Val | Ala | Lys | Leu | Leu | Lys | Leu |
|     |     | 35  |     |     |     |     | 40  |     |

&lt;210&gt; 67

&lt;211&gt; 40

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 67

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Ala | Glu | Arg | Ala | Ala | Leu | Glu | Glu | Leu | Val | Lys | Leu | Gln | Gly | Glu |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     | 15  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Val | Arg | Gly | Leu | Lys | Gln | Gln | Lys | Ala | Ser | Ala | Glu | Leu | Ile | Glu |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Glu | Val | Ala | Lys | Leu | Leu | Lys |
|     |     | 35  |     |     |     |     | 40  |

&lt;210&gt; 68



&lt;211&gt; 79

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 68

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Glu | Arg | Ala | Ala | Leu | Glu | Glu | Leu | Val | Lys | Leu | Gln | Gly | Glu | Arg |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Arg | Gly | Leu | Lys | Gln | Gln | Lys | Ala | Ser | Ala | Glu | Leu | Ile | Glu | Glu |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Val | Ala | Lys | Leu | Leu | Lys | Leu | Lys | Ala | Gln | Leu | Gly | Pro | Asp | Glu |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Lys | Gln | Lys | Phe | Val | Leu | Lys | Thr | Pro | Lys | Gly | Thr | Arg | Asp | Tyr |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| Ser | Pro | Arg | Gln | Met | Ala | Val | Arg | Glu | Lys | Val | Phe | Asp | Val | Ile |  |
| 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     |  |

&lt;210&gt; 69

&lt;211&gt; 78

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 69

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Arg | Ala | Ala | Leu | Glu | Glu | Leu | Val | Lys | Leu | Gln | Gly | Glu | Arg | Val |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Gly | Leu | Lys | Gln | Gln | Lys | Ala | Ser | Ala | Glu | Leu | Ile | Glu | Glu | Glu |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Ala | Lys | Leu | Leu | Lys | Leu | Lys | Ala | Gln | Leu | Gly | Pro | Asp | Glu | Ser |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | Gln | Lys | Phe | Val | Leu | Lys | Thr | Pro | Lys | Gly | Thr | Arg | Asp | Tyr | Ser |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|--|
| Pro | Arg | Gln | Met | Ala | Val | Arg | Glu | Lys | Val | Phe | Asp | Val | Ile |  |  |
| 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |  |  |

&lt;210&gt; 70

&lt;211&gt; 77

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 70

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Ala | Ala | Leu | Glu | Glu | Leu | Val | Lys | Leu | Gln | Gly | Glu | Arg | Val | Arg |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gly | Leu | Lys | Gln | Gln | Lys | Ala | Ser | Ala | Glu | Leu | Ile | Glu | Glu | Glu | Val |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Lys | Leu | Leu | Lys | Leu | Lys | Ala | Gln | Leu | Gly | Pro | Asp | Glu | Ser | Lys |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Lys | Phe | Val | Leu | Lys | Thr | Pro | Lys | Gly | Thr | Arg | Asp | Tyr | Ser | Pro |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |  |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|--|--|
| Arg | Gln | Met | Ala | Val | Arg | Glu | Lys | Val | Phe | Asp | Val | Ile |  |  |  |
| 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |     |  |  |  |

&lt;210&gt; 71



&lt;211&gt; 76

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 71

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Ala | Leu | Glu | Glu | Leu | Val | Lys | Leu | Gln | Gly | Glu | Arg | Val | Arg | Gly |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Lys | Gln | Gln | Lys | Ala | Ser | Ala | Glu | Leu | Ile | Glu | Glu | Glu | Val | Ala |
|     |     | 20  |     |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | Leu | Leu | Lys | Leu | Lys | Ala | Gln | Leu | Gly | Pro | Asp | Glu | Ser | Lys | Gln |
|     | 35  |     |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | Phe | Val | Leu | Lys | Thr | Pro | Lys | Gly | Thr | Arg | Asp | Tyr | Ser | Pro | Arg |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Met | Ala | Val | Arg | Glu | Lys | Val | Phe | Asp | Val | Ile |
| 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |

&lt;210&gt; 72

&lt;211&gt; 75

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 72

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Leu | Glu | Glu | Leu | Val | Lys | Leu | Gln | Gly | Glu | Arg | Val | Arg | Gly | Leu |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | Gln | Gln | Lys | Ala | Ser | Ala | Glu | Leu | Ile | Glu | Glu | Glu | Val | Ala | Lys |
|     |     | 20  |     |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Leu | Lys | Leu | Lys | Ala | Gln | Leu | Gly | Pro | Asp | Glu | Ser | Lys | Gln | Lys |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Phe | Val | Leu | Lys | Thr | Pro | Lys | Gly | Thr | Arg | Asp | Tyr | Ser | Pro | Arg | Gln |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Ala | Val | Arg | Glu | Lys | Val | Phe | Asp | Val | Ile |
| 65  |     |     |     |     | 70  |     |     |     |     | 75  |

&lt;210&gt; 73

&lt;211&gt; 74

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 73

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Glu | Glu | Leu | Val | Lys | Leu | Gln | Gly | Glu | Arg | Val | Arg | Gly | Leu | Lys |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Gln | Lys | Ala | Ser | Ala | Glu | Leu | Ile | Glu | Glu | Glu | Val | Ala | Lys | Leu |
|     |     | 20  |     |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Lys | Leu | Lys | Ala | Gln | Leu | Gly | Pro | Asp | Glu | Ser | Lys | Gln | Lys | Phe |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Leu | Lys | Thr | Pro | Lys | Gly | Thr | Arg | Asp | Tyr | Ser | Pro | Arg | Gln | Met |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Val | Arg | Glu | Lys | Val | Phe | Asp | Val | Ile |
| 65  |     |     |     |     | 70  |     |     |     |     |

&lt;210&gt; 74



&lt;211&gt; 73

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 74

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Glu | Leu | Val | Lys | Leu | Gln | Gly | Glu | Arg | Val | Arg | Gly | Leu | Lys | Gln |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Lys | Ala | Ser | Ala | Glu | Leu | Ile | Glu | Glu | Glu | Val | Ala | Lys | Leu | Leu |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | Leu | Lys | Ala | Gln | Leu | Gly | Pro | Asp | Glu | Ser | Lys | Gln | Lys | Phe | Val |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Lys | Thr | Pro | Lys | Gly | Thr | Arg | Asp | Tyr | Ser | Pro | Arg | Gln | Met | Ala |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |

|     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Arg | Glu | Lys | Val | Phe | Asp | Val | Ile |
| 65  |     |     |     |     | 70  |     |     |     |

&lt;210&gt; 75

&lt;211&gt; 72

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 75

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Leu | Val | Lys | Leu | Gln | Gly | Glu | Arg | Val | Arg | Gly | Leu | Lys | Gln | Gln |
| 1   |     |     | 5   |     |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | Ala | Ser | Ala | Glu | Leu | Ile | Glu | Glu | Glu | Val | Ala | Lys | Leu | Leu | Lys |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Lys | Ala | Gln | Leu | Gly | Pro | Asp | Glu | Ser | Lys | Gln | Lys | Phe | Val | Leu |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | Thr | Pro | Lys | Gly | Thr | Arg | Asp | Tyr | Ser | Pro | Arg | Gln | Met | Ala | Val |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |

|     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Glu | Lys | Val | Phe | Asp | Val | Ile |
| 65  |     |     |     |     | 70  |     |     |

&lt;210&gt; 76

&lt;211&gt; 71

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 76

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Val | Lys | Leu | Gln | Gly | Glu | Arg | Val | Arg | Gly | Leu | Lys | Gln | Gln | Lys |
| 1   |     |     | 5   |     |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Ser | Ala | Glu | Leu | Ile | Glu | Glu | Glu | Val | Ala | Lys | Leu | Leu | Lys | Leu |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | Ala | Gln | Leu | Gly | Pro | Asp | Glu | Ser | Lys | Gln | Lys | Phe | Val | Leu | Lys |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thr | Pro | Lys | Gly | Thr | Arg | Asp | Tyr | Ser | Pro | Arg | Gln | Met | Ala | Val | Arg |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |

|     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|
| Glu | Lys | Val | Phe | Asp | Val | Ile |
| 65  |     |     |     |     | 70  |     |

&lt;210&gt; 77



&lt;211&gt; 70

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 77

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Lys | Leu | Gln | Gly | Glu | Arg | Val | Arg | Gly | Leu | Lys | Gln | Gln | Lys | Ala |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Ala | Glu | Leu | Ile | Glu | Glu | Glu | Val | Ala | Lys | Leu | Leu | Lys | Leu | Lys |
|     |     | 20  |     |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Gln | Leu | Gly | Pro | Asp | Glu | Ser | Lys | Gln | Lys | Phe | Val | Leu | Lys | Thr |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Lys | Gly | Thr | Arg | Asp | Tyr | Ser | Pro | Arg | Gln | Met | Ala | Val | Arg | Glu |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |

|     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|
| Lys | Val | Phe | Asp | Val | Ile |
| 65  |     |     |     |     | 70  |

&lt;210&gt; 78

&lt;211&gt; 69

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 78

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | Leu | Gln | Gly | Glu | Arg | Val | Arg | Gly | Leu | Lys | Gln | Gln | Lys | Ala | Ser |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Glu | Leu | Ile | Glu | Glu | Glu | Val | Ala | Lys | Leu | Leu | Lys | Leu | Lys | Ala |
|     |     | 20  |     |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Leu | Gly | Pro | Asp | Glu | Ser | Lys | Gln | Lys | Phe | Val | Leu | Lys | Thr | Pro |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | Gly | Thr | Arg | Asp | Tyr | Ser | Pro | Arg | Gln | Met | Ala | Val | Arg | Glu | Lys |
|     | 50  |     |     |     |     | 55  |     |     |     | 60  |     |     |     |     |     |

|     |     |     |     |     |
|-----|-----|-----|-----|-----|
| Val | Phe | Asp | Val | Ile |
| 65  |     |     |     |     |

&lt;210&gt; 79

&lt;211&gt; 68

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 79

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Gln | Gly | Glu | Arg | Val | Arg | Gly | Leu | Lys | Gln | Gln | Lys | Ala | Ser | Ala |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Leu | Ile | Glu | Glu | Glu | Val | Ala | Lys | Leu | Leu | Lys | Leu | Lys | Ala | Gln |
|     |     | 20  |     |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Gly | Pro | Asp | Glu | Ser | Lys | Gln | Lys | Phe | Val | Leu | Lys | Thr | Pro | Lys |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gly | Thr | Arg | Asp | Tyr | Ser | Pro | Arg | Gln | Met | Ala | Val | Arg | Glu | Lys | Val |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |

|     |     |     |     |
|-----|-----|-----|-----|
| Phe | Asp | Val | Ile |
| 65  |     |     |     |

&lt;210&gt; 80



&lt;211&gt; 67

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 80

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Gly | Glu | Arg | Val | Arg | Gly | Leu | Lys | Gln | Gln | Lys | Ala | Ser | Ala | Glu |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Ile | Glu | Glu | Glu | Val | Ala | Lys | Leu | Leu | Lys | Leu | Lys | Ala | Gln | Leu |
|     |     | 20  |     |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gly | Pro | Asp | Glu | Ser | Lys | Gln | Lys | Phe | Val | Leu | Lys | Thr | Pro | Lys | Gly |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thr | Arg | Asp | Tyr | Ser | Pro | Arg | Gln | Met | Ala | Val | Arg | Glu | Lys | Val | Phe |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |

|     |     |     |
|-----|-----|-----|
| Asp | Val | Ile |
| 65  |     |     |

&lt;210&gt; 81

&lt;211&gt; 66

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 81

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gly | Glu | Arg | Val | Arg | Gly | Leu | Lys | Gln | Gln | Lys | Ala | Ser | Ala | Glu | Leu |
| 1   |     |     | 5   |     |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ile | Glu | Glu | Glu | Val | Ala | Lys | Leu | Leu | Lys | Leu | Lys | Ala | Gln | Leu | Gly |
|     |     | 20  |     |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Asp | Glu | Ser | Lys | Gln | Lys | Phe | Val | Leu | Lys | Thr | Pro | Lys | Gly | Thr |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Asp | Tyr | Ser | Pro | Arg | Gln | Met | Ala | Val | Arg | Glu | Lys | Val | Phe | Asp |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |

|     |     |
|-----|-----|
| Val | Ile |
| 65  |     |

&lt;210&gt; 82

&lt;211&gt; 64

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 82

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Val | Arg | Gly | Leu | Lys | Gln | Gln | Lys | Ala | Ser | Ala | Glu | Leu | Ile | Glu |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Glu | Val | Ala | Lys | Leu | Leu | Lys | Leu | Lys | Ala | Gln | Leu | Gly | Pro | Asp |
|     |     | 20  |     |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Ser | Lys | Gln | Lys | Phe | Val | Leu | Lys | Thr | Pro | Lys | Gly | Thr | Arg | Asp |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Tyr | Ser | Pro | Arg | Gln | Met | Ala | Val | Arg | Glu | Lys | Val | Phe | Asp | Val | Ile |
|     | 50  |     |     |     | 55  |     |     |     |     |     | 60  |     |     |     |     |

&lt;210&gt; 83

&lt;211&gt; 63

&lt;212&gt; PRT



&lt;213&gt; Homo sapiens

&lt;400&gt; 83

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Arg | Gly | Leu | Lys | Gln | Gln | Lys | Ala | Ser | Ala | Glu | Leu | Ile | Glu | Glu |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Val | Ala | Lys | Leu | Leu | Lys | Leu | Lys | Ala | Gln | Leu | Gly | Pro | Asp | Glu |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Lys | Gln | Lys | Phe | Val | Leu | Lys | Thr | Pro | Lys | Gly | Thr | Arg | Asp | Tyr |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Pro | Arg | Gln | Met | Ala | Val | Arg | Glu | Lys | Val | Phe | Asp | Val | Ile |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |

&lt;210&gt; 84

&lt;211&gt; 62

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 84

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Gly | Leu | Lys | Gln | Gln | Lys | Ala | Ser | Ala | Glu | Leu | Ile | Glu | Glu | Glu |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Ala | Lys | Leu | Leu | Lys | Leu | Lys | Ala | Gln | Leu | Gly | Pro | Asp | Glu | Ser |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | Gln | Lys | Phe | Val | Leu | Lys | Thr | Pro | Lys | Gly | Thr | Arg | Asp | Tyr | Ser |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Arg | Gln | Met | Ala | Val | Arg | Glu | Lys | Val | Phe | Asp | Val | Ile |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |

&lt;210&gt; 85

&lt;211&gt; 61

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 85

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gly | Leu | Lys | Gln | Gln | Lys | Ala | Ser | Ala | Glu | Leu | Ile | Glu | Glu | Glu | Val |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Lys | Leu | Leu | Lys | Leu | Lys | Ala | Gln | Leu | Gly | Pro | Asp | Glu | Ser | Lys |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Lys | Phe | Val | Leu | Lys | Thr | Pro | Lys | Gly | Thr | Arg | Asp | Tyr | Ser | Pro |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Gln | Met | Ala | Val | Arg | Glu | Lys | Val | Phe | Asp | Val | Ile |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |

&lt;210&gt; 86

&lt;211&gt; 60

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 86

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Lys | Gln | Gln | Lys | Ala | Ser | Ala | Glu | Leu | Ile | Glu | Glu | Glu | Val | Ala |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | Leu | Leu | Lys | Leu | Lys | Ala | Gln | Leu | Gly | Pro | Asp | Glu | Ser | Lys | Gln |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |



Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp Tyr Ser Pro Arg  
 35 40 45

Gln Met Ala Val Arg Glu Lys Val Phe Asp Val Ile

50 55 60

<210> 87

<211> 59

<212> PRT

<213> Homo sapiens

<400> 87

Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu Glu Glu Val Ala Lys  
 1 5 10 15

Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp Glu Ser Lys Gln Lys  
 20 25 30

Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp Tyr Ser Pro Arg Gln  
 35 40 45

Met Ala Val Arg Glu Lys Val Phe Asp Val Ile  
 50 55

<210> 88

<211> 58

<212> PRT

<213> Homo sapiens

<400> 88

Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu Glu Glu Val Ala Lys Leu  
 1 5 10 15

Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp Glu Ser Lys Gln Lys Phe  
 20 25 30

Val Leu Lys Thr Pro Lys Gly Thr Arg Asp Tyr Ser Pro Arg Gln Met  
 35 40 45

Ala Val Arg Glu Lys Val Phe Asp Val Ile  
 50 55

<210> 89

<211> 57

<212> PRT

<213> Homo sapiens

<400> 89

Gln Lys Ala Ser Ala Glu Leu Ile Glu Glu Glu Val Ala Lys Leu Leu  
 1 5 10 15

Lys Leu Lys Ala Gln Leu Gly Pro Asp Glu Ser Lys Gln Lys Phe Val  
 20 25 30

Leu Lys Thr Pro Lys Gly Thr Arg Asp Tyr Ser Pro Arg Gln Met Ala  
 35 40 45

Val Arg Glu Lys Val Phe Asp Val Ile  
 50 55

<210> 90

<211> 56



&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 90

Lys Ala Ser Ala Glu Leu Ile Glu Glu Glu Val Ala Lys Leu Leu Lys  
 1 5 10 15

Leu Lys Ala Gln Leu Gly Pro Asp Glu Ser Lys Gln Lys Phe Val Leu  
 20 25 30

Lys Thr Pro Lys Gly Thr Arg Asp Tyr Ser Pro Arg Gln Met Ala Val  
 35 40 45

Arg Glu Lys Val Phe Asp Val Ile  
 50 55

&lt;210&gt; 91

&lt;211&gt; 55

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 91

Ala Ser Ala Glu Leu Ile Glu Glu Glu Val Ala Lys Leu Leu Lys Leu  
 1 5 10 15

Lys Ala Gln Leu Gly Pro Asp Glu Ser Lys Gln Lys Phe Val Leu Lys  
 20 25 30

Thr Pro Lys Gly Thr Arg Asp Tyr Ser Pro Arg Gln Met Ala Val Arg  
 35 40 45

Glu Lys Val Phe Asp Val Ile  
 50 55

&lt;210&gt; 92

&lt;211&gt; 54

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 92

Ser Ala Glu Leu Ile Glu Glu Glu Val Ala Lys Leu Leu Lys Leu Lys

1 5 10 15

Ala Gln Leu Gly Pro Asp Glu Ser Lys Gln Lys Phe Val Leu Lys Thr  
 20 25 30

Pro Lys Gly Thr Arg Asp Tyr Ser Pro Arg Gln Met Ala Val Arg Glu  
 35 40 45

Lys Val Phe Asp Val Ile  
 50

&lt;210&gt; 93

&lt;211&gt; 53

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 93

Ala Glu Leu Ile Glu Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala  
 1 5 10 15

Glu Leu Gly Pro Arg Asp Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro



Gln Leu Gly Phe Asp Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Phe  
20 25 30

Lys Gly Thr Arg Asp Tyr Ser Pro Arg Gln Met Ala Val Arg Glu Lys  
35 40 45

Val Phe Asp Val Ile  
50

<210> 94

<211> 52

<212> PRT

<213> Homo sapiens

<400> 94

Glu Leu Ile Glu Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln  
1 5 10 15

Leu Gly Pro Asp Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys  
20 25 30

Gly Thr Arg Asp Tyr Ser Pro Arg Gln Met Ala Val Arg Glu Lys Val  
35 40 45

Phe Asp Val Ile  
50

<210> 95

<211> 51

<212> PRT

<213> Homo sapiens

<400> 95

Leu Ile Glu Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu  
1 5 10 15

Gly Pro Asp Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly  
20 25 30

Thr Arg Asp Tyr Ser Pro Arg Gln Met Ala Val Arg Glu Lys Val Phe  
35 40 45

Asp Val Ile  
50

<210> 96

<211> 50

<212> PRT

<213> Homo sapiens

<400> 96

Ile Glu Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly  
1 5 10 15

Pro Asp Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr  
20 25 30

Arg Asp Tyr Ser Pro Arg Gln Met Ala Val Arg Glu Lys Val Phe Asp  
35 40 45

Val Ile  
50

<210> 97



&lt;211&gt; 49

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 97

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Glu | Glu | Val | Ala | Lys | Leu | Leu | Lys | Leu | Lys | Ala | Gln | Leu | Gly | Pro |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asp | Glu | Ser | Lys | Gln | Lys | Phe | Val | Leu | Lys | Thr | Pro | Lys | Gly | Thr | Arg |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asp | Tyr | Ser | Pro | Arg | Gln | Met | Ala | Val | Arg | Glu | Lys | Val | Phe | Asp | Val |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

Ile

&lt;210&gt; 98

&lt;211&gt; 48

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 98

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Glu | Val | Ala | Lys | Leu | Leu | Lys | Leu | Lys | Ala | Gln | Leu | Gly | Pro | Asp |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Ser | Lys | Gln | Lys | Phe | Val | Leu | Lys | Thr | Pro | Lys | Gly | Thr | Arg | Asp |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Tyr | Ser | Pro | Arg | Gln | Met | Ala | Val | Arg | Glu | Lys | Val | Phe | Asp | Val | Ile |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

&lt;210&gt; 99

&lt;211&gt; 47

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 99

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Val | Ala | Lys | Leu | Leu | Lys | Leu | Lys | Ala | Gln | Leu | Gly | Pro | Asp | Glu |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Lys | Gln | Lys | Phe | Val | Leu | Lys | Thr | Pro | Lys | Gly | Thr | Arg | Asp | Tyr |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Pro | Arg | Gln | Met | Ala | Val | Arg | Glu | Lys | Val | Phe | Asp | Val | Ile |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |

&lt;210&gt; 100

&lt;211&gt; 46

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 100

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Ala | Lys | Leu | Leu | Lys | Leu | Lys | Ala | Gln | Leu | Gly | Pro | Asp | Glu | Ser |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | Gln | Lys | Phe | Val | Leu | Lys | Thr | Pro | Lys | Gly | Thr | Arg | Asp | Tyr | Ser |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Arg | Gln | Met | Ala | Val | Arg | Glu | Lys | Val | Phe | Asp | Val | Ile |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |



&lt;210&gt; 101

&lt;211&gt; 45

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 101

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Lys | Leu | Leu | Lys | Leu | Lys | Ala | Gln | Leu | Gly | Pro | Asp | Glu | Ser | Lys |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Lys | Phe | Val | Leu | Lys | Thr | Pro | Lys | Gly | Thr | Arg | Asp | Tyr | Ser | Pro |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Gln | Met | Ala | Val | Arg | Glu | Lys | Val | Phe | Asp | Val | Ile |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |

&lt;210&gt; 102

&lt;211&gt; 44

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 102

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | Leu | Leu | Lys | Leu | Lys | Ala | Gln | Leu | Gly | Pro | Asp | Glu | Ser | Lys | Gln |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | Phe | Val | Leu | Lys | Thr | Pro | Lys | Gly | Thr | Arg | Asp | Tyr | Ser | Pro | Arg |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Met | Ala | Val | Arg | Glu | Lys | Val | Phe | Asp | Val | Ile |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     |

&lt;210&gt; 103

&lt;211&gt; 43

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 103

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Leu | Lys | Leu | Lys | Ala | Gln | Leu | Gly | Pro | Asp | Glu | Ser | Lys | Gln | Lys |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Phe | Val | Leu | Lys | Thr | Pro | Lys | Gly | Thr | Arg | Asp | Tyr | Ser | Pro | Arg | Gln |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Ala | Val | Arg | Glu | Lys | Val | Phe | Asp | Val | Ile |
|     |     | 35  |     |     |     |     | 40  |     |     |     |

&lt;210&gt; 104

&lt;211&gt; 42

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 104

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Lys | Leu | Lys | Ala | Gln | Leu | Gly | Pro | Asp | Glu | Ser | Lys | Gln | Lys | Phe |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|

|   |  |  |  |   |  |  |  |  |  |    |  |  |  |  |    |
|---|--|--|--|---|--|--|--|--|--|----|--|--|--|--|----|
| 1 |  |  |  | 5 |  |  |  |  |  | 10 |  |  |  |  | 15 |
|---|--|--|--|---|--|--|--|--|--|----|--|--|--|--|----|

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Leu | Lys | Thr | Pro | Lys | Gly | Thr | Arg | Asp | Tyr | Ser | Pro | Arg | Gln | Met |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Val | Arg | Glu | Lys | Val | Phe | Asp | Val | Ile |
|     |     | 35  |     |     |     |     | 40  |     |     |

&lt;210&gt; 105



&lt;211&gt; 41

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 105

Lys Leu Lys Ala Gln Leu Gly Pro Asp Glu Ser Lys Gln Lys Phe Val  
 1 5 10 15

Leu Lys Thr Pro Lys Gly Thr Arg Asp Tyr Ser Pro Arg Gln Met Ala  
 20 25 30

Val Arg Glu Lys Val Phe Asp Val Ile  
 35 40

&lt;210&gt; 106

&lt;211&gt; 40

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 106

Leu Lys Ala Gln Leu Gly Pro Asp Glu Ser Lys Gln Lys Phe Val Leu  
 1 5 10 15

Lys Thr Pro Lys Gly Thr Arg Asp Tyr Ser Pro Arg Gln Met Ala Val  
 20 25 30

Arg Glu Lys Val Phe Asp Val Ile  
 35 40

&lt;210&gt; 107

&lt;211&gt; 275

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; HRS-Fc fusion protein (C-terminal Fc fusion)

&lt;400&gt; 107

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
 1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
 20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
 35 40 45

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly  
 50 55 60

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met  
 65 70 75 80

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His  
 85 90 95

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val  
 100 105 110

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr  
 115 120 125

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly



130                      135                      140  
 Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile  
 145                      150                      155                      160  
 Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val  
 165                      170                      175  
 Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser  
 180                      185                      190  
 Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu  
 195                      200                      205  
 Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro  
 210                      215                      220  
 Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val  
 225                      230                      235                      240  
 Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met  
 245                      250                      255  
 His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser  
 260                      265                      270  
 Pro Gly Lys  
 275

&lt;210&gt; 108

&lt;211&gt; 275

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; HRS-Fc fusion protein (N-terminal Fc fusion)

&lt;400&gt; 108

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



Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val  
145 150 155 160

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro  
165 170 175

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr  
180 185 190

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val  
195 200 205

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu  
210 215 220

Ser Pro Gly Lys Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu  
225 230 235 240

Gln Gly Glu Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu  
245 250 255

Leu Ile Glu Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu  
260 265 270

Gly Pro Asp  
275

<210> 109

<211> 733

<212> PRT

<213> Artificial Sequence

<220>

<223> HRS-Fc fusion protein (C-terminal Fc fusion)

<400> 109

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp  
50 55 60

Tyr Ser Pro Arg Gln Met Ala Val Arg Glu Lys Val Phe Asp Val Ile  
65 70 75 80

Ile Arg Cys Phe Lys Arg His Gly Ala Glu Val Ile Asp Thr Pro Val  
85 90 95

Phe Glu Leu Lys Glu Thr Leu Met Gly Lys Tyr Gly Glu Asp Ser Lys  
100 105 110

Leu Ile Tyr Asp Leu Lys Asp Gln Gly Gly Glu Leu Leu Ser Leu Arg

115

120

125

Tyr Asp Leu Thr Val Pro Phe Ala Arg Tyr Leu Ala Met Asn Lys Leu  
130 135 140



Thr Asn Ile Lys Arg Tyr His Ile Ala Lys Val Tyr Arg Arg Asp Asn  
 145 150 155 160  
 Pro Ala Met Thr Arg Gly Arg Tyr Arg Glu Phe Tyr Gln Cys Asp Phe  
 165 170 175  
 Asp Ile Ala Gly Asn Phe Asp Pro Met Ile Pro Asp Ala Glu Cys Leu  
 180 185 190  
 Lys Ile Met Cys Glu Ile Leu Ser Ser Leu Gln Ile Gly Asp Phe Leu  
 195 200 205  
 Val Lys Val Asn Asp Arg Arg Ile Leu Asp Gly Met Phe Ala Ile Cys  
 210 215 220  
 Gly Val Ser Asp Ser Lys Phe Arg Thr Ile Cys Ser Ser Val Asp Lys  
 225 230 235 240  
 Leu Asp Lys Val Ser Trp Glu Glu Val Lys Asn Glu Met Val Gly Glu  
 245 250 255  
 Lys Gly Leu Ala Pro Glu Val Ala Asp Arg Ile Gly Asp Tyr Val Gln  
 260 265 270  
 Gln His Gly Gly Val Ser Leu Val Glu Gln Leu Leu Gln Asp Pro Lys  
 275 280 285  
 Leu Ser Gln Asn Lys Gln Ala Leu Glu Gly Leu Gly Asp Leu Lys Leu  
 290 295 300  
 Leu Phe Glu Tyr Leu Thr Leu Phe Gly Ile Asp Asp Lys Ile Ser Phe  
 305 310 315 320  
 Asp Leu Ser Leu Ala Arg Gly Leu Asp Tyr Tyr Thr Gly Val Ile Tyr  
 325 330 335  
 Glu Ala Val Leu Leu Gln Thr Pro Ala Gln Ala Gly Glu Glu Pro Leu  
 340 345 350  
 Gly Val Gly Ser Val Ala Ala Gly Gly Arg Tyr Asp Gly Leu Val Gly  
 355 360 365  
 Met Phe Asp Pro Lys Gly Arg Lys Val Pro Cys Val Gly Leu Ser Ile  
 370 375 380  
 Gly Val Glu Arg Ile Phe Ser Ile Val Glu Gln Arg Leu Glu Ala Leu  
 385 390 395 400  
 Glu Glu Lys Ile Arg Thr Thr Glu Thr Gln Val Leu Val Ala Ser Ala  
 405 410 415  
 Gln Lys Lys Leu Leu Glu Glu Arg Leu Lys Leu Val Ser Glu Leu Trp  
 420 425 430  
 Asp Ala Gly Ile Lys Ala Glu Leu Leu Tyr Lys Lys Asn Pro Lys Leu  
 435 440 445  
 Leu Asn Gln Leu Gln Tyr Cys Glu Glu Ala Gly Ile Pro Leu Val Ala  
 450 455 460  
 Ile Ile Gly Glu Gln Glu Leu Lys Asp Gly Val Ile Lys Leu Arg Ser  
 465 470 475 480  
 Val Thr Ser Arg Glu Glu Val Asp Val Arg Arg Glu Asp Leu Val Glu  
 485 490 495



Glu Ile Lys Arg Arg Thr Gly Gln Pro Leu Asp Lys Thr His Thr Cys  
500 505 510

Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu  
515 520 525

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu  
530 535 540

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys  
545 550 555 560

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys  
565 570 575

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu  
580 585 590

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys  
595 600 605

Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys  
610 615 620

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser  
625 630 635 640

Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys  
645 650 655

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln  
660 665 670

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly  
675 680 685

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln  
690 695 700

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn  
705 710 715 720

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys  
725 730

<210> 110

<211> 733

<212> PRT

<213> Artificial Sequence

<220>

<223> HRS-Fc fusion protein (N-terminal Fc fusion)

<400> 110

Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu  
1 5 10 15

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu  
20 25 30

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser  
35 40 45

His Glu Asn Pro Glu Val Lys Phe Asn Thr Thr Val Asn Glu Val Glu



His Glu Asp Pro Glu Val Lys Phe Asn Ile Tyr Val Asp Gly Val Glu  
 50 55 60  
 Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr  
 65 70 75 80  
 Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn  
 85 90 95  
 Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro  
 100 105 110  
 Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln  
 115 120 125  
 Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val  
 130 135 140  
 Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val  
 145 150 155 160  
 Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro  
 165 170 175  
 Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr  
 180 185 190  
 Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val  
 195 200 205  
 Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu  
 210 215 220  
 Ser Pro Gly Lys Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu  
 225 230 235 240  
 Gln Gly Glu Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu  
 245 250 255  
 Leu Ile Glu Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu  
 260 265 270  
 Gly Pro Asp Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly  
 275 280 285  
 Thr Arg Asp Tyr Ser Pro Arg Gln Met Ala Val Arg Glu Lys Val Phe  
 290 295 300  
 Asp Val Ile Ile Arg Cys Phe Lys Arg His Gly Ala Glu Val Ile Asp  
 305 310 315 320  
 Thr Pro Val Phe Glu Leu Lys Glu Thr Leu Met Gly Lys Tyr Gly Glu  
 325 330 335  
 Asp Ser Lys Leu Ile Tyr Asp Leu Lys Asp Gln Gly Gly Glu Leu Leu  
 340 345 350  
 Ser Leu Arg Tyr Asp Leu Thr Val Pro Phe Ala Arg Tyr Leu Ala Met  
 355 360 365  
 Asn Lys Leu Thr Asn Ile Lys Arg Tyr His Ile Ala Lys Val Tyr Arg  
 370 375 380  
 Arg Asp Asn Pro Ala Met Thr Arg Gly Arg Tyr Arg Glu Phe Tyr Gln  
 385 390 395 400  
 Cys Asp Phe Asp Ile Ala Gly Asn Phe Asp Pro Met Ile Pro Asp Ala  
 405 410 415 420 425 430 435 440 445 450



| 405                                                                                | 410 | 415 |
|------------------------------------------------------------------------------------|-----|-----|
| Glu Cys Leu Lys Ile Met Cys Glu Ile Leu Ser Ser Leu Gln Ile Gly<br>420 425 430     |     |     |
| Asp Phe Leu Val Lys Val Asn Asp Arg Arg Ile Leu Asp Gly Met Phe<br>435 440 445     |     |     |
| Ala Ile Cys Gly Val Ser Asp Ser Lys Phe Arg Thr Ile Cys Ser Ser<br>450 455 460     |     |     |
| Val Asp Lys Leu Asp Lys Val Ser Trp Glu Glu Val Lys Asn Glu Met<br>465 470 475 480 |     |     |
| Val Gly Glu Lys Gly Leu Ala Pro Glu Val Ala Asp Arg Ile Gly Asp<br>485 490 495     |     |     |
| Tyr Val Gln Gln His Gly Gly Val Ser Leu Val Glu Gln Leu Leu Gln<br>500 505 510     |     |     |
| Asp Pro Lys Leu Ser Gln Asn Lys Gln Ala Leu Glu Gly Leu Gly Asp<br>515 520 525     |     |     |
| Leu Lys Leu Leu Phe Glu Tyr Leu Thr Leu Phe Gly Ile Asp Asp Lys<br>530 535 540     |     |     |
| Ile Ser Phe Asp Leu Ser Leu Ala Arg Gly Leu Asp Tyr Tyr Thr Gly<br>545 550 555 560 |     |     |
| Val Ile Tyr Glu Ala Val Leu Leu Gln Thr Pro Ala Gln Ala Gly Glu<br>565 570 575     |     |     |
| Glu Pro Leu Gly Val Gly Ser Val Ala Ala Gly Gly Arg Tyr Asp Gly<br>580 585 590     |     |     |
| Leu Val Gly Met Phe Asp Pro Lys Gly Arg Lys Val Pro Cys Val Gly<br>595 600 605     |     |     |
| Leu Ser Ile Gly Val Glu Arg Ile Phe Ser Ile Val Glu Gln Arg Leu<br>610 615 620     |     |     |
| Glu Ala Leu Glu Glu Lys Ile Arg Thr Thr Glu Thr Gln Val Leu Val<br>625 630 635 640 |     |     |
| Ala Ser Ala Gln Lys Lys Leu Leu Glu Glu Arg Leu Lys Leu Val Ser<br>645 650 655     |     |     |
| Glu Leu Trp Asp Ala Gly Ile Lys Ala Glu Leu Leu Tyr Lys Lys Asn<br>660 665 670     |     |     |
| Pro Lys Leu Leu Asn Gln Leu Gln Tyr Cys Glu Glu Ala Gly Ile Pro<br>675 680 685     |     |     |
| Leu Val Ala Ile Ile Gly Glu Gln Glu Leu Lys Asp Gly Val Ile Lys<br>690 695 700     |     |     |
| Leu Arg Ser Val Thr Ser Arg Glu Glu Val Asp Val Arg Arg Glu Asp<br>705 710 715 720 |     |     |
| Leu Val Glu Glu Ile Lys Arg Arg Thr Gly Gln Pro Leu<br>725 730                     |     |     |

&lt;210&gt; 111

&lt;211&gt; 1527

&lt;212&gt; DNA



<213> Artificial Sequence

<220>

<223> Codon optimized version of HRS

<400> 111

|                                                                    |      |
|--------------------------------------------------------------------|------|
| atggcagagc gtgcggcgct ggaggagctg gtgaaacttc agggagagcg cgtgcgaggc  | 60   |
| ctcaagcagc agaaggccag cgccgagctg atcgaggagg aggtggcgaa actcctgaaa  | 120  |
| ctgaaggcac agctgggtcc tgatgaaagc aaacagaaat ttgtgctcaa aacccccaa   | 180  |
| ggcacaagag actatagtc cggcgagatg gcagttcgcg agaaggtgtt tgacgtaatc   | 240  |
| atccgttgct tcaagcgcca cgggtgcagaa gtcattgata cacctgtatt tgaactaaag | 300  |
| gaaacactga tgggaaagta tggggaagac tccaagctta tctatgacct gaaggaccag  | 360  |
| ggcggggagc tcctgtccct tcgctatgac ctactgttc cttttgctcg gtatttggca   | 420  |
| atgaataaac tgaccaacat taaacgctac cacatagcaa aggtatatcg gcgggataac  | 480  |
| ccagccatga cccgtggccg ataccgggaa ttctaccagt gtgattttga cattgctggg  | 540  |
| aactttgatc ccatgatccc tgatgcagag tgcctgaaga tcatgtgcga gatcctgagt  | 600  |
| tcacttcaga taggcgactt cctggtcaag gtaaacgac gacgcattct agatgggatg   | 660  |
| tttgctatct gtggtgtttc tgacagcaag ttccgtacca tctgctcctc agtagacaag  | 720  |
| ctggacaagg tgtcctggga agaggtgaag aatgagatgg tgggagagaa gggccttgca  | 780  |
| cctgaggtgg ctgaccgat tggggactat gtccagcaac atggtggggt atccctggtg   | 840  |
| gaacagctgc tccaggatcc taaactatcc caaaacaagc aggccttgga gggcctggga  | 900  |
| gacctgaagt tgctctttga gtacctgacc ctatttggca ttgatgacaa aatctccttt  | 960  |
| gacctgagcc ttgctcgagg gctggattac tacactgggg tgatctatga ggcagtgtg   | 1020 |
| ctacagaccc cagccagggc aggggaagag cccctgggtg tgggcagtgt ggctgctgga  | 1080 |
| ggacgctatg atgggctagt gggcatgttc gaccccaaag ggcgcaaggt gccatgtgtg  | 1140 |
| gggctcagca ttgggggtgga gcggattttc tccatcgtgg aacagagact agaggctttg | 1200 |
| gaggagaaga tacggaccac ggagacacag gtgcttgtgg catctgcaca gaagaagctg  | 1260 |
| ctagaggaaa gactaaagct tgtctcagaa ctgtgggatg ctgggatcaa ggctgagctg  | 1320 |
| ctgtacaaga agaaccocaa gctactgaac cagttacagt actgtgagga ggcaggcatc  | 1380 |
| ccactggtgg ctatcatcgg cgagcaggaa ctcaaggatg gggcatcaa gctccgttca   | 1440 |
| gtgacgagca gggaagaggt ggatgtccga agagaagacc ttgtggagga aatcaaaagg  | 1500 |
| agaacaggcc agcccctctg catctgc                                      | 1527 |

<210> 112

<211> 423

<212> DNA

<213> Artificial Sequence

<220>

<223> Codon optimized version of HRS

<400> 112

|                                                                   |     |
|-------------------------------------------------------------------|-----|
| atggcagaac gtgccgccct ggaagagctg gtaaaactgc aaggcgagcg tgttcgtggt | 60  |
| ctgaaacagc agaaagcaag cgctgaactg atcgaagaag aagtggcgaa actgctgaaa | 120 |
| ctgaaagcac agctgggtcc tgatgaatca aaacaaaaat tcgtcctgaa aactccgaaa | 180 |
| ggaacccgtg actattctcc tcgtcaaatg gccgtccgtg aaaaagtgtt cgacgtgatc | 240 |
| attcgtgct ttaaacgcca tgggtgccga gtgattgata ccccggtgtt tgagctgaaa  | 300 |



gagacactga tgggcaaata tgggtgaggac agcaaactga tttatgacct gaaagatcag 360  
 ggtggtgaac tgctgagtct gcgctatgat ctgacagttc cgtttgcccg ttatctggca 420  
 atg 423

<210> 113

<211> 1224

<212> DNA

<213> Artificial Sequence

<220>

<223> Codon optimized version of HRS

<400> 113

atggcagaac gtgccgccct ggaagagctg gtaaaactgc aaggcgagcg tgttcgtggt 60  
 ctgaaacagc agaaagcaag cgctgaactg atcgaagaag aagtggcgaa actgctgaaa 120  
 ctgaaagcac agctgggtcc tgatgaatca aaacaaaaat tcgtcctgaa aactccgaaa 180  
 ggaaccogtg actattctcc tcgtcaaactg gccgtccgtg aaaaagtgtt cgacgtgatc 240  
 attcgtctgt ttaaacgcca tgggtgccga gtgattgata ccccggtgtt tgagctgaaa 300  
 gagacactga tgggcaaata tgggtgaggac agcaaactga tttatgacct gaaagatcag 360  
 ggtggtgaac tgctgagtct gcgctatgat ctgacagttc cgtttgcccg ttatctggca 420  
 atgaataaac tgaccaacat taaacgctat cacattgcta aagtctatcg ccgtgacaat 480  
 cctgctatga cccgtggtcg ttatcgtgag ttctatcagt gtgacttcga tattgccggc 540  
 aactttgatc cgatgatccc ggatgctgaa tgcctgaaaa tcatgtgtga gatcctgagc 600  
 agtctgcaga ttggcgatct cctgggtgaaa gtcaacgata gccgtattct ggatggcatg 660  
 ttccgcatct gtggtgttag cgactccaaa ttccgtacca tctgtagtag tgtggacaaa 720  
 ctggataaag tgagctggga ggaggtgaaa aacgaaatgg tgggcgagaa aggtctggct 780  
 cctgaagtgg ctgaccgtat tgggtgattat gtccagcagc acggtggagt atcactggtt 840  
 gagcaactgc tgcaagacct taaactgagt cagaataaac aggccttga gggactggga 900  
 gatctgaaac tgctgttcga gtatctgacc ctgttcggta tcgatgacaa aatctccttt 960  
 gacctgtcac tggctcgtgg actggactat tataccggcg tgatctatga agctgtactg 1020  
 ctgcaaactc cagcacaagc aggtgaagag cctctgggtg tgggtagtgt agccgctggg 1080  
 ggacgttatg atggactggt ggggatgttc gaccctaaag gccgtaaagt tccgtgtgtg 1140  
 ggtctgagta tcggtgttga gcgtatcttt tccatcgtcg agcaacgtct ggaagcactg 1200  
 gaggaaaaaa tcogtacgac cgaa 1224

<210> 114

<211> 339

<212> DNA

<213> Artificial Sequence

<220>

<223> Codon optimized version of HRS

<400> 114

atggcagaac gtgccgccct ggaagagctg gtaaaactgc aaggcgagcg tgttcgtggt 60  
 ctgaaacagc agaaagcaag cgctgaactg atcgaagaag aagtggcgaa actgctgaaa 120  
 ctgaaagcac agctgggtcc tgatgaatca aaacaaaaat tcgtcctgaa aactccgaaa 180  
 ggaaccogtg actattctcc tcgtcaaactg gccgtccgtg aaaaagtgtt cgacgtgatc 240  
 attcgtctgt ttaaacgcca tgggtgccga gtgattgata ccccggtgtt tgagctgaaa 300



attcgctgct ttaaacgcca tggcgccgaa gtgattgata ccccggtggt tgagctgaaa 300  
gagacactga tgggcaaata tgggtgaggac agcaaactg 339

<210> 115

<211> 180

<212> DNA

<213> Artificial Sequence

<220>

<223> Codon optimized version of HRS

<400> 115

atggcagaac gtgccgccct ggaagagctg gtaaaactgc aaggcgagcg tgttcgtggt 60  
ctgaaacagc agaaagcaag cgctgaactg atcgaagaag aagtggcgaa actgctgaaa 120  
ctgaaagcac agctgggtcc tgatgaatca aaacaaaaat tcgtcctgaa aactccgaag 180

<210> 116

<211> 1518

<212> DNA

<213> Artificial Sequence

<220>

<223> Codon optimized version of HRS

<400> 116

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ctgaaggcac agctgggtcc tgatgaaagc aaacagaaat ttgtgctcaa aacccccaaag 180  
ggcacaagag actatagtc cgggcagatg gcagttcgcg agaaggtgtt tgacgtaatc 240  
atccgttgct tcaagcgcca cgggtgcagaa gtcattgata cacctgtatt tgaactaaag 300  
gaaacactga tgggaaagta tggggaagac tccaagctta tctatgacct gaaggaccag 360  
ggcgggggagc tcctgtccct tcgctatgac ctcaactgttc cttttgctcg gtatttgga 420  
atgaataaac tgaccaacat taaacgctac cacatagcaa aggtatatcg gcgggataac 480  
ccagccatga cccgtggccg ataccgggaa ttctaccagt gtgattttga cattgctggg 540  
aactttgatc ccatgatccc tgatgcagag tgccctgaaga tcatgtgcga gatcctgagt 600  
tcacttcaga taggcgactt cctgggtcaag gtaaaccgac gacgcattct agatgggatg 660  
tttgctatct gtggtgtttc tgacagcaag ttccgtacca tctgctcctc agtagacaag 720  
ctggacaagg tgcctggga agaggtgaag aatgagatgg tgggagagaa gggccttgca 780  
cctgaggtgg ctgaccgat tggggactat gtccagcaac atggtgggggt atccctggtg 840  
gaacagctgc tccaggatcc taaactatcc caaaacaagc aggccttgga gggcctggga 900  
gacctgaagt tgctctttga gtacctgacc ctatttgga ttgatgacaa aatctccttt 960  
gacctgagcc ttgctcgagg gctggattac tacactgggg tgatctatga ggcagtgctg 1020  
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gaggagaaga tacggaccac ggagacacag gtgcttgtgg catctgcaca gaagaagctg 1260  
ctagaggaaa gactaaagct tgtctcagaa ctgtgggatg ctgggatcaa ggctgagctg 1320  
ctgtacaaga agaaccctaaa gctactgaac cagttacagt actgtgagga ggcaggcatc 1380  
ccactggtgg ctatcatcgg cgagcaggaa ctcaaggatg gggcatcaa gctccgttca 1440



gtgacgagca ggggaagaggt ggatgtccga agagaagacc ttgtggagga aatcaaaagg 1500  
agaacaggcc agccccc 1518

<210> 117  
<211> 144  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Codon Optimized version of HRS

<400> 117  
atggcagaac gtgccgccct ggaagagctg gtaaaactgc aaggcgagcg tgttcgtggt 60  
ctgaaacagc agaaagcaag cgctgaactg atcgaagaag aagtggcgaa actgctgaaa 120  
ctgaaagcac agctgggtcc tgat 144

<210> 118  
<211> 429  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Codon optimized version of HRS

<400> 118  
tgccctgaaa tcatgtgtga gacccctgagt agtctgcaaa ttggcgactt tctggtcaaa 60  
gtgaacgata gccgtattct ggatggcatg ttccgccatct gtggtgtag cgactccaaa 120  
ttccgtacaa tctgtagcag cgtggacaaa ctggataaag tgtcctggga agaggtgaaa 180  
aacgaaatgg tgggtgaaaa aggtctggct ccggagggtg ctgaccgtat cgggtgattat 240  
gttcagcagc acggcggtgt tagtctggtt gaacaactgc tgcaagacc gaaactgtct 300  
cagaacaaac aggcctgga aggactggga gatctgaaac tgctgttcga gtatctgacg 360  
ctgttcggca ttgatgaaa aatttctttc gacctgtcac tggcacgtgg actggactat 420  
tataccggt 429

<210> 119  
<211> 315  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Codon optimized version of HRS

<400> 119  
cgtaccaccg aaacccaagt tctggttgcc tcagctcaga aaaaactgct ggaagaacgc 60  
ctgaaactgg ttagogaact gtgggatgct ggcattaaag ccgaactgct gtataaaaaa 120  
aacccgaaac tgctgaatca gctgcagtat tgtgaggaag cgggtattcc tctggtggcc 180  
attatcggag aacaggaact gaaagacggc gttattaaac tgcgtagcgt gacctctcgt 240  
gaagaagttg acgttcgccg tgaagatctg gtcgaggaaa tcaaacgtcg taccggtcaa 300  
cctctgtgta ttg 315

<210> 120  
<211> 810



&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Codon optimized version of HRS

&lt;400&gt; 120

|                                                                     |     |
|---------------------------------------------------------------------|-----|
| atggcagaac gtgccgccct ggaagagctg gtaaaactgc aaggcgagcg tgttcgtggt   | 60  |
| ctgaaacagc agaaagcaag cgctgaactg atcgaagaag aagtggcgaa actgctgaaa   | 120 |
| ctgaaagcac agctgggtcc tgatgaatca aaacaaaaat tcgtcctgaa aactccgaaa   | 180 |
| ggaacccgtg actattctcc tcgtcaaatg gccgtccgtg aaaaagtgtt cgacgtgac    | 240 |
| attcgctgct ttaaacgcca tgggtgccga gtgattgata ccccggtgtt tgagctgaaa   | 300 |
| gagacactga tgggcaaata tgggtgaggac agcaaactga tctatgacct gaaagaccaa  | 360 |
| ggcgggtgaac tgctgtccct gcgttatgat ctgactgtgc cgtttgcccg ttatctggcc  | 420 |
| atgaataaac tgacgaacat taaacgctat cacattgcc aagtgtatcg ccgtgacaat    | 480 |
| cctgctatga ctcggtgacg ttatcgtaga ttctatcagt gtgacttcga tattgccggc   | 540 |
| aacttcgacc ctatgattcc ggatgctgaa tgcccgaaaa tcatgtgtga gatcctgagc   | 600 |
| agcctgcaaa ttgggtgactt cctgggtgaaa gtgaatgacc gtcgtatcct ggatggcatg | 660 |
| tttgccattt gtggtgtgag cgattccaaa ttccgtacca tctgtagtag tgtggacaaa   | 720 |
| ctggataaag tgggctatcc gtggtggaac tctttagacc gtattctgaa ctatcctaaa   | 780 |
| accagccgcc cgtggcgtgc ttgggaaact                                    | 810 |

&lt;210&gt; 121

&lt;211&gt; 1185

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Codon optimized version of HRS

&lt;400&gt; 121

|                                                                   |      |
|-------------------------------------------------------------------|------|
| atggcagaac gtgccgccct ggaagagctg gtaaaactgc aaggcgagcg tgttcgtggt | 60   |
| ctgaaacagc agaaagcaag cgctgaactg atcgaagaag aagtggcgaa actgctgaaa | 120  |
| ctgaaagcac agctgggtcc tgatgaatca aaacaaaaat tcgtcctgaa aactccgaaa | 180  |
| gacttcgata ttgccgggaa ttttgacct atgatccctg atgccgaatg tctgaaaatc  | 240  |
| atgtgtgaga tcctgagcag tctgcagatt ggtgacttcc tggtgaaagt gaacgatcgc | 300  |
| cgtattctgg atggaatgtt tgccatttgt ggcgtgtctg acagcaaatt tcgtacgatc | 360  |
| tgtagcagcg tggataaact ggataaagtg agctgggagg aggtgaaaaa tgagatggtg | 420  |
| ggcgaaaaag gtctggcacc tgaagtggct gaccgtatcg gtgattatgt tcagcaacat | 480  |
| ggcgggtgtt ctctggtcga acagctgctg caagacccaa aactgagcca gaacaaacag | 540  |
| gcactggaag gactgggtga tctgaaactg ctgtttgagt atctgacgct gtttggcatc | 600  |
| gatgacaaaa tctcgtttga cctgagcctg gcacgtggtc tggattatta taccggcgtg | 660  |
| atctatgaag ccgtcctgct gcaaactcca gcacaagcag gtgaagaacc tctgggtgtt | 720  |
| ggtagtgtag cggcaggcgg acgttatgat ggactggtgg ggatgtttga tccgaaaggc | 780  |
| cgtaaagttc cgtgtgtcgg tctgagtatc ggggttgagc gtatctttag cattgtggag | 840  |
| caacgtctgg aagctctgga ggaaaaaatc cgtaccaccg aaaccaagt tctggttgcc  | 900  |
| tcagctcaga aaaaactgct ggaagaagc ctgaaactgg ttagcgaact gtgggatgct  | 960  |
| ggcattaaag ccgaactgct gtataaaaaa aaccgaaac tgctgaatca gctgcagtat  | 1020 |



|                                                                   |      |
|-------------------------------------------------------------------|------|
| tgtgaggaag cgggtattcc tctggtggcc attatcggag aacaggaact gaaagacggc | 1080 |
| gttattaaac tgcgtagcgt gacctctcgt gaagaagttg acgttcgccg tgaagatctg | 1140 |
| gtcgaggaaa tcaaacgtcg taccgggtcaa cctctgtgta ttigc                | 1185 |

&lt;210&gt; 122

&lt;211&gt; 1077

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Codon optimized version of HRS

&lt;400&gt; 122

|                                                                   |      |
|-------------------------------------------------------------------|------|
| atggcagaac gtgccgccct ggaagagctg gtaaaactgc aaggcgagcg tgttcgtggt | 60   |
| ctgaaacagc agaaagcaag cgctgaactg atcgaagaag aagtggcgaa actgctgaaa | 120  |
| ctgaaagcac agctgggtcc tgatgaatca aaacaaaaat tcgtcctgaa aactccgaaa | 180  |
| gtgaatgata gccgtatcct ggatggcatg ttggccattt gtggtgtgag cgactcgaaa | 240  |
| ttccgtacga ttgctctag cgtcgataaa ctggacaaag tgcctggga agaggtgaaa   | 300  |
| aacgagatgg tgggtgagaa aggtctggct cctgaagttg ccgaccgat tggtgattat  | 360  |
| gttcagcagc atggcggtgt ttcaactggt gaacaactgc tgcaagacc gaaactgtct  | 420  |
| cagaataaac aggcgctgga aggactggga gatctgaaac tgctgtttga gtatctgacc | 480  |
| ctgttcggca ttgatgaaa aatcagcttc gacctgagcc tggcacgtgg tctggattat  | 540  |
| tataccggcg tgatctatga agccgttctg ctgcagacac cagcacaagc aggcgaagaa | 600  |
| cctctgggtg ttggttctgt ggcagccggg ggtcgttatg atggactggt aggcattgtc | 660  |
| gatccgaaag gccgtaaagt tccgtgtgtg ggactgagta tcggtgttga gcgtatcttt | 720  |
| agcatcgtgg aacaacgtct ggaagcgctg gaggagaaaa ttcgtaccac cgaaacccaa | 780  |
| gttctggttg cctcagctca gaaaaaactg ctggaagaac gcctgaaact ggtagcgaa  | 840  |
| ctgtgggatg ctggcattaa agccgaactg ctgtataaaa aaaacccgaa actgctgaat | 900  |
| cagctgcagt attgtgagga agcgggtatt cctctggtgg ccattatcgg agaacaggaa | 960  |
| ctgaaagacg gcgttattaa actgcgtagc gtgacctctc gtgaagaagt tgacgttcgc | 1020 |
| cgtgaagatc tggtcgagga aatcaaactg cgtaccggtc aacctctgtg tatttgc    | 1077 |

&lt;210&gt; 123

&lt;211&gt; 1197

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Codon optimized version of HRS

&lt;400&gt; 123

|                                                                   |     |
|-------------------------------------------------------------------|-----|
| atggcagaac gtgccgccct ggaagagctg gtaaaactgc aaggcgagcg tgttcgtggt | 60  |
| ctgaaacagc agaaagcaag cgctgaactg atcgaagaag aagtggcgaa actgctgaaa | 120 |
| ctgaaagcac agctgggtcc tgatgaatca aaacaaaaat tcgtcctgaa aactccgaaa | 180 |
| ggaactcgtg attatagccc tcgccagatg gctgtccgtg aaaaagtgtt cgatgtgata | 240 |
| attcgtctgt tcaaactgca tgggtgccga gtcattgata ccccggtgtt cgagctgaaa | 300 |
| gtgaacgata gccgtattct ggatggcatg ttccgccatt gtggtgttag cgatagcaaa | 360 |
| ttccgtacaa tctgctctag cgtggacaaa ctggacaaag tgagctggga agaggtgaaa | 420 |



|                                                                   |      |
|-------------------------------------------------------------------|------|
| aacgagatgg tgggtgagaa aggcctggct cctgaagttg ccgaccgtat cggagattat | 480  |
| gttcagcagc atggcggagt ttcactggtt gaacaactgc tgcaagaccg gaaactgtct | 540  |
| cagaacaaac aggcactgga aggtctggga gatctgaaac tgctgttcga gtatctgacg | 600  |
| ctgttcggta ttgacgacaa aatttccttc gacctgtcgc tggcacgtgg tctggattat | 660  |
| tatacaggcg tgatctatga ggctgtactg ctgcagacac cagcacaagc aggtgaagag | 720  |
| cctctgggtg ttggttcagt tgctgccggg ggacgttatg acggactggg agggatgttt | 780  |
| gacccaaaag gccgtaaagt cccgtgtgta ggactgtcta ttggcgttga gcgtatcttt | 840  |
| agcatcgtgg agcaacgtct ggaagctctg gaggagaaaa tccgtaccac cgaaacccaa | 900  |
| gttctgggtg cctcagctca gaaaaaactg ctggaagaac gcctgaaact ggtagcgaa  | 960  |
| ctgtgggatg ctggcattaa agccgaactg ctgtataaaa aaaacccgaa actgctgaat | 1020 |
| cagctgcagt attgtgagga agcgggtatt cctctgggtg ccattatcgg agaacaggaa | 1080 |
| ctgaaagacg gcgttattaa actgcgtagc gtgacctctc gtgaagaagt tgacgttcgc | 1140 |
| cgtgaagatc tggtcgagga aatcaaactg cgtaccgggc aacctctgtg tatttgc    | 1197 |

&lt;210&gt; 124

&lt;211&gt; 1419

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Codon optimized version of HRS

&lt;400&gt; 124

|                                                                     |      |
|---------------------------------------------------------------------|------|
| atggcagaac gtgccgccct ggaagagctg gtaaaactgc aaggcgagcg tgttcgtggg   | 60   |
| ctgaaacagc agaaagcaag cgctgaactg atcgaagaag aagtggcgaa actgctgaaa   | 120  |
| ctgaaagcac agctgggtcc tgatgaatca aaacaaaaat tcgtcctgaa aactccgaaa   | 180  |
| ggaactcgtg attatagccc tcgccagatg gctgtccgtg aaaaagtgtt cgatgtgatc   | 240  |
| attcgtctgt tcaaacgtca tgggtgccga gtcattgata ccccggtgtt cgagctgaaa   | 300  |
| gaaaccctga tgggcaaata tggggaagat tccaaactga tctatgacct gaaagaccag   | 360  |
| ggaggtgaac tgctgtctct gcgctatgac ctgactgttc cttttgctcg ctatctggcc   | 420  |
| atgaataaac tgaccaacat caaacgctat catatcgcca aagtgtatcg ccgtgacaat   | 480  |
| ccagcaatga cccgtgggtc ttatcgtgaa ttttatcagt gtgtgaacga tcgccgtatt   | 540  |
| ctggacggca tgttcgccat ttgtggtgtg tctgactcca aatttcgtac gatctgctca   | 600  |
| agcgtggaca aactggacaa agtgagctgg gaagaggtga aaaacgagat ggtgggtgag   | 660  |
| aaaggcctgg ctctgaagt tgccgaccgt atcggagatt atgttcagca gcatggcgga    | 720  |
| gtttcactgg ttgaacaact gctgcaagac ccgaaactgt cacagaacaa acaggcactg   | 780  |
| gaaggtctgg gggatctgaa actgctgttc gagtatctga cgctgttcgg tattgacgac   | 840  |
| aaaatcagct tcgatctgag cctggcacgt ggtctggact attataccgg cgtgatttat   | 900  |
| gaagccgttc tgctgcagac tccagcacia gcaggtgaag agcctctggg tgttggaagt   | 960  |
| gtggcagccg gtggccgtta tgatggtctg gttggcatgt ttgacccgaa aggccgtaaa   | 1020 |
| gtcccgtgtg taggaactgt tateggcgtg gagcgtatct ttagcatcgt ggaacaacgc   | 1080 |
| ctggaagctc tgggaagagaa aatccgtacc accgaaaccc aagttctggg tgccctcagct | 1140 |
| cagaaaaaac tgctggaaga acgcctgaaa ctggttagcg aactgtggga tgctggcatt   | 1200 |
| aaagccgaac tgctgtataa aaaaaacccg aaactgctga atcagctgca gtattgtgag   | 1260 |
| gaagcgggta ttctctgggt ggccattatc ggagaacagg aactgaaaga cggcgttatt   | 1320 |



aaactgcgta gcgtgacctc tcgtgaagaa gttgacgttc gccgtgaaga tctggtcgag 1380  
gaaatcaaac gtctgaccgg tcaacctctg tgtattttgc 1419

<210> 125

<211> 1407

<212> DNA

<213> Artificial Sequence

<220>

<223> Codon optimized version of HRS

<400> 125

atggcagaac gtgccgccct ggaagagctg gtaaaactgc aaggcgagcg tgttcgtggg 60  
ctgaaacagc agaaagcaag cgctgaactg atcgaagaag aagtggcgaa actgctgaaa 120  
ctgaaagcac agctgggtcc tgatgaatca aaacaaaaat tcgtcctgaa aactccgaaa 180  
gaaaccctga tgggcaaata tggcgaagat tccaaactga tctatgacct gaaagaccaa 240  
ggcgggtgaac tgctgtccct gcgttatgac ctgactgttc cgtttgctcg ttatctggcc 300  
atgaataaac tgaccaacat taaacgctat cacattgcc aagtgtatcg ccgtgacaat 360  
cctgctatga ctctgtgacg ttatcgtgaa ttctatcagt gtgacttcga tattgccggc 420  
aacttcgacc ctatgattcc ggatgctgaa tgcctgaaaa tcatgtgtga gatcctgagc 480  
agcctgcaaa ttggtgactt cctgggtgaaa gtgaatgacc gtcgtatcct ggatggcatg 540  
ttcgccatit gtggtgttag cgattccaaa ttccgtacca tctgtagtag tgtggacaaa 600  
ctggataaag tgagctggga agaggtgaaa aacgaaatgg tgggcgaaaa aggtctggca 660  
cctgaggttg ctgatcgtat cgggtgactat gtccagcagc atggaggtgt ttcactggtt 720  
gagcaactgc tgcaagatcc gaaactgtct cagaacaaac aggccttgga aggactgggt 780  
gatctgaaac tgctgttcga gtatctgacg ctgttcggta ttgatgacaa aatctcgttc 840  
gacctgtctc tggctcgtgg actggattat tatacggggc taatctatga agctgtcctg 900  
ctgcagacac cagcacaagc aggtgaagag cctctgggtg ttggaagtgt tgctgccggg 960  
ggtcgctatg acggactggt tggcatgttc gatccgaaag gccgtaaagt tccgtgtgta 1020  
ggactgagca ttggcggttg gcgtatcttt tccatcgttg agcaacgtct ggaagcactg 1080  
gaagagaaaa tccgtaccac cgaaacccaa gttctggttg cctcagctca gaaaaactg 1140  
ctggaagaac gcctgaaact ggtagcgaa ctgtgggatg ctggcattaa agccgaactg 1200  
ctgtataaaa aaaaccggaa actgctgaat cagctgcagt attgtgagga agcgggtatt 1260  
cctctggtgg ccattatcgg agaacaggaa ctgaaagacg gcgttattaa actgcgtagc 1320  
gtgacctctc gtgaagaagt tgacgttcgc cgtgaagatc tggtcgagga aatcaaacgt 1380  
cgtaccggtc aacctctgtg tattttgc 1407

<210> 126

<211> 1305

<212> DNA

<213> Artificial Sequence

<220>

<223> Codon optimized version of HRS

<400> 126

atggcagaac gtgccgccct ggaagagctg gtaaaactgc aaggcgagcg tgttcgtggg 60  
ctgaaacagc agaaagcaag cgctgaactg atcgaagaag aagtggcgaa actgctgaaa 120  
ctgaaagcac agctgggtcc tgatgaatca aaacaaaaat tcgtcctgaa aactccgaaa 180



```

ctgaaagcac agctgggtcc tgatgaatca aaacaaaaat tcttcctgaa aactccgaaa      180
ggaactcgtg attatagccc tcgccagatg gctgtccgtg aaaaagtgtt cgatgtgatc      240
attcgtctgt tcaaacgtca tggtagccgaa gtcattgata ccccggtgtt cgagctgaaa      300
gatttcgata ttgccggcaa ctttgatccg atgattccgg atgctgagtg tctgaaaatc      360
atgtgtgaga tcctgagtag tctgcagatt ggggatttcc tggtgaaagt gaacgatcgc      420
cgtattctgg acggcatgtt tgccatttgt ggcgttagcg atagcaaatt ccgtacgatc      480
tgtagcagtg tggacaaact ggataaagtc tcttggaag aggtcaaaaa cgagatggtt      540
ggtgagaaag gcctggctcc tgaagtggct gaccgtattg gtgattatgt ccagcagcat      600
ggtggtgttt cactggttga acaactgctg caagacccga aactgtctca gaacaaacag      660
gcactggaag gtctgggtga tctgaaactg ctgttcgagt atctgacgct gttcgggtatt      720
gacgacaaaa ttctcttoga cctgtcactg gcacgtggtc tggattatta tacaggcgta      780
atctatgagg ctgtactgct gcaaactcca gcacaagcag gtgaagaacc tctgggagtt      840
ggtagtgtag cggcaggggg tcgttatgat gggctggtcg ggatgttcga tccaaaaggc      900
cgtaaagtcc cgtgtgttgg tctgtctatt ggcgttgagc gtatcttctc catcgtggag      960
caacgtctgg aagctctgga agaaaaaatc cgtaccaccg aaaccaagt tctggttgcc     1020
tcagctcaga aaaaactgct ggaagaacgc ctgaaactgg ttagcgaact gtgggatgct     1080
ggcattaaag ccgaactgct gtataaaaaa aacccgaaac tgctgaatca gctgcagtat     1140
tgtgaggaag cgggtattcc tctggtggcc attatcggag aacaggaact gaaagacggc     1200
gttattaaac tgcgtagcgt gacctctcgt gaagaagttg acgttcgccg tgaagatctg     1260
gtcgaggaaa tcaaacgtcg taccgggtcaa cctctgtgta tttgc                      1305

```

&lt;210&gt; 127

&lt;211&gt; 423

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Codon optimized version of HRS

&lt;400&gt; 127

```

atgttcgacc caaaaggccg taaagttccg tgtgtagggc tgtctatcgg tgttgagcgt      60
atcttctcca tcgttgagca gcgtctggaa gcactggagg aaaaaatccg tacgaccgag     120
actcaagtcc tggttgctag tgcccagaaa aaactgctgg aagagcgcct gaaactggtt     180
agtgagctgt gggatgccgg tattaagcc gaactgctgt ataaaaaaaa cccgaaactg     240
ctgaatcagc tgcagtattg tgaagaagcg ggcattccgc tggtagcgat tatcggggaa     300
caagaactga aagatggcgt gatcaaactg cgtagcgtta caagccgtga ggaagtggac     360
gtccgcctg aggatctggt tgaagagatt aaacgccgta caggtcagcc tctgtgtatt     420
tgc                                          423

```

&lt;210&gt; 128

&lt;211&gt; 19

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; MISC\_FEATURE

&lt;222&gt; (1)..(19)

&lt;223&gt; Human IgA1 hinge region



&lt;400&gt; 128

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Pro | Ser | Thr | Pro | Pro | Thr | Pro | Ser | Pro | Ser | Thr | Pro | Pro | Thr | Pro |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

Ser Pro Ser

&lt;210&gt; 129

&lt;211&gt; 101

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; MISC\_FEATURE

&lt;222&gt; (1)..(101)

&lt;223&gt; Human IgA1 CH2 region

&lt;400&gt; 129

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Cys | Cys | His | Pro | Arg | Leu | Ser | Leu | His | Arg | Pro | Ala | Leu | Glu | Asp | Leu |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Leu | Gly | Ser | Glu | Ala | Asn | Leu | Thr | Cys | Thr | Leu | Thr | Gly | Leu | Arg |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asp | Ala | Ser | Gly | Val | Thr | Phe | Thr | Trp | Thr | Pro | Ser | Ser | Gly | Lys | Ser |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Val | Gln | Gly | Pro | Pro | Glu | Arg | Asp | Leu | Cys | Gly | Cys | Tyr | Ser | Val |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Ser | Val | Leu | Pro | Gly | Cys | Ala | Glu | Pro | Trp | Asn | His | Gly | Lys | Thr |
| 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Phe | Thr | Cys | Thr | Ala | Ala | Tyr | Pro | Glu | Ser | Lys | Thr | Pro | Leu | Thr | Ala |
|     |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |

|     |     |     |     |     |
|-----|-----|-----|-----|-----|
| Thr | Leu | Ser | Lys | Ser |
|     |     |     | 100 |     |

&lt;210&gt; 130

&lt;211&gt; 131

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; MISC\_FEATURE

&lt;222&gt; (1)..(131)

&lt;223&gt; human IgA1 CH3 region

&lt;400&gt; 130

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gly | Asn | Thr | Phe | Arg | Pro | Glu | Val | His | Leu | Leu | Pro | Pro | Pro | Ser | Glu |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Leu | Ala | Leu | Asn | Glu | Leu | Val | Thr | Leu | Thr | Cys | Leu | Ala | Arg | Gly |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Phe | Ser | Pro | Lys | Asp | Val | Leu | Val | Arg | Trp | Leu | Gln | Gly | Ser | Gln | Glu |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Pro | Arg | Glu | Lys | Tyr | Leu | Thr | Trp | Ala | Ser | Arg | Gln | Glu | Pro | Ser |
|     | 50  |     |     |     |     | 55  |     |     |     | 60  |     |     |     |     |     |

Gln Gly Thr Thr Thr Phe Ala Val Thr Ser Ile Leu Arg Val Ala Ala



[illegible]

<210> 131  
<211> 6  
<212> PRT  
<213> Homo sapiens  
  
<220>  
<221> MISC\_FEATURE  
<222> (1)..(6)  
<223> human IgA2 hinge region

```
<400> 131
Val Pro Pro Pro Pro Pro
1          5
```

<210> 132  
<211> 101  
<212> PRT  
<213> Homo sapiens

<220>  
<221> MISC\_FEATURE  
<222> (1)..(101)  
<223> human IgA2 CH2

<400> 132

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Cys | Cys | His | Pro | Arg | Leu | Ser | Leu | His | Arg | Pro | Ala | Leu | Glu | Asp | Leu |
| 1   |     |     |     | 5   |     |     |     | 10  |     |     |     |     |     | 15  |     |

Leu Leu Gly Ser Glu Ala Asn Leu Thr Cys Thr Leu Thr Gly Leu Arg  
20 25 30

Asp Ala Ser Gly Ala Thr Phe Thr Trp Thr Pro Ser Ser Gly Lys Ser  
35 40 45

Ala Val Gln Gly Pro Pro Glu Arg Asp Leu Cys Gly Cys Tyr Ser Val  
50 55 60

Ser Ser Val Leu Pro Gly Cys Ala Gln Pro Trp Asn His Gly Glu Thr  
65 70 75 80

Phe Thr Cys Thr Ala Ala His Pro Glu Leu Lys Thr Pro Leu Thr Ala  
85 90 95

Asn Ile Thr Lys Ser  
100

$\langle 210 \rangle$  133  
 $\langle 211 \rangle$  131



<212> PRT

<213> Homo sapiens

<220>

<221> MISC\_FEATURE

<222> (1)..(131)

<223> human IgA2 CH3 region

<400> 133

Gly Asn Thr Phe Arg Pro Glu Val His Leu Leu Pro Pro Pro Ser Glu  
1 5 10 15

Glu Leu Ala Leu Asn Glu Leu Val Thr Leu Thr Cys Leu Ala Arg Gly  
20 25 30

Phe Ser Pro Lys Asp Val Leu Val Arg Trp Leu Gln Gly Ser Gln Glu  
35 40 45

Leu Pro Arg Glu Lys Tyr Leu Thr Trp Ala Ser Arg Gln Glu Pro Ser  
50 55 60

Gln Gly Thr Thr Thr Phe Ala Val Thr Ser Ile Leu Arg Val Ala Ala  
65 70 75 80

Glu Asp Trp Lys Lys Gly Asp Thr Phe Ser Cys Met Val Gly His Glu  
85 90 95

Ala Leu Pro Leu Ala Phe Thr Gln Lys Thr Ile Asp Arg Leu Ala Gly  
100 105 110

Lys Pro Thr His Val Asn Val Ser Val Val Met Ala Glu Val Asp Gly  
115 120 125

Thr Cys Tyr  
130

<210> 134

<211> 58

<212> PRT

<213> Homo sapiens

<220>

<221> MISC\_FEATURE

<222> (1)..(58)

<223> human IgD hinge region

<400> 134

Glu Ser Pro Lys Ala Gln Ala Ser Ser Val Pro Thr Ala Gln Pro Gln  
1 5 10 15

Ala Glu Gly Ser Leu Ala Lys Ala Thr Thr Ala Pro Ala Thr Thr Arg  
20 25 30

Asn Thr Gly Arg Gly Gly Glu Glu Lys Lys Lys Glu Lys Glu Lys Glu  
35 40 45

Glu Gln Glu Glu Arg Glu Thr Lys Thr Pro  
50 55

<210> 135

<211> 108



&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; MISC\_FEATURE

&lt;222&gt; (1)..(108)

&lt;223&gt; human IgD CH2 region

&lt;400&gt; 135

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Cys | Pro | Ser | His | Thr | Gln | Pro | Leu | Gly | Val | Tyr | Leu | Leu | Thr | Pro |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Val | Gln | Asp | Leu | Trp | Leu | Arg | Asp | Lys | Ala | Thr | Phe | Thr | Cys | Phe |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Val | Gly | Ser | Asp | Leu | Lys | Asp | Ala | His | Leu | Thr | Trp | Glu | Val | Ala |
|     |     | 35  |     |     |     | 40  |     |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gly | Lys | Val | Pro | Thr | Gly | Gly | Val | Glu | Glu | Gly | Leu | Leu | Glu | Arg | His |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Asn | Gly | Ser | Gln | Ser | Gln | His | Ser | Arg | Leu | Thr | Leu | Pro | Arg | Ser |
| 65  |     |     |     |     | 70  |     |     |     | 75  |     |     |     |     |     | 80  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Trp | Asn | Ala | Gly | Thr | Ser | Val | Thr | Cys | Thr | Leu | Asn | His | Pro | Ser |
|     |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Pro | Pro | Gln | Arg | Leu | Met | Ala | Leu | Arg | Glu | Pro |
|     |     |     | 100 |     |     |     |     | 105 |     |     |     |

&lt;210&gt; 136

&lt;211&gt; 117

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; MISC\_FEATURE

&lt;222&gt; (1)..(117)

&lt;223&gt; human IgD CH3 region

&lt;400&gt; 136

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Ala | Gln | Ala | Pro | Val | Lys | Leu | Ser | Leu | Asn | Leu | Leu | Ala | Ser | Ser |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asp | Pro | Pro | Glu | Ala | Ala | Ser | Trp | Leu | Leu | Cys | Glu | Val | Ser | Gly | Phe |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Pro | Pro | Asn | Ile | Leu | Leu | Met | Trp | Leu | Glu | Asp | Gln | Arg | Glu | Val |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asn | Thr | Ser | Gly | Phe | Ala | Pro | Ala | Arg | Pro | Pro | Pro | Gln | Pro | Arg | Ser |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thr | Thr | Phe | Trp | Ala | Trp | Ser | Val | Leu | Arg | Val | Pro | Ala | Pro | Pro | Ser |
| 65  |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     |     | 80  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Gln | Pro | Ala | Thr | Tyr | Thr | Cys | Val | Val | Ser | His | Glu | Asp | Ser | Arg |
|     |     |     |     | 85  |     |     |     | 90  |     |     |     |     |     | 95  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thr | Leu | Leu | Asn | Ala | Ser | Arg | Ser | Leu | Glu | Val | Ser | Tyr | Val | Thr | Asp |
|     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |



His Gly Pro Met Lys  
115

<210> 137

<211> 107

<212> PRT

<213> Homo sapiens

<220>

<221> MISC\_FEATURE

<222> (1)..(107)

<223> human IgE CH2 region

<400> 137

Val Cys Ser Arg Asp Phe Thr Pro Pro Thr Val Lys Ile Leu Gln Ser  
1 5 10 15

Ser Cys Asp Gly Gly Gly His Phe Pro Pro Thr Ile Gln Leu Leu Cys  
20 25 30

Leu Val Ser Gly Tyr Thr Pro Gly Thr Ile Asn Ile Thr Trp Leu Glu  
35 40 45

Asp Gly Gln Val Met Asp Val Asp Leu Ser Thr Ala Ser Thr Thr Gln  
50 55 60

Glu Gly Glu Leu Ala Ser Thr Gln Ser Glu Leu Thr Leu Ser Gln Lys  
65 70 75 80

His Trp Leu Ser Asp Arg Thr Tyr Thr Cys Gln Val Thr Tyr Gln Gly  
85 90 95

His Thr Phe Glu Asp Ser Thr Lys Lys Cys Ala  
100 105

<210> 138

<211> 108

<212> PRT

<213> Homo sapiens

<220>

<221> MISC\_FEATURE

<222> (1)..(108)

<223> human IgE CH3 region

<400> 138

Asp Ser Asn Pro Arg Gly Val Ser Ala Tyr Leu Ser Arg Pro Ser Pro  
1 5 10 15

Phe Asp Leu Phe Ile Arg Lys Ser Pro Thr Ile Thr Cys Leu Val Val  
20 25 30

Asp Leu Ala Pro Ser Lys Gly Thr Val Asn Leu Thr Trp Ser Arg Ala  
35 40 45

Ser Gly Lys Pro Val Asn His Ser Thr Arg Lys Glu Glu Lys Gln Arg  
50 55 60

Asn Gly Thr Leu Thr Val Thr Ser Thr Leu Pro Val Gly Thr Arg Asp  
65 70 75 80

Trp Ile Glu Gly Glu Thr Tyr Gln Cys Arg Val Thr His Pro His Leu



859095

Pro Arg Ala Leu Met Arg Ser Thr Thr Lys Thr Ser  
100105

<210> 139  
<211> 110  
<212> PRT  
<213> Homo sapiens  
  
<220>  
<221> MISC\_FEATURE  
<222> (1)..(110)  
<223> human IgE CH4 region  
  
<400> 139  
Gly Pro Arg Ala Ala Pro Glu Val Tyr Ala Phe Ala Thr Pro Glu Trp  
151015  
  
Pro Gly Ser Arg Asp Lys Arg Thr Leu Ala Cys Leu Ile Gln Asn Phe  
202530  
  
Met Pro Glu Asp Ile Ser Val Gln Trp Leu His Asn Glu Val Gln Leu  
354045  
  
Pro Asp Ala Arg His Ser Thr Thr Gln Pro Arg Lys Thr Lys Gly Ser  
505560  
  
Gly Phe Phe Val Phe Ser Arg Leu Glu Val Thr Arg Ala Glu Trp Glu  
65707580  
  
Gln Lys Asp Glu Phe Ile Cys Arg Ala Val His Glu Ala Ala Ser Pro  
859095  
  
Ser Gln Thr Val Gln Arg Ala Val Ser Val Asn Pro Gly Lys  
100105110

<210> 140  
<211> 15  
<212> PRT  
<213> Homo sapiens  
  
<220>  
<221> MISC\_FEATURE  
<222> (1)..(15)  
<223> human IgG1 hinge region  
  
<400> 140  
Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro  
1551015

<210> 141  
<211> 110  
<212> PRT  
<213> Homo sapiens  
  
<220>  
<221> MISC\_FEATURE  
<222> (1)..(110)  
<223> human IgG1 CH2 region



&lt;400&gt; 141

Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys  
 1 5 10 15

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val  
 20 25 30

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr  
 35 40 45

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu  
 50 55 60

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His  
 65 70 75 80

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys  
 85 90 95

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys  
 100 105 110

&lt;210&gt; 142

&lt;211&gt; 107

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; MISC\_FEATURE

&lt;222&gt; (1)..(107)

&lt;223&gt; human IgG1 CH3 region

&lt;400&gt; 142

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp  
 1 5 10 15

Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe  
 20 25 30

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu  
 35 40 45

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe  
 50 55 60

Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly  
 65 70 75 80

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr  
 85 90 95

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys  
 100 105

&lt;210&gt; 143

&lt;211&gt; 12

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; MISC\_FEATURE



<222> (1)..(12)

<223> human IgG2 hinge region

<400> 143

|     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Arg | Lys | Cys | Cys | Val | Glu | Cys | Pro | Pro | Cys | Pro |
| 1   |     |     | 5   |     |     |     |     | 10  |     |     |     |

<210> 144

<211> 109

<212> PRT

<213> Homo sapiens

<220>

<221> MISC\_FEATURE

<222> (1)..(109)

<223> human IgG2 CH2 region

<400> 144

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Pro | Pro | Val | Ala | Gly | Pro | Ser | Val | Phe | Leu | Phe | Pro | Pro | Lys | Pro |
| 1   |     |     | 5   |     |     |     |     | 10  |     |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | Asp | Thr | Leu | Met | Ile | Ser | Arg | Thr | Pro | Glu | Val | Thr | Cys | Val | Val |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Asp | Val | Ser | His | Glu | Asp | Pro | Glu | Val | Gln | Phe | Asn | Trp | Tyr | Val |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asp | Gly | Val | Glu | Val | His | Asn | Ala | Lys | Thr | Lys | Pro | Arg | Glu | Glu | Gln |
|     | 50  |     |     |     |     | 55  |     |     |     | 60  |     |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Phe | Asn | Ser | Thr | Phe | Arg | Val | Val | Ser | Val | Leu | Thr | Val | Val | His | Gln |
| 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     | 80  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asp | Trp | Leu | Asn | Gly | Lys | Glu | Tyr | Lys | Cys | Lys | Val | Ser | Asn | Lys | Gly |
|     |     |     | 85  |     |     |     |     |     | 90  |     |     |     |     | 95  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Pro | Ala | Pro | Ile | Glu | Lys | Thr | Ile | Ser | Lys | Thr | Lys |
|     |     |     | 100 |     |     |     |     | 105 |     |     |     |     |

<210> 145

<211> 107

<212> PRT

<213> Homo sapiens

<220>

<221> MISC\_FEATURE

<222> (1)..(107)

<223> human IgG2 CH3 region

<400> 145

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gly | Gln | Pro | Arg | Glu | Pro | Gln | Val | Tyr | Thr | Leu | Pro | Pro | Ser | Arg | Glu |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Met | Thr | Lys | Asn | Gln | Val | Ser | Leu | Thr | Cys | Leu | Val | Lys | Gly | Phe |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Tyr | Pro | Ser | Asp | Ile | Ala | Val | Glu | Trp | Glu | Ser | Asn | Gly | Gln | Pro | Glu |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asn | Asn | Tyr | Lys | Thr | Thr | Pro | Pro | Met | Leu | Asp | Ser | Asp | Gly | Ser | Phe |
|     | 50  |     |     |     |     |     | 55  |     |     |     | 60  |     |     |     |     |



Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly  
65 70 75 80

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr  
85 90 95

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys  
100 105

<210> 146

<211> 62

<212> PRT

<213> Homo sapiens

<220>

<221> MISC\_FEATURE

<222> (1)..(62)

<223> human IgG3 hinge region

<400> 146

Glu Leu Lys Thr Pro Leu Gly Asp Thr Thr His Thr Cys Pro Arg Cys

1 5 10 15

Pro Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro  
20 25 30

Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro Glu  
35 40 45

Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro  
50 55 60

<210> 147

<211> 110

<212> PRT

<213> Homo sapiens

<220>

<221> MISC\_FEATURE

<222> (1)..(110)

<223> human IgG3 CH2 region

<400> 147

Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys  
1 5 10 15

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val  
20 25 30

Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe Lys Trp Tyr  
35 40 45

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu  
50 55 60

Gln Tyr Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Leu His  
65 70 75 80

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys  
85 90 95



Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys  
100 105 110

<210> 148  
<211> 107  
<212> PRT  
<213> Homo sapiens

<220>  
<221> MISC\_FEATURE  
<222> (1)..(107)  
<223> human IgG3 CH3 region

<400> 148  
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu  
1 5 10 15

Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe  
20 25 30

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Ser Gly Gln Pro Glu  
35 40 45

Asn Asn Tyr Asn Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe  
50 55 60

Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly  
65 70 75 80

Asn Ile Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn Arg Phe  
85 90 95

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys  
100 105

<210> 149  
<211> 12  
<212> PRT  
<213> Homo sapiens

<220>  
<221> MISC\_FEATURE  
<222> (1)..(12)  
<223> human IgG4 hinge region

<400> 149  
Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro  
1 5 10

<210> 150  
<211> 110  
<212> PRT  
<213> Homo sapiens

<220>  
<221> MISC\_FEATURE  
<222> (1)..(110)  
<223> human IgG4 CH2 region



&lt;400&gt; 150

Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys  
 1 5 10 15

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val  
 20 25 30

Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr  
 35 40 45

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu  
 50 55 60

Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His  
 65 70 75 80

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys  
 85 90 95

Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys  
 100 105 110

&lt;210&gt; 151

&lt;211&gt; 107

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; MISC\_FEATURE

&lt;222&gt; (1)..(107)

&lt;223&gt; human IgG4 CH3 region

&lt;400&gt; 151

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu  
 1 5 10 15

Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe  
 20 25 30

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu  
 35 40 45

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe  
 50 55 60

Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly  
 65 70 75 80

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr  
 85 90 95

Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys  
 100 105

&lt;210&gt; 152

&lt;211&gt; 112

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;



&lt;221&gt; MISC\_FEATURE

&lt;222&gt; (1)..(112)

&lt;223&gt; human IgM CH2 region

&lt;400&gt; 152

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Ile | Ala | Glu | Leu | Pro | Pro | Lys | Val | Ser | Val | Phe | Val | Pro | Pro | Arg |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asp | Gly | Phe | Phe | Gly | Asn | Pro | Arg | Lys | Ser | Lys | Leu | Ile | Cys | Gln | Ala |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thr | Gly | Phe | Ser | Pro | Arg | Gln | Ile | Gln | Val | Ser | Trp | Leu | Arg | Glu | Gly |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | Gln | Val | Gly | Ser | Gly | Val | Thr | Thr | Asp | Gln | Val | Gln | Ala | Glu | Ala |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | Glu | Ser | Gly | Pro | Thr | Thr | Tyr | Lys | Val | Thr | Ser | Thr | Leu | Thr | Ile |
| 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | Glu | Ser | Asp | Trp | Leu | Gly | Gln | Ser | Met | Phe | Thr | Cys | Arg | Val | Asp |
|     |     |     | 85  |     |     |     |     |     | 90  |     |     |     |     | 95  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| His | Arg | Gly | Leu | Thr | Phe | Gln | Gln | Asn | Ala | Ser | Ser | Met | Cys | Val | Pro |
|     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |

&lt;210&gt; 153

&lt;211&gt; 106

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; MISC\_FEATURE

&lt;222&gt; (1)..(106)

&lt;223&gt; human IgM CH3 region

&lt;400&gt; 153

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asp | Gln | Asp | Thr | Ala | Ile | Arg | Val | Phe | Ala | Ile | Pro | Pro | Ser | Phe | Ala |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Ile | Phe | Leu | Thr | Lys | Ser | Thr | Lys | Leu | Thr | Cys | Leu | Val | Thr | Asp |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Thr | Thr | Tyr | Asp | Ser | Val | Thr | Ile | Ser | Trp | Thr | Arg | Gln | Asn | Gly |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Ala | Val | Lys | Thr | His | Thr | Asn | Ile | Ser | Glu | Ser | His | Pro | Asn | Ala |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thr | Phe | Ser | Ala | Val | Gly | Glu | Ala | Ser | Ile | Cys | Glu | Asp | Asp | Trp | Asn |
| 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Gly | Glu | Arg | Phe | Thr | Cys | Thr | Val | Thr | His | Thr | Asp | Leu | Pro | Ser |
|     |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |

|     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Leu | Lys | Gln | Thr | Ile | Ser | Arg | Pro | Lys |
|     |     |     | 100 |     |     |     |     | 105 |     |

&lt;210&gt; 154

&lt;211&gt; 131

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens



&lt;220&gt;

&lt;221&gt; MISC\_FEATURE

&lt;222&gt; (1)..(131)

&lt;223&gt; human IgM CH4 region

&lt;400&gt; 154

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gly | Val | Ala | Leu | His | Arg | Pro | Asp | Val | Tyr | Leu | Leu | Pro | Pro | Ala | Arg |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Gln | Leu | Asn | Leu | Arg | Glu | Ser | Ala | Thr | Ile | Thr | Cys | Leu | Val | Thr |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gly | Phe | Ser | Pro | Ala | Asp | Val | Phe | Val | Gln | Trp | Met | Gln | Arg | Gly | Gln |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Leu | Ser | Pro | Glu | Lys | Tyr | Val | Thr | Ser | Ala | Pro | Met | Pro | Glu | Pro |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Ala | Pro | Gly | Arg | Tyr | Phe | Ala | His | Ser | Ile | Leu | Thr | Val | Ser | Glu |
| 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Glu | Trp | Asn | Thr | Gly | Glu | Thr | Tyr | Thr | Cys | Val | Val | Ala | His | Glu |
|     |     |     | 85  |     |     |     |     |     | 90  |     |     |     |     | 95  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Leu | Pro | Asn | Arg | Val | Thr | Glu | Arg | Thr | Val | Asp | Lys | Ser | Thr | Gly |
|     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | Pro | Thr | Leu | Tyr | Asn | Val | Ser | Leu | Val | Met | Ser | Asp | Thr | Ala | Gly |
|     |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |

|     |     |     |
|-----|-----|-----|
| Thr | Cys | Tyr |
|     | 130 |     |

&lt;210&gt; 155

&lt;211&gt; 230

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Fc variant

&lt;220&gt;

&lt;221&gt; VARIANT

&lt;222&gt; (1)..(1)

&lt;223&gt; Xaa is Ala or absent

&lt;220&gt;

&lt;221&gt; VARIANT

&lt;222&gt; (16)..(16)

&lt;223&gt; Xaa is Pro or Glu

&lt;220&gt;

&lt;221&gt; VARIANT

&lt;222&gt; (17)..(17)

&lt;223&gt; Xaa is Phe, Val or Ala

&lt;220&gt;

&lt;221&gt; VARIANT

&lt;222&gt; (18)..(18)



<223> Xaa is Leu, Glu or Ala

<220>

<221> VARIANT

<222> (80)..(80)

<223> Xaa is Asn or Ala

<220>

<221> VARIANT

<222> (230)..(230)

<223> Xaa is Lys or absent

<400> 155

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Xaa | Glu | Ser | Lys | Tyr | Gly | Pro | Pro | Cys | Pro | Pro | Cys | Pro | Ala | Pro | Xaa |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Xaa | Xaa | Gly | Gly | Pro | Ser | Val | Phe | Leu | Phe | Pro | Pro | Lys | Pro | Lys | Asp |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thr | Leu | Met | Ile | Ser | Arg | Thr | Pro | Glu | Val | Thr | Cys | Val | Val | Val | Asp |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Ser | Gln | Glu | Asp | Pro | Glu | Val | Gln | Phe | Asn | Trp | Tyr | Val | Asp | Gly |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Glu | Val | His | Asn | Ala | Lys | Thr | Lys | Pro | Arg | Glu | Glu | Gln | Phe | Xaa |
| 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Thr | Tyr | Arg | Val | Val | Ser | Val | Leu | Thr | Val | Leu | His | Gln | Asp | Trp |
|     |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Asn | Gly | Lys | Glu | Tyr | Lys | Cys | Lys | Val | Ser | Asn | Lys | Gly | Leu | Pro |
|     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Ser | Ile | Glu | Lys | Thr | Ile | Ser | Lys | Ala | Lys | Gly | Gln | Pro | Arg | Glu |
|     |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Gln | Val | Tyr | Thr | Leu | Pro | Pro | Ser | Gln | Glu | Glu | Met | Thr | Lys | Asn |
|     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Val | Ser | Leu | Thr | Cys | Leu | Val | Lys | Gly | Phe | Tyr | Pro | Ser | Asp | Ile |
| 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Val | Glu | Trp | Glu | Ser | Asn | Gly | Gln | Pro | Glu | Asn | Asn | Tyr | Lys | Thr |
|     |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thr | Pro | Pro | Val | Leu | Asp | Ser | Asp | Gly | Ser | Phe | Phe | Leu | Tyr | Ser | Arg |
|     |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Thr | Val | Asp | Lys | Ser | Arg | Trp | Gln | Glu | Gly | Asn | Val | Phe | Ser | Cys |
|     |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Val | Met | His | Glu | Ala | Leu | His | Asn | His | Tyr | Thr | Gln | Lys | Ser | Leu |
|     | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |

|     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|
| Ser | Leu | Ser | Leu | Gly | Xaa |
| 225 |     |     |     |     | 230 |

<210> 156

<211> 209

<212> PRT

<213> Homo sapiens



&lt;400&gt; 156

Cys His Pro Arg Leu Ser Leu His Arg Pro Ala Leu Glu Asp Leu Leu  
1 5 10 15

Leu Gly Ser Glu Ala Asn Leu Thr Cys Thr Leu Thr Gly Leu Arg Asp  
20 25 30

Ala Ser Gly Val Thr Phe Thr Trp Thr Pro Ser Ser Gly Lys Ser Ala  
35 40 45

Val Gln Gly Pro Pro Glu Arg Asp Leu Cys Gly Cys Tyr Ser Val Ser  
50 55 60

Ser Val Leu Pro Gly Cys Ala Glu Pro Trp Asn His Gly Lys Thr Phe  
65 70 75 80

Thr Cys Thr Ala Ala Tyr Pro Glu Ser Lys Thr Pro Leu Thr Ala Thr  
85 90 95

Leu Ser Lys Ser Gly Asn Thr Phe Arg Pro Glu Val His Leu Leu Pro  
100 105 110

Pro Pro Ser Glu Glu Leu Ala Leu Asn Glu Leu Val Thr Leu Thr Cys  
115 120 125

Leu Ala Arg Gly Phe Ser Pro Lys Asp Val Leu Val Arg Trp Leu Gln  
130 135 140

Gly Ser Gln Glu Leu Pro Arg Glu Lys Tyr Leu Thr Trp Ala Ser Arg  
145 150 155 160

Gln Glu Pro Ser Gln Gly Thr Thr Thr Phe Ala Val Thr Ser Ile Leu  
165 170 175

Arg Val Ala Ala Glu Asp Trp Lys Lys Gly Asp Thr Phe Ser Cys Met  
180 185 190

Val Gly His Glu Ala Leu Pro Leu Ala Phe Thr Gln Lys Thr Ile Asp  
195 200 205

Arg

&lt;210&gt; 157

&lt;211&gt; 209

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 157

Cys His Pro Arg Leu Ser Leu His Arg Pro Ala Leu Glu Asp Leu Leu  
1 5 10 15

Leu Gly Ser Glu Ala Asn Leu Thr Cys Thr Leu Thr Gly Leu Arg Asp  
20 25 30

Ala Ser Gly Ala Thr Phe Thr Trp Thr Pro Ser Ser Gly Lys Ser Ala  
35 40 45

Val Gln Gly Pro Pro Glu Arg Asp Leu Cys Gly Cys Tyr Ser Val Ser  
50 55 60

Ser Val Leu Pro Gly Cys Ala Gln Pro Trp Asn His Gly Glu Thr Phe  
65 70 75 80

Met Gln Thr Ala Ala His Pro Glu Tyr Tyr Met Pro Tyr Met Ala Ser



Thr Cys Thr Ala Ala His Pro Glu Leu Lys Thr Pro Leu Thr Ala Asn  
85 90 95

Ile Thr Lys Ser Gly Asn Thr Phe Arg Pro Glu Val His Leu Leu Pro  
100 105 110

Pro Pro Ser Glu Glu Leu Ala Leu Asn Glu Leu Val Thr Leu Thr Cys  
115 120 125

Leu Ala Arg Gly Phe Ser Pro Lys Asp Val Leu Val Arg Trp Leu Gln  
130 135 140

Gly Ser Gln Glu Leu Pro Arg Glu Lys Tyr Leu Thr Trp Ala Ser Arg  
145 150 155 160

Gln Glu Pro Ser Gln Gly Thr Thr Thr Phe Ala Val Thr Ser Ile Leu  
165 170 175

Arg Val Ala Ala Glu Asp Trp Lys Lys Gly Asp Thr Phe Ser Cys Met  
180 185 190

Val Gly His Glu Ala Leu Pro Leu Ala Phe Thr Gln Lys Thr Ile Asp  
195 200 205

Arg

<210> 158

<211> 212

<212> PRT

<213> Homo sapiens

<400> 158

Thr Ala Ile Arg Val Phe Ala Ile Pro Pro Ser Phe Ala Ser Ile Phe  
1 5 10 15

Leu Thr Lys Ser Thr Lys Leu Thr Cys Leu Val Thr Asp Leu Thr Thr  
20 25 30

Tyr Asp Ser Val Thr Ile Ser Trp Thr Arg Gln Asn Gly Glu Ala Val  
35 40 45

Lys Thr His Thr Asn Ile Ser Glu Ser His Pro Asn Ala Thr Phe Ser  
50 55 60

Ala Val Gly Glu Ala Ser Ile Cys Glu Asp Asp Trp Asn Ser Gly Glu  
65 70 75 80

Arg Phe Thr Cys Thr Val Thr His Thr Asp Leu Pro Ser Pro Leu Lys  
85 90 95

Gln Thr Ile Ser Arg Pro Lys Gly Val Ala Leu His Arg Pro Asp Val  
100 105 110

Tyr Leu Leu Pro Pro Ala Arg Glu Gln Leu Asn Leu Arg Glu Ser Ala  
115 120 125

Thr Ile Thr Cys Leu Val Thr Gly Phe Ser Pro Ala Asp Val Phe Val  
130 135 140

Gln Trp Met Gln Arg Gly Gln Pro Leu Ser Pro Glu Lys Tyr Val Thr  
145 150 155 160

Ser Ala Pro Met Pro Glu Pro Gln Ala Pro Gly Arg Tyr Phe Ala His  
165 170 175



Ser Ile Leu Thr Val Ser Glu Glu Glu Trp Asn Thr Gly Glu Thr Tyr  
180 185 190

Thr Cys Val Val Ala His Glu Ala Leu Pro Asn Arg Val Thr Glu Arg  
195 200 205

Thr Val Asp Lys  
210

<210> 159

<211> 208

<212> PRT

<213> Homo sapiens

<400> 159

Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu  
1 5 10 15

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser  
20 25 30

His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Met Glu  
35 40 45

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr  
50 55 60

Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn  
65 70 75 80

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro  
85 90 95

Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln  
100 105 110

Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val  
115 120 125

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val  
130 135 140

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro  
145 150 155 160

Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr  
165 170 175

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val  
180 185 190

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu  
195 200 205

<210> 160

<211> 208

<212> PRT

<213> Homo sapiens

<400> 160

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu  
1 5 10 15



Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser  
20 25 30

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu  
35 40 45

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr  
50 55 60

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn  
65 70 75 80

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro  
85 90 95

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln  
100 105 110

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val  
115 120 125

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val  
130 135 140

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro  
145 150 155 160

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr  
165 170 175

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val  
180 185 190

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu  
195 200 205

<210> 161

<211> 208

<212> PRT

<213> Homo sapiens

<400> 161

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu  
1 5 10 15

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser  
20 25 30

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu  
35 40 45

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr  
50 55 60

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn  
65 70 75 80

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro  
85 90 95

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln  
100 105 110

Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val  
115 120 125



110 120 130  
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val  
130 135 140  
  
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro  
145 150 155 160  
  
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr  
165 170 175  
  
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val  
180 185 190  
  
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu  
195 200 205

<210> 162  
<211> 208  
<212> PRT  
<213> Homo sapiens

<220>  
<221> VARIANT  
<222> (80)..(80)  
<223> Xaa = any amino acid

<220>  
<221> VARIANT  
<222> (90)..(90)  
<223> Xaa = any amino acid

<220>  
<221> VARIANT  
<222> (103)..(103)  
<223> Xaa = any amino acid

<220>  
<221> VARIANT  
<222> (105)..(105)  
<223> Xaa = any amino acid

<220>  
<221> VARIANT  
<222> (181)..(181)  
<223> Xaa = any amino acid

<400> 162  
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu  
1 5 10 15  
  
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser  
20 25 30  
  
Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu  
35 40 45  
  
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr  
50 55 60



Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Xaa  
65 70 75 80

Gly Lys Glu Tyr Lys Cys Lys Val Ser Xaa Lys Gly Leu Pro Ser Ser  
85 90 95

Ile Glu Lys Thr Ile Ser Xaa Ala Xaa Gly Gln Pro Arg Glu Pro Gln  
100 105 110

Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val  
115 120 125

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val  
130 135 140

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro  
145 150 155 160

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr  
165 170 175

Val Asp Lys Ser Xaa Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val  
180 185 190

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu  
195 200 205

<210> 163

<211> 210

<212> PRT

<213> Homo sapiens

<400> 163

Arg Gly Val Ser Ala Tyr Leu Ser Arg Pro Ser Pro Phe Asp Leu Phe  
1 5 10 15

Ile Arg Lys Ser Pro Thr Ile Thr Cys Leu Val Val Asp Leu Ala Pro  
20 25 30

Ser Lys Gly Thr Val Gln Leu Thr Trp Ser Arg Ala Ser Gly Lys Pro  
35 40 45

Val Asn His Ser Thr Arg Lys Glu Glu Lys Gln Arg Asn Gly Thr Leu  
50 55 60

Thr Val Thr Ser Thr Leu Pro Val Gly Thr Arg Asp Trp Ile Glu Gly  
65 70 75 80

Glu Thr Tyr Gln Cys Arg Val Thr His Pro His Leu Pro Arg Ala Leu  
85 90 95

Met Arg Ser Thr Thr Lys Thr Ser Gly Pro Arg Ala Ala Pro Glu Val  
100 105 110

Tyr Ala Phe Ala Thr Pro Glu Trp Pro Gly Ser Arg Asp Lys Arg Thr  
115 120 125

Leu Ala Cys Leu Ile Gln Asn Phe Met Pro Glu Asp Ile Ser Val Gln  
130 135 140

Trp Leu His Asn Glu Val Gln Leu Pro Asp Ala Arg His Ser Thr Thr  
145 150 155 160

Gln Pro Arg Lys Thr Lys Gly Ser Gly Phe Phe Val Phe Ser Arg Leu



- - 165 - - - 170 175

Glu Val Thr Arg Ala Glu Trp Glu Gln Lys Asp Glu Phe Ile Cys Arg  
180 185 190

Ala Val His Glu Ala Ala Ser Pro Ser Gln Thr Val Gln Arg Ala Val  
195 200 205

Ser Val  
210

<210> 164  
<211> 15  
<212> DNA  
<213> Homo sapiens

<400> 164  
aaacaaaaca aaaca 15

<210> 165  
<211> 10  
<212> DNA  
<213> Homo sapiens

<400> 165  
aaacaaaaca 10

<210> 166  
<211> 10  
<212> DNA  
<213> Homo sapiens

<400> 166  
caaaacaaaa 10

<210> 167  
<211> 15  
<212> DNA  
<213> Homo sapiens

<400> 167  
caaaacaaaa caaaa 15

<210> 168  
<211> 10  
<212> DNA  
<213> Homo sapiens

<400> 168  
aaacaaaaca 10

<210> 169  
<211> 10  
<212> DNA  
<213> Homo sapiens

<400> 169



acaaaacaaa 10

<210> 170

<211> 500

<212> PRT

<213> Homo sapiens

<400> 170

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu

20

25

30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp  
50 55 60

Tyr Ser Pro Arg Gln Met Ala Val Arg Glu Lys Val Phe Asp Val Ile  
65 70 75 80

Ile Arg Cys Phe Lys Arg His Gly Ala Glu Val Ile Asp Thr Pro Val  
85 90 95

Phe Glu Leu Lys Glu Thr Leu Met Gly Lys Tyr Gly Glu Asp Ser Lys  
100 105 110

Leu Ile Tyr Asp Leu Lys Asp Gln Gly Gly Glu Leu Leu Ser Leu Arg  
115 120 125

Tyr Asp Leu Thr Val Pro Phe Ala Arg Tyr Leu Ala Met Asn Lys Leu  
130 135 140

Thr Asn Ile Lys Arg Tyr His Ile Ala Lys Val Tyr Arg Arg Asp Asn  
145 150 155 160

Pro Ala Met Thr Arg Gly Arg Tyr Arg Glu Phe Tyr Gln Cys Asp Phe  
165 170 175

Asp Ile Ala Gly Asn Phe Asp Pro Met Ile Pro Asp Ala Glu Cys Leu  
180 185 190

Lys Ile Met Cys Glu Ile Leu Ser Ser Leu Gln Ile Gly Asp Phe Leu  
195 200 205

Val Lys Val Asn Asp Arg Arg Ile Leu Asp Gly Met Phe Ala Ile Cys  
210 215 220

Gly Val Ser Asp Ser Lys Phe Arg Thr Ile Cys Ser Ser Val Asp Lys  
225 230 235 240

Leu Asp Lys Val Ser Trp Glu Glu Val Lys Asn Glu Met Val Gly Glu  
245 250 255

Lys Gly Leu Ala Pro Glu Val Ala Asp Arg Ile Gly Asp Tyr Val Gln  
260 265 270

Gln His Gly Gly Val Ser Leu Val Glu Gln Leu Leu Gln Asp Pro Lys  
275 280 285

Leu Ser Gln Asn Lys Gln Ala Leu Glu Gly Leu Gly Asp Leu Lys Leu



290                      295                      300  
 Leu Phe Glu Tyr Leu Thr Leu Phe Gly Ile Asp Asp Lys Ile Ser Phe  
 305                      310                      315                      320  
 Asp Leu Ser Leu Ala Arg Gly Leu Asp Tyr Tyr Thr Gly Val Ile Tyr  
                     325                      330                      335  
 Glu Ala Val Leu Leu Gln Thr Pro Ala Gln Ala Gly Glu Glu Pro Leu  
                     340                      345                      350  
 Gly Val Gly Ser Val Ala Ala Gly Gly Arg Tyr Asp Gly Leu Val Gly  
                     355                      360                      365  
 Met Phe Asp Pro Lys Gly Arg Lys Val Pro Cys Val Gly Leu Ser Ile  
                     370                      375                      380  
 Gly Val Glu Arg Ile Phe Ser Ile Val Glu Gln Arg Leu Glu Ala Leu  
 385                      390                      395                      400  
 Glu Glu Lys Ile Arg Thr Thr Glu Thr Gln Val Leu Val Ala Ser Ala  
                     405                      410                      415  
 Gln Lys Lys Leu Leu Glu Glu Arg Leu Lys Leu Val Ser Glu Leu Trp  
                     420                      425                      430  
 Asp Ala Gly Ile Lys Ala Glu Leu Leu Tyr Lys Lys Asn Pro Lys Leu  
                     435                      440                      445  
 Leu Asn Gln Leu Gln Tyr Cys Glu Glu Ala Gly Ile Pro Leu Val Ala  
                     450                      455                      460  
 Ile Ile Gly Glu Gln Glu Leu Lys Asp Gly Val Ile Lys Leu Arg Ser  
 465                      470                      475                      480  
 Val Thr Ser Arg Glu Glu Val Asp Val Arg Arg Glu Asp Leu Val Glu  
                     485                      490                      495  
 Glu Ile Lys Arg  
                     500  
 <210> 171  
 <211> 501  
 <212> PRT  
 <213> Homo sapiens  
 <400> 171  
 Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
 1                      5                      10                      15  
 Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
                     20                      25                      30  
 Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
                     35                      40                      45  
 Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp  
                     50                      55                      60  
 Tyr Ser Pro Arg Gln Met Ala Val Arg Glu Lys Val Phe Asp Val Ile  
 65                      70                      75                      80  
 Ile Arg Cys Phe Lys Arg His Gly Ala Glu Val Ile Asp Thr Pro Val  
                     85                      90                      95



Phe Glu Leu Lys Glu Thr Leu Met Gly Lys Tyr Gly Glu Asp Ser Lys  
100 105 110

Leu Ile Tyr Asp Leu Lys Asp Gln Gly Gly Glu Leu Leu Ser Leu Arg  
115 120 125

Tyr Asp Leu Thr Val Pro Phe Ala Arg Tyr Leu Ala Met Asn Lys Leu  
130 135 140

Thr Asn Ile Lys Arg Tyr His Ile Ala Lys Val Tyr Arg Arg Asp Asn  
145 150 155 160

Pro Ala Met Thr Arg Gly Arg Tyr Arg Glu Phe Tyr Gln Cys Asp Phe  
165 170 175

Asp Ile Ala Gly Asn Phe Asp Pro Met Ile Pro Asp Ala Glu Cys Leu  
180 185 190

Lys Ile Met Cys Glu Ile Leu Ser Ser Leu Gln Ile Gly Asp Phe Leu  
195 200 205

Val Lys Val Asn Asp Arg Arg Ile Leu Asp Gly Met Phe Ala Ile Cys  
210 215 220

Gly Val Ser Asp Ser Lys Phe Arg Thr Ile Cys Ser Ser Val Asp Lys  
225 230 235 240

Leu Asp Lys Val Ser Trp Glu Glu Val Lys Asn Glu Met Val Gly Glu  
245 250 255

Lys Gly Leu Ala Pro Glu Val Ala Asp Arg Ile Gly Asp Tyr Val Gln  
260 265 270

Gln His Gly Gly Val Ser Leu Val Glu Gln Leu Leu Gln Asp Pro Lys  
275 280 285

Leu Ser Gln Asn Lys Gln Ala Leu Glu Gly Leu Gly Asp Leu Lys Leu  
290 295 300

Leu Phe Glu Tyr Leu Thr Leu Phe Gly Ile Asp Asp Lys Ile Ser Phe  
305 310 315 320

Asp Leu Ser Leu Ala Arg Gly Leu Asp Tyr Tyr Thr Gly Val Ile Tyr  
325 330 335

Glu Ala Val Leu Leu Gln Thr Pro Ala Gln Ala Gly Glu Glu Pro Leu  
340 345 350

Gly Val Gly Ser Val Ala Ala Gly Gly Arg Tyr Asp Gly Leu Val Gly  
355 360 365

Met Phe Asp Pro Lys Gly Arg Lys Val Pro Cys Val Gly Leu Ser Ile  
370 375 380

Gly Val Glu Arg Ile Phe Ser Ile Val Glu Gln Arg Leu Glu Ala Leu  
385 390 395 400

Glu Glu Lys Ile Arg Thr Thr Glu Thr Gln Val Leu Val Ala Ser Ala  
405 410 415

Gln Lys Lys Leu Leu Glu Glu Arg Leu Lys Leu Val Ser Glu Leu Trp  
420 425 430

Asp Ala Gly Ile Lys Ala Glu Leu Leu Tyr Lys Lys Asn Pro Lys Leu  
435 440 445



Leu Asn Gln Leu Gln Tyr Cys Glu Glu Ala Gly Ile Pro Leu Val Ala  
 450 455 460

Ile Ile Gly Glu Gln Glu Leu Lys Asp Gly Val Ile Lys Leu Arg Ser  
 465 470 475 480

Val Thr Ser Arg Glu Glu Val Asp Val Arg Arg Glu Asp Leu Val Glu  
 485 490 495

Glu Ile Lys Arg Arg  
 500

<210> 172

<211> 502

<212> PRT

<213> Homo sapiens

<400> 172

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
 1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
 20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
 35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp  
 50 55 60

Tyr Ser Pro Arg Gln Met Ala Val Arg Glu Lys Val Phe Asp Val Ile  
 65 70 75 80

Ile Arg Cys Phe Lys Arg His Gly Ala Glu Val Ile Asp Thr Pro Val  
 85 90 95

Phe Glu Leu Lys Glu Thr Leu Met Gly Lys Tyr Gly Glu Asp Ser Lys  
 100 105 110

Leu Ile Tyr Asp Leu Lys Asp Gln Gly Gly Glu Leu Leu Ser Leu Arg  
 115 120 125

Tyr Asp Leu Thr Val Pro Phe Ala Arg Tyr Leu Ala Met Asn Lys Leu  
 130 135 140

Thr Asn Ile Lys Arg Tyr His Ile Ala Lys Val Tyr Arg Arg Asp Asn  
 145 150 155 160

Pro Ala Met Thr Arg Gly Arg Tyr Arg Glu Phe Tyr Gln Cys Asp Phe  
 165 170 175

Asp Ile Ala Gly Asn Phe Asp Pro Met Ile Pro Asp Ala Glu Cys Leu  
 180 185 190

Lys Ile Met Cys Glu Ile Leu Ser Ser Leu Gln Ile Gly Asp Phe Leu  
 195 200 205

Val Lys Val Asn Asp Arg Arg Ile Leu Asp Gly Met Phe Ala Ile Cys  
 210 215 220

Gly Val Ser Asp Ser Lys Phe Arg Thr Ile Cys Ser Ser Val Asp Lys  
 225 230 235 240



Leu Asp Lys Val Ser Trp Glu Glu Val Lys Asn Glu Met Val Gly Glu  
245 250 255

Lys Gly Leu Ala Pro Glu Val Ala Asp Arg Ile Gly Asp Tyr Val Gln  
260 265 270

Gln His Gly Gly Val Ser Leu Val Glu Gln Leu Leu Gln Asp Pro Lys  
275 280 285

Leu Ser Gln Asn Lys Gln Ala Leu Glu Gly Leu Gly Asp Leu Lys Leu  
290 295 300

Leu Phe Glu Tyr Leu Thr Leu Phe Gly Ile Asp Asp Lys Ile Ser Phe  
305 310 315 320

Asp Leu Ser Leu Ala Arg Gly Leu Asp Tyr Tyr Thr Gly Val Ile Tyr  
325 330 335

Glu Ala Val Leu Leu Gln Thr Pro Ala Gln Ala Gly Glu Glu Pro Leu  
340 345 350

Gly Val Gly Ser Val Ala Ala Gly Gly Arg Tyr Asp Gly Leu Val Gly  
355 360 365

Met Phe Asp Pro Lys Gly Arg Lys Val Pro Cys Val Gly Leu Ser Ile  
370 375 380

Gly Val Glu Arg Ile Phe Ser Ile Val Glu Gln Arg Leu Glu Ala Leu  
385 390 395 400

Glu Glu Lys Ile Arg Thr Thr Glu Thr Gln Val Leu Val Ala Ser Ala  
405 410 415

Gln Lys Lys Leu Leu Glu Glu Arg Leu Lys Leu Val Ser Glu Leu Trp  
420 425 430

Asp Ala Gly Ile Lys Ala Glu Leu Leu Tyr Lys Lys Asn Pro Lys Leu  
435 440 445

Leu Asn Gln Leu Gln Tyr Cys Glu Glu Ala Gly Ile Pro Leu Val Ala

**450**

Ile Ile Gly Glu Gln Glu Leu Lys Asp Gly Val Ile Lys Leu Arg Ser  
465 470 475 480

Val Thr Ser Arg Glu Glu Val Asp Val Arg Arg Glu Asp Leu Val Glu  
485 490 495

Glu Ile Lys Arg Arg Thr  
500

<210> 173

**<211> 503**

&lt;212&gt; PRT

<213> Homo sapiens

<400> 173

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

[illegible]



Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
 35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp  
 50 55 60

Tyr Ser Pro Arg Gln Met Ala Val Arg Glu Lys Val Phe Asp Val Ile  
 65 70 75 80

Ile Arg Cys Phe Lys Arg His Gly Ala Glu Val Ile Asp Thr Pro Val  
 85 90 95

Phe Glu Leu Lys Glu Thr Leu Met Gly Lys Tyr Gly Glu Asp Ser Lys  
 100 105 110

Leu Ile Tyr Asp Leu Lys Asp Gln Gly Gly Glu Leu Leu Ser Leu Arg  
 115 120 125

Tyr Asp Leu Thr Val Pro Phe Ala Arg Tyr Leu Ala Met Asn Lys Leu  
 130 135 140

Thr Asn Ile Lys Arg Tyr His Ile Ala Lys Val Tyr Arg Arg Asp Asn  
 145 150 155 160

Pro Ala Met Thr Arg Gly Arg Tyr Arg Glu Phe Tyr Gln Cys Asp Phe  
 165 170 175

Asp Ile Ala Gly Asn Phe Asp Pro Met Ile Pro Asp Ala Glu Cys Leu  
 180 185 190

Lys Ile Met Cys Glu Ile Leu Ser Ser Leu Gln Ile Gly Asp Phe Leu  
 195 200 205

Val Lys Val Asn Asp Arg Arg Ile Leu Asp Gly Met Phe Ala Ile Cys  
 210 215 220

Gly Val Ser Asp Ser Lys Phe Arg Thr Ile Cys Ser Ser Val Asp Lys  
 225 230 235 240

Leu Asp Lys Val Ser Trp Glu Glu Val Lys Asn Glu Met Val Gly Glu  
 245 250 255

Lys Gly Leu Ala Pro Glu Val Ala Asp Arg Ile Gly Asp Tyr Val Gln  
 260 265 270

Gln His Gly Gly Val Ser Leu Val Glu Gln Leu Leu Gln Asp Pro Lys  
 275 280 285

Leu Ser Gln Asn Lys Gln Ala Leu Glu Gly Leu Gly Asp Leu Lys Leu  
 290 295 300

Leu Phe Glu Tyr Leu Thr Leu Phe Gly Ile Asp Asp Lys Ile Ser Phe  
 305 310 315 320

Asp Leu Ser Leu Ala Arg Gly Leu Asp Tyr Tyr Thr Gly Val Ile Tyr  
 325 330 335

Glu Ala Val Leu Leu Gln Thr Pro Ala Gln Ala Gly Glu Glu Pro Leu  
 340 345 350

Gly Val Gly Ser Val Ala Ala Gly Gly Arg Tyr Asp Gly Leu Val Gly  
 355 360 365

Met Phe Asp Pro Lys Glv Arg Lys Val Pro Cys Val Glv Leu Ser Ile



370 375 380  
 Gly Val Glu Arg Ile Phe Ser Ile Val Glu Gln Arg Leu Glu Ala Leu  
 385 390 395 400  
 Glu Glu Lys Ile Arg Thr Thr Glu Thr Gln Val Leu Val Ala Ser Ala  
 405 410 415  
 Gln Lys Lys Leu Leu Glu Glu Arg Leu Lys Leu Val Ser Glu Leu Trp  
 420 425 430  
 Asp Ala Gly Ile Lys Ala Glu Leu Leu Tyr Lys Lys Asn Pro Lys Leu  
 435 440 445  
 Leu Asn Gln Leu Gln Tyr Cys Glu Glu Ala Gly Ile Pro Leu Val Ala  
 450 455 460  
 Ile Ile Gly Glu Gln Glu Leu Lys Asp Gly Val Ile Lys Leu Arg Ser  
 465 470 475 480  
 Val Thr Ser Arg Glu Glu Val Asp Val Arg Arg Glu Asp Leu Val Glu  
 485 490 495  
 Glu Ile Lys Arg Arg Thr Gly  
 500  
 <210> 174  
 <211> 504  
 <212> PRT  
 <213> Homo sapiens  
 <400> 174  
 Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
 1 5 10 15  
 Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
 20 25 30  
 Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
 35 40 45  
 Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp  
 50 55 60  
 Tyr Ser Pro Arg Gln Met Ala Val Arg Glu Lys Val Phe Asp Val Ile  
 65 70 75 80  
 Ile Arg Cys Phe Lys Arg His Gly Ala Glu Val Ile Asp Thr Pro Val  
 85 90 95  
 Phe Glu Leu Lys Glu Thr Leu Met Gly Lys Tyr Gly Glu Asp Ser Lys  
 100 105 110  
 Leu Ile Tyr Asp Leu Lys Asp Gln Gly Gly Glu Leu Leu Ser Leu Arg  
 115 120 125  
 Tyr Asp Leu Thr Val Pro Phe Ala Arg Tyr Leu Ala Met Asn Lys Leu  
 130 135 140  
 Thr Asn Ile Lys Arg Tyr His Ile Ala Lys Val Tyr Arg Arg Asp Asn  
 145 150 155 160  
 Pro Ala Met Thr Arg Gly Arg Tyr Arg Glu Phe Tyr Gln Cys Asp Phe  
 165 170 175



Asp Ile Ala Gly Asn Phe Asp Pro Met Ile Pro Asp Ala Glu Cys Leu  
180 185 190

Lys Ile Met Cys Glu Ile Leu Ser Ser Leu Gln Ile Gly Asp Phe Leu  
195 200 205

Val Lys Val Asn Asp Arg Arg Ile Leu Asp Gly Met Phe Ala Ile Cys  
210 215 220

Gly Val Ser Asp Ser Lys Phe Arg Thr Ile Cys Ser Ser Val Asp Lys  
225 230 235 240

Leu Asp Lys Val Ser Trp Glu Glu Val Lys Asn Glu Met Val Gly Glu  
245 250 255

Lys Gly Leu Ala Pro Glu Val Ala Asp Arg Ile Gly Asp Tyr Val Gln  
260 265 270

Gln His Gly Gly Val Ser Leu Val Glu Gln Leu Leu Gln Asp Pro Lys  
275 280 285

Leu Ser Gln Asn Lys Gln Ala Leu Glu Gly Leu Gly Asp Leu Lys Leu  
290 295 300

Leu Phe Glu Tyr Leu Thr Leu Phe Gly Ile Asp Asp Lys Ile Ser Phe  
305 310 315 320

Asp Leu Ser Leu Ala Arg Gly Leu Asp Tyr Tyr Thr Gly Val Ile Tyr  
325 330 335

Glu Ala Val Leu Leu Gln Thr Pro Ala Gln Ala Gly Glu Glu Pro Leu  
340 345 350

Gly Val Gly Ser Val Ala Ala Gly Gly Arg Tyr Asp Gly Leu Val Gly  
355 360 365

Met Phe Asp Pro Lys Gly Arg Lys Val Pro Cys Val Gly Leu Ser Ile  
370 375 380

Gly Val Glu Arg Ile Phe Ser Ile Val Glu Gln Arg Leu Glu Ala Leu  
385 390 395 400

Glu Glu Lys Ile Arg Thr Thr Glu Thr Gln Val Leu Val Ala Ser Ala  
405 410 415

Gln Lys Lys Leu Leu Glu Glu Arg Leu Lys Leu Val Ser Glu Leu Trp  
420 425 430

Asp Ala Gly Ile Lys Ala Glu Leu Leu Tyr Lys Lys Asn Pro Lys Leu  
435 440 445

Leu Asn Gln Leu Gln Tyr Cys Glu Glu Ala Gly Ile Pro Leu Val Ala  
450 455 460

Ile Ile Gly Glu Gln Glu Leu Lys Asp Gly Val Ile Lys Leu Arg Ser  
465 470 475 480

Val Thr Ser Arg Glu Glu Val Asp Val Arg Arg Glu Asp Leu Val Glu  
485 490 495

Glu Ile Lys Arg Arg Thr Gly Gln  
500

<210> 175

<211> 505



&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 175

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Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu
1           5           10           15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu
                20           25           30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp
          35           40           45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp
50           55           60

Tyr Ser Pro Arg Gln Met Ala Val Arg Glu Lys Val Phe Asp Val Ile
65           70           75           80

Ile Arg Cys Phe Lys Arg His Gly Ala Glu Val Ile Asp Thr Pro Val
                85           90           95

Phe Glu Leu Lys Glu Thr Leu Met Gly Lys Tyr Gly Glu Asp Ser Lys
          100           105           110

Leu Ile Tyr Asp Leu Lys Asp Gln Gly Gly Glu Leu Leu Ser Leu Arg
          115           120           125

Tyr Asp Leu Thr Val Pro Phe Ala Arg Tyr Leu Ala Met Asn Lys Leu
130           135           140

Thr Asn Ile Lys Arg Tyr His Ile Ala Lys Val Tyr Arg Arg Asp Asn
145           150           155           160

Pro Ala Met Thr Arg Gly Arg Tyr Arg Glu Phe Tyr Gln Cys Asp Phe
          165           170           175

Asp Ile Ala Gly Asn Phe Asp Pro Met Ile Pro Asp Ala Glu Cys Leu
          180           185           190

Lys Ile Met Cys Glu Ile Leu Ser Ser Leu Gln Ile Gly Asp Phe Leu
          195           200           205

Val Lys Val Asn Asp Arg Arg Ile Leu Asp Gly Met Phe Ala Ile Cys
210           215           220

Gly Val Ser Asp Ser Lys Phe Arg Thr Ile Cys Ser Ser Val Asp Lys
225           230           235           240

Leu Asp Lys Val Ser Trp Glu Glu Val Lys Asn Glu Met Val Gly Glu
          245           250           255

Lys Gly Leu Ala Pro Glu Val Ala Asp Arg Ile Gly Asp Tyr Val Gln
          260           265           270

Gln His Gly Gly Val Ser Leu Val Glu Gln Leu Leu Gln Asp Pro Lys
          275           280           285

Leu Ser Gln Asn Lys Gln Ala Leu Glu Gly Leu Gly Asp Leu Lys Leu
290           295           300

Leu Phe Glu Tyr Leu Thr Leu Phe Gly Ile Asp Asp Lys Ile Ser Phe
305           310           315           320

Asp Leu Ser Leu Ala Arg Gly Leu Asp Tyr Tyr Thr Gly Val Ile Tyr
          325           330           335

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Glu Ala Val Leu Leu Gln Thr Pro Ala Gln Ala Gly Glu Glu Pro Leu  
340 345 350

Gly Val Gly Ser Val Ala Ala Gly Gly Arg Tyr Asp Gly Leu Val Gly  
355 360 365

Met Phe Asp Pro Lys Gly Arg Lys Val Pro Cys Val Gly Leu Ser Ile  
370 375 380

Gly Val Glu Arg Ile Phe Ser Ile Val Glu Gln Arg Leu Glu Ala Leu  
385 390 395 400

Glu Glu Lys Ile Arg Thr Thr Glu Thr Gln Val Leu Val Ala Ser Ala  
405 410 415

Gln Lys Lys Leu Leu Glu Glu Arg Leu Lys Leu Val Ser Glu Leu Trp  
420 425 430

Asp Ala Gly Ile Lys Ala Glu Leu Leu Tyr Lys Lys Asn Pro Lys Leu  
435 440 445

Leu Asn Gln Leu Gln Tyr Cys Glu Glu Ala Gly Ile Pro Leu Val Ala  
450 455 460

Ile Ile Gly Glu Gln Glu Leu Lys Asp Gly Val Ile Lys Leu Arg Ser  
465 470 475 480

Val Thr Ser Arg Glu Glu Val Asp Val Arg Arg Glu Asp Leu Val Glu  
485 490 495

Glu Ile Lys Arg Arg Thr Gly Gln Pro  
500 505

<210> 176

<211> 507

<212> PRT

<213> Homo sapiens

<400> 176

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp  
50 55 60

Tyr Ser Pro Arg Gln Met Ala Val Arg Glu Lys Val Phe Asp Val Ile  
65 70 75 80

Ile Arg Cys Phe Lys Arg His Gly Ala Glu Val Ile Asp Thr Pro Val  
85 90 95

Phe Glu Leu Lys Glu Thr Leu Met Gly Lys Tyr Gly Glu Asp Ser Lys  
100 105 110

Leu Ile Tyr Asp Leu Lys Asp Gln Gly Gly Glu Leu Leu Ser Leu Arg  
115 120 125



Tyr Asp Leu Thr Val Pro Phe Ala Arg Tyr Leu Ala Met Asn Lys Leu  
 130 135 140  
 Thr Asn Ile Lys Arg Tyr His Ile Ala Lys Val Tyr Arg Arg Asp Asn  
 145 150 155 160  
 Pro Ala Met Thr Arg Gly Arg Tyr Arg Glu Phe Tyr Gln Cys Asp Phe  
 165 170 175  
 Asp Ile Ala Gly Asn Phe Asp Pro Met Ile Pro Asp Ala Glu Cys Leu  
 180 185 190  
 Lys Ile Met Cys Glu Ile Leu Ser Ser Leu Gln Ile Gly Asp Phe Leu  
 195 200 205  
 Val Lys Val Asn Asp Arg Arg Ile Leu Asp Gly Met Phe Ala Ile Cys  
 210 215 220  
 Gly Val Ser Asp Ser Lys Phe Arg Thr Ile Cys Ser Ser Val Asp Lys  
 225 230 235 240  
 Leu Asp Lys Val Ser Trp Glu Glu Val Lys Asn Glu Met Val Gly Glu  
 245 250 255  
 Lys Gly Leu Ala Pro Glu Val Ala Asp Arg Ile Gly Asp Tyr Val Gln  
 260 265 270  
 Gln His Gly Gly Val Ser Leu Val Glu Gln Leu Leu Gln Asp Pro Lys  
 275 280 285  
 Leu Ser Gln Asn Lys Gln Ala Leu Glu Gly Leu Gly Asp Leu Lys Leu  
 290 295 300  
 Leu Phe Glu Tyr Leu Thr Leu Phe Gly Ile Asp Asp Lys Ile Ser Phe  
 305 310 315 320  
 Asp Leu Ser Leu Ala Arg Gly Leu Asp Tyr Tyr Thr Gly Val Ile Tyr  
 325 330 335  
 Glu Ala Val Leu Leu Gln Thr Pro Ala Gln Ala Gly Glu Glu Pro Leu  
 340 345 350  
 Gly Val Gly Ser Val Ala Ala Gly Gly Arg Tyr Asp Gly Leu Val Gly  
 355 360 365  
 Met Phe Asp Pro Lys Gly Arg Lys Val Pro Cys Val Gly Leu Ser Ile  
 370 375 380  
 Gly Val Glu Arg Ile Phe Ser Ile Val Glu Gln Arg Leu Glu Ala Leu  
 385 390 395 400  
 Glu Glu Lys Ile Arg Thr Thr Glu Thr Gln Val Leu Val Ala Ser Ala  
 405 410 415  
 Gln Lys Lys Leu Leu Glu Glu Arg Leu Lys Leu Val Ser Glu Leu Trp  
 420 425 430  
 Asp Ala Gly Ile Lys Ala Glu Leu Leu Tyr Lys Lys Asn Pro Lys Leu  
 435 440 445  
 Leu Asn Gln Leu Gln Tyr Cys Glu Glu Ala Gly Ile Pro Leu Val Ala  
 450 455 460  
 Ile Ile Gly Glu Gln Glu Leu Lys Asp Gly Val Ile Lys Leu Arg Ser



465                      470                      475                      480  
 Val Thr Ser Arg Glu Glu Val Asp Val Arg Arg Glu Asp Leu Val Glu  
                                  485                                   490                                   495  
  
 Glu Ile Lys Arg Arg Thr Gly Gln Pro Leu Cys  
                                  500                                   505  
  
 <210> 177  
 <211> 508  
 <212> PRT  
 <213> Homo sapiens  
  
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 Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
  
                                  20                                   25                                   30  
  
 Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
                                  35                                   40                                   45  
  
 Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp  
                                  50                                   55                                   60  
  
 Tyr Ser Pro Arg Gln Met Ala Val Arg Glu Lys Val Phe Asp Val Ile  
 65                                   70                                   75                                   80  
  
 Ile Arg Cys Phe Lys Arg His Gly Ala Glu Val Ile Asp Thr Pro Val  
                                  85                                   90                                   95  
  
 Phe Glu Leu Lys Glu Thr Leu Met Gly Lys Tyr Gly Glu Asp Ser Lys  
                                  100                                   105                                   110  
  
 Leu Ile Tyr Asp Leu Lys Asp Gln Gly Gly Glu Leu Leu Ser Leu Arg  
                                  115                                   120                                   125  
  
 Tyr Asp Leu Thr Val Pro Phe Ala Arg Tyr Leu Ala Met Asn Lys Leu  
                                  130                                   135                                   140  
  
 Thr Asn Ile Lys Arg Tyr His Ile Ala Lys Val Tyr Arg Arg Asp Asn  
 145                                   150                                   155                                   160  
  
 Pro Ala Met Thr Arg Gly Arg Tyr Arg Glu Phe Tyr Gln Cys Asp Phe  
                                  165                                   170                                   175  
  
 Asp Ile Ala Gly Asn Phe Asp Pro Met Ile Pro Asp Ala Glu Cys Leu  
                                  180                                   185                                   190  
  
 Lys Ile Met Cys Glu Ile Leu Ser Ser Leu Gln Ile Gly Asp Phe Leu  
                                  195                                   200                                   205  
  
 Val Lys Val Asn Asp Arg Arg Ile Leu Asp Gly Met Phe Ala Ile Cys  
                                  210                                   215                                   220  
  
 Gly Val Ser Asp Ser Lys Phe Arg Thr Ile Cys Ser Ser Val Asp Lys  
 225                                   230                                   235                                   240  
  
 Leu Asp Lys Val Ser Trp Glu Glu Val Lys Asn Glu Met Val Gly Glu  
                                  245                                   250                                   255  
  
 Lys Gly Leu Ala Pro Glu Val Ala Asp Arg Ile Gly Asp Tyr Val Gln



|                                                                 |     |     |
|-----------------------------------------------------------------|-----|-----|
| 260                                                             | 265 | 270 |
| Gln His Gly Gly Val Ser Leu Val Glu Gln Leu Leu Gln Asp Pro Lys |     |     |
| 275                                                             | 280 | 285 |
| Leu Ser Gln Asn Lys Gln Ala Leu Glu Gly Leu Gly Asp Leu Lys Leu |     |     |
| 290                                                             | 295 | 300 |
| Leu Phe Glu Tyr Leu Thr Leu Phe Gly Ile Asp Asp Lys Ile Ser Phe |     |     |
| 305                                                             | 310 | 315 |
| Asp Leu Ser Leu Ala Arg Gly Leu Asp Tyr Tyr Thr Gly Val Ile Tyr |     |     |
| 325                                                             | 330 | 335 |
| Glu Ala Val Leu Leu Gln Thr Pro Ala Gln Ala Gly Glu Glu Pro Leu |     |     |
| 340                                                             | 345 | 350 |
| Gly Val Gly Ser Val Ala Ala Gly Gly Arg Tyr Asp Gly Leu Val Gly |     |     |
| 355                                                             | 360 | 365 |
| Met Phe Asp Pro Lys Gly Arg Lys Val Pro Cys Val Gly Leu Ser Ile |     |     |
| 370                                                             | 375 | 380 |
| Gly Val Glu Arg Ile Phe Ser Ile Val Glu Gln Arg Leu Glu Ala Leu |     |     |
| 385                                                             | 390 | 395 |
| Glu Glu Lys Ile Arg Thr Thr Glu Thr Gln Val Leu Val Ala Ser Ala |     |     |
| 405                                                             | 410 | 415 |
| Gln Lys Lys Leu Leu Glu Glu Arg Leu Lys Leu Val Ser Glu Leu Trp |     |     |
| 420                                                             | 425 | 430 |
| Asp Ala Gly Ile Lys Ala Glu Leu Leu Tyr Lys Lys Asn Pro Lys Leu |     |     |
| 435                                                             | 440 | 445 |
| Leu Asn Gln Leu Gln Tyr Cys Glu Glu Ala Gly Ile Pro Leu Val Ala |     |     |
| 450                                                             | 455 | 460 |
| Ile Ile Gly Glu Gln Glu Leu Lys Asp Gly Val Ile Lys Leu Arg Ser |     |     |
| 465                                                             | 470 | 475 |
| Val Thr Ser Arg Glu Glu Val Asp Val Arg Arg Glu Asp Leu Val Glu |     |     |
| 485                                                             | 490 | 495 |
| Glu Ile Lys Arg Arg Thr Gly Gln Pro Leu Cys Ile                 |     |     |
| 500                                                             | 505 |     |

<210> 178  
 <211> 509  
 <212> PRT  
 <213> Homo sapiens

|                                                                 |
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| <400> 178                                                       |
| Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu |
| 1 5 10 15                                                       |
| Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu |
| 20 25 30                                                        |
| Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp |
| 35 40 45                                                        |
| Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp |
| 50 55 60                                                        |



Tyr Ser Pro Arg Gln Met Ala Val Arg Glu Lys Val Phe Asp Val Ile  
65 70 75 80

Ile Arg Cys Phe Lys Arg His Gly Ala Glu Val Ile Asp Thr Pro Val  
85 90 95

Phe Glu Leu Lys Glu Thr Leu Met Gly Lys Tyr Gly Glu Asp Ser Lys  
100 105 110

Leu Ile Tyr Asp Leu Lys Asp Gln Gly Gly Glu Leu Leu Ser Leu Arg  
115 120 125

Tyr Asp Leu Thr Val Pro Phe Ala Arg Tyr Leu Ala Met Asn Lys Leu  
130 135 140

Thr Asn Ile Lys Arg Tyr His Ile Ala Lys Val Tyr Arg Arg Asp Asn  
145 150 155 160

Pro Ala Met Thr Arg Gly Arg Tyr Arg Glu Phe Tyr Gln Cys Asp Phe  
165 170 175

Asp Ile Ala Gly Asn Phe Asp Pro Met Ile Pro Asp Ala Glu Cys Leu  
180 185 190

Lys Ile Met Cys Glu Ile Leu Ser Ser Leu Gln Ile Gly Asp Phe Leu  
195 200 205

Val Lys Val Asn Asp Arg Arg Ile Leu Asp Gly Met Phe Ala Ile Cys  
210 215 220

Gly Val Ser Asp Ser Lys Phe Arg Thr Ile Cys Ser Ser Val Asp Lys  
225 230 235 240

Leu Asp Lys Val Ser Trp Glu Glu Val Lys Asn Glu Met Val Gly Glu  
245 250 255

Lys Gly Leu Ala Pro Glu Val Ala Asp Arg Ile Gly Asp Tyr Val Gln  
260 265 270

Gln His Gly Gly Val Ser Leu Val Glu Gln Leu Leu Gln Asp Pro Lys  
275 280 285

Leu Ser Gln Asn Lys Gln Ala Leu Glu Gly Leu Gly Asp Leu Lys Leu  
290 295 300

Leu Phe Glu Tyr Leu Thr Leu Phe Gly Ile Asp Asp Lys Ile Ser Phe  
305 310 315 320

Asp Leu Ser Leu Ala Arg Gly Leu Asp Tyr Tyr Thr Gly Val Ile Tyr  
325 330 335

Glu Ala Val Leu Leu Gln Thr Pro Ala Gln Ala Gly Glu Glu Pro Leu  
340 345 350

Gly Val Gly Ser Val Ala Ala Gly Gly Arg Tyr Asp Gly Leu Val Gly  
355 360 365

Met Phe Asp Pro Lys Gly Arg Lys Val Pro Cys Val Gly Leu Ser Ile  
370 375 380

Gly Val Glu Arg Ile Phe Ser Ile Val Glu Gln Arg Leu Glu Ala Leu  
385 390 395 400

Glu Glu Lys Ile Arg Thr Thr Glu Thr Gln Val Leu Val Ala Ser Ala  
405 410 415



Gln Lys Lys Leu Leu Glu Glu Arg Leu Lys Leu Val Ser Glu Leu Trp  
420 425 430

Asp Ala Gly Ile Lys Ala Glu Leu Leu Tyr Lys Lys Asn Pro Lys Leu  
435 440 445

Leu Asn Gln Leu Gln Tyr Cys Glu Glu Ala Gly Ile Pro Leu Val Ala  
450 455 460

Ile Ile Gly Glu Gln Glu Leu Lys Asp Gly Val Ile Lys Leu Arg Ser  
465 470 475 480

Val Thr Ser Arg Glu Glu Val Asp Val Arg Arg Glu Asp Leu Val Glu  
485 490 495

Glu Ile Lys Arg Arg Thr Gly Gln Pro Leu Cys Ile Cys  
500 505

<210> 179

<211> 61

<212> PRT

<213> Artificial Sequence

<220>

<223> Cysteine modified HRS polypeptide

<400> 179

Met Cys Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly  
1 5 10 15

Glu Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile  
20 25 30

Glu Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro  
35 40 45

Asp Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys  
50 55 60

<210> 180

<211> 60

<212> PRT

<213> Artificial Sequence

<220>

<223> Cysteine modified HRS polypeptide

<400> 180

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Cys Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys  
50 55 60

<210> 181

<211> 61



<212> PRT

### <213> Artificial Sequence

**<220>**

<223> Cysteine modified HRS polypeptide

**<400> 181**

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Cys  
50 55 60

<210> 182

<211> 183

## <212> DNA

### <213> Artificial Sequence

**<220>**

<223> Polynucleotide encoding cyteine modified HRS

<400> 182

|                                                                    |     |
|--------------------------------------------------------------------|-----|
| atgtgtgcag aaagagccgc cctggaagag ttagttaagt tgcaagggtga acgtgtccgt | 60  |
| ggctctgaagc agcagaaggc tagcgcggag ctgatcgaag aagaggtggc caaactgctg | 120 |
| aagctgaagg cgcagctggg cccggacgag agcaaacaaa agttcgtcct gaaaaccccg  | 180 |
| aaa                                                                | 183 |

**<210> 183**

**<211> 180**

## <212> DNA

### <213> Artificial Sequence

**<220>**

<223> Polynucleotide encoding cyteine modified HRS

**<400> 183**

|            |            |            |            |            |            |     |
|------------|------------|------------|------------|------------|------------|-----|
| atggcagaac | gtgcggcatt | ggaagaattg | gttaaactgc | aaggtgaacg | tgttcgtggt | 60  |
| ctgaagcagc | agaagtgcag | cgcggagctg | atcgaagaag | aggtggccaa | actgctgaag | 120 |
| ctgaaggcgc | agctgggccc | ggacgagagc | aaacaaaagt | tctctctgaa | aaccccgaaa | 180 |

<210> 184

<211> 183

<212> DNA

### <213> Artificial Sequence

**<220>**

<223> Polynucleotide encoding cyteine modified HRS

<400> 184

atggcagaac gtgcggcatt ggaagaattg gttaaactgc aaggtgaacg tgttcgtggt 60  
ctgaagcagc agaaggctag cgcggagctg atcgaagaag aggtggccaa actgctgaag 120



ctgaaggcgc agctgggccc ggacgagagc aaacaaaagt tcgtcctgaa aaccccgaag 180  
tgc 183

<210> 185  
<211> 506  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Cysteine mutated HRS polypeptide

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



275                      280                      285  
 Leu Ser Gln Asn Lys Gln Ala Leu Glu Gly Leu Gly Asp Leu Lys Leu  
 290                      295                      300  
 Leu Phe Glu Tyr Leu Thr Leu Phe Gly Ile Asp Asp Lys Ile Ser Phe  
 305                      310                      315                      320  
 Asp Leu Ser Leu Ala Arg Gly Leu Asp Tyr Tyr Thr Gly Val Ile Tyr  
 325                      330                      335  
 Glu Ala Val Leu Leu Gln Thr Pro Ala Gln Ala Gly Glu Glu Pro Leu  
 340                      345                      350  
 Gly Val Gly Ser Val Ala Ala Gly Gly Arg Tyr Asp Gly Leu Val Gly  
 355                      360                      365  
 Met Phe Asp Pro Lys Gly Arg Lys Val Pro Cys Val Gly Leu Ser Ile  
 370                      375                      380  
 Gly Val Glu Arg Ile Phe Ser Ile Val Glu Gln Arg Leu Glu Ala Leu  
 385                      390                      395                      400  
 Glu Glu Lys Ile Arg Thr Thr Glu Thr Gln Val Leu Val Ala Ser Ala  
 405                      410                      415  
 Gln Lys Lys Leu Leu Glu Glu Arg Leu Lys Leu Val Ser Glu Leu Trp  
 420                      425                      430  
 Asp Ala Gly Ile Lys Ala Glu Leu Leu Tyr Lys Lys Asn Pro Lys Leu  
 435                      440                      445  
 Leu Asn Gln Leu Gln Tyr Cys Glu Glu Ala Gly Ile Pro Leu Val Ala  
 450                      455                      460  
 Ile Ile Gly Glu Gln Glu Leu Lys Asp Gly Val Ile Lys Leu Arg Ser  
 465                      470                      475                      480  
 Val Thr Ser Arg Glu Glu Val Asp Val Arg Arg Glu Asp Leu Val Glu  
 485                      490                      495  
 Glu Ile Lys Arg Arg Thr Gly Gln Pro Leu  
 500                      505  
 <210> 186  
 <211> 506  
 <212> PRT  
 <213> Artificial Sequence  
 <220>  
 <223> Cysteine mutated HRS polypeptide  
 <400> 186  
 Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
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 Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
 20                      25                      30  
 Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
 35                      40                      45  
 Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp  
 50                      55                      60



Tyr Ser Pro Arg Gln Met Ala Val Arg Glu Lys Val Phe Asp Val Ile  
65 70 75 80

Ile Arg Cys Phe Lys Arg His Gly Ala Glu Val Ile Asp Thr Pro Val  
85 90 95

Phe Glu Leu Lys Glu Thr Leu Met Gly Lys Tyr Gly Glu Asp Ser Lys  
100 105 110

Leu Ile Tyr Asp Leu Lys Asp Gln Gly Gly Glu Leu Leu Ser Leu Arg  
115 120 125

Tyr Asp Leu Thr Val Pro Phe Ala Arg Tyr Leu Ala Met Asn Lys Leu  
130 135 140

Thr Asn Ile Lys Arg Tyr His Ile Ala Lys Val Tyr Arg Arg Asp Asn  
145 150 155 160

Pro Ala Met Thr Arg Gly Arg Tyr Arg Glu Phe Tyr Gln Val Asp Phe  
165 170 175

Asp Ile Ala Gly Asn Phe Asp Pro Met Ile Pro Asp Ala Glu Cys Leu  
180 185 190

Lys Ile Met Cys Glu Ile Leu Ser Ser Leu Gln Ile Gly Asp Phe Leu  
195 200 205

Val Lys Val Asn Asp Arg Arg Ile Leu Asp Gly Met Phe Ala Ile Cys  
210 215 220

Gly Val Ser Asp Ser Lys Phe Arg Thr Ile Cys Ser Ser Val Asp Lys  
225 230 235 240

Leu Asp Lys Val Ser Trp Glu Glu Val Lys Asn Glu Met Val Gly Glu  
245 250 255

Lys Gly Leu Ala Pro Glu Val Ala Asp Arg Ile Gly Asp Tyr Val Gln  
260 265 270

Gln His Gly Gly Val Ser Leu Val Glu Gln Leu Leu Gln Asp Pro Lys  
275 280 285

Leu Ser Gln Asn Lys Gln Ala Leu Glu Gly Leu Gly Asp Leu Lys Leu  
290 295 300

Leu Phe Glu Tyr Leu Thr Leu Phe Gly Ile Asp Asp Lys Ile Ser Phe  
305 310 315 320

Asp Leu Ser Leu Ala Arg Gly Leu Asp Tyr Tyr Thr Gly Val Ile Tyr  
325 330 335

Glu Ala Val Leu Leu Gln Thr Pro Ala Gln Ala Gly Glu Glu Pro Leu  
340 345 350

Gly Val Gly Ser Val Ala Ala Gly Gly Arg Tyr Asp Gly Leu Val Gly  
355 360 365

Met Phe Asp Pro Lys Gly Arg Lys Val Pro Cys Val Gly Leu Ser Ile  
370 375 380

Gly Val Glu Arg Ile Phe Ser Ile Val Glu Gln Arg Leu Glu Ala Leu  
385 390 395 400

Glu Glu Lys Ile Arg Thr Thr Glu Thr Gln Val Leu Val Ala Ser Ala  
405 410 415



Gln Lys Lys Leu Leu Glu Glu Arg Leu Lys Leu Val Ser Glu Leu Trp  
420 425 430

Asp Ala Gly Ile Lys Ala Glu Leu Leu Tyr Lys Lys Asn Pro Lys Leu  
435 440 445

Leu Asn Gln Leu Gln Tyr Cys Glu Glu Ala Gly Ile Pro Leu Val Ala  
450 455 460

Ile Ile Gly Glu Gln Glu Leu Lys Asp Gly Val Ile Lys Leu Arg Ser  
465 470 475 480

Val Thr Ser Arg Glu Glu Val Asp Val Arg Arg Glu Asp Leu Val Glu  
485 490 495

Glu Ile Lys Arg Arg Thr Gly Gln Pro Leu  
500 505

<210> 187

<211> 506

<212> PRT

<213> Artificial Sequence

<220>

<223> Cysteine mutated HRS polypeptide

<400> 187

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp  
50 55 60

Tyr Ser Pro Arg Gln Met Ala Val Arg Glu Lys Val Phe Asp Val Ile  
65 70 75 80

Ile Arg Cys Phe Lys Arg His Gly Ala Glu Val Ile Asp Thr Pro Val  
85 90 95

Phe Glu Leu Lys Glu Thr Leu Met Gly Lys Tyr Gly Glu Asp Ser Lys  
100 105 110

Leu Ile Tyr Asp Leu Lys Asp Gln Gly Gly Glu Leu Leu Ser Leu Arg  
115 120 125

Tyr Asp Leu Thr Val Pro Phe Ala Arg Tyr Leu Ala Met Asn Lys Leu  
130 135 140

Thr Asn Ile Lys Arg Tyr His Ile Ala Lys Val Tyr Arg Arg Asp Asn  
145 150 155 160

Pro Ala Met Thr Arg Gly Arg Tyr Arg Glu Phe Tyr Gln Cys Asp Phe  
165 170 175

Asp Ile Ala Gly Asn Phe Asp Pro Met Ile Pro Asp Ala Glu Ala Leu  
180 185 190

Lys Ile Met Cys Glu Ile Leu Ser Ser Leu Gln Ile Gly Asp Phe Leu  
195 200 205



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195              200              205
Val Lys Val Asn Asp Arg Arg Ile Leu Asp Gly Met Phe Ala Ile Cys
210              215              220

Gly Val Ser Asp Ser Lys Phe Arg Thr Ile Cys Ser Ser Val Asp Lys
225              230              235              240

Leu Asp Lys Val Ser Trp Glu Glu Val Lys Asn Glu Met Val Gly Glu
245              250              255

Lys Gly Leu Ala Pro Glu Val Ala Asp Arg Ile Gly Asp Tyr Val Gln
260              265              270

Gln His Gly Gly Val Ser Leu Val Glu Gln Leu Leu Gln Asp Pro Lys
275              280              285

Leu Ser Gln Asn Lys Gln Ala Leu Glu Gly Leu Gly Asp Leu Lys Leu
290              295              300

Leu Phe Glu Tyr Leu Thr Leu Phe Gly Ile Asp Asp Lys Ile Ser Phe
305              310              315              320

Asp Leu Ser Leu Ala Arg Gly Leu Asp Tyr Tyr Thr Gly Val Ile Tyr
325              330              335

Glu Ala Val Leu Leu Gln Thr Pro Ala Gln Ala Gly Glu Glu Pro Leu
340              345              350

Gly Val Gly Ser Val Ala Ala Gly Gly Arg Tyr Asp Gly Leu Val Gly
355              360              365

Met Phe Asp Pro Lys Gly Arg Lys Val Pro Cys Val Gly Leu Ser Ile
370              375              380

Gly Val Glu Arg Ile Phe Ser Ile Val Glu Gln Arg Leu Glu Ala Leu
385              390              395              400

Glu Glu Lys Ile Arg Thr Thr Glu Thr Gln Val Leu Val Ala Ser Ala
405              410              415

Gln Lys Lys Leu Leu Glu Glu Arg Leu Lys Leu Val Ser Glu Leu Trp
420              425              430

Asp Ala Gly Ile Lys Ala Glu Leu Leu Tyr Lys Lys Asn Pro Lys Leu
435              440              445

Leu Asn Gln Leu Gln Tyr Cys Glu Glu Ala Gly Ile Pro Leu Val Ala
450              455              460

Ile Ile Gly Glu Gln Glu Leu Lys Asp Gly Val Ile Lys Leu Arg Ser
465              470              475              480

Val Thr Ser Arg Glu Glu Val Asp Val Arg Arg Glu Asp Leu Val Glu
485              490              495

Glu Ile Lys Arg Arg Thr Gly Gln Pro Leu
500              505

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&lt;210&gt; 188

&lt;211&gt; 506

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;



&lt;223&gt; Cysteine mutated HRS polypeptide

&lt;400&gt; 188

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Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu
1          5          10          15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu
          20          25          30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp
          35          40          45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp
          50          55          60

Tyr Ser Pro Arg Gln Met Ala Val Arg Glu Lys Val Phe Asp Val Ile
65          70          75          80

Ile Arg Cys Phe Lys Arg His Gly Ala Glu Val Ile Asp Thr Pro Val
          85          90          95

Phe Glu Leu Lys Glu Thr Leu Met Gly Lys Tyr Gly Glu Asp Ser Lys
          100          105          110

Leu Ile Tyr Asp Leu Lys Asp Gln Gly Gly Glu Leu Leu Ser Leu Arg
          115          120          125

Tyr Asp Leu Thr Val Pro Phe Ala Arg Tyr Leu Ala Met Asn Lys Leu
          130          135          140

Thr Asn Ile Lys Arg Tyr His Ile Ala Lys Val Tyr Arg Arg Asp Asn
145          150          155          160

Pro Ala Met Thr Arg Gly Arg Tyr Arg Glu Phe Tyr Gln Cys Asp Phe
          165          170          175

Asp Ile Ala Gly Asn Phe Asp Pro Met Ile Pro Asp Ala Glu Ser Leu
          180          185          190

Lys Ile Met Cys Glu Ile Leu Ser Ser Leu Gln Ile Gly Asp Phe Leu
          195          200          205

Val Lys Val Asn Asp Arg Arg Ile Leu Asp Gly Met Phe Ala Ile Cys
          210          215          220

Gly Val Ser Asp Ser Lys Phe Arg Thr Ile Cys Ser Ser Val Asp Lys
225          230          235          240

Leu Asp Lys Val Ser Trp Glu Glu Val Lys Asn Glu Met Val Gly Glu
          245          250          255

Lys Gly Leu Ala Pro Glu Val Ala Asp Arg Ile Gly Asp Tyr Val Gln
          260          265          270

Gln His Gly Gly Val Ser Leu Val Glu Gln Leu Leu Gln Asp Pro Lys
          275          280          285

Leu Ser Gln Asn Lys Gln Ala Leu Glu Gly Leu Gly Asp Leu Lys Leu
          290          295          300

Leu Phe Glu Tyr Leu Thr Leu Phe Gly Ile Asp Asp Lys Ile Ser Phe
305          310          315          320

Asp Leu Ser Leu Ala Arg Gly Leu Asp Tyr Tyr Thr Gly Val Ile Tyr
          325          330          335

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Glu Ala Val Leu Leu Gln Thr Pro Ala Gln Ala Gly Glu Glu Pro Leu  
340 345 350

Gly Val Gly Ser Val Ala Ala Gly Gly Arg Tyr Asp Gly Leu Val Gly  
355 360 365

Met Phe Asp Pro Lys Gly Arg Lys Val Pro Cys Val Gly Leu Ser Ile  
370 375 380

Gly Val Glu Arg Ile Phe Ser Ile Val Glu Gln Arg Leu Glu Ala Leu  
385 390 395 400

Glu Glu Lys Ile Arg Thr Thr Glu Thr Gln Val Leu Val Ala Ser Ala  
405 410 415

Gln Lys Lys Leu Leu Glu Glu Arg Leu Lys Leu Val Ser Glu Leu Trp  
420 425 430

Asp Ala Gly Ile Lys Ala Glu Leu Leu Tyr Lys Lys Asn Pro Lys Leu  
435 440 445

Leu Asn Gln Leu Gln Tyr Cys Glu Glu Ala Gly Ile Pro Leu Val Ala  
450 455 460

Ile Ile Gly Glu Gln Glu Leu Lys Asp Gly Val Ile Lys Leu Arg Ser  
465 470 475 480

Val Thr Ser Arg Glu Glu Val Asp Val Arg Arg Glu Asp Leu Val Glu  
485 490 495

Glu Ile Lys Arg Arg Thr Gly Gln Pro Leu  
500 505

<210> 189

<211> 506

<212> PRT

<213> Artificial Sequence

<220>

<223> Cysteine mutated HRS polypeptide

<400> 189

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp  
50 55 60

Tyr Ser Pro Arg Gln Met Ala Val Arg Glu Lys Val Phe Asp Val Ile  
65 70 75 80

Ile Arg Cys Phe Lys Arg His Gly Ala Glu Val Ile Asp Thr Pro Val  
85 90 95

Phe Glu Leu Lys Glu Thr Leu Met Gly Lys Tyr Gly Glu Asp Ser Lys  
100 105 110

Leu Ile Thr Asn Leu Lys Asn Gln Glu Glu Glu Leu Leu Ser Leu Arg



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Leu Ile Tyr Asp Leu Lys Asp Gln Gly Gly Glu Leu Leu Ser Leu Arg
115                      120                      125

Tyr Asp Leu Thr Val Pro Phe Ala Arg Tyr Leu Ala Met Asn Lys Leu
130                      135                      140

Thr Asn Ile Lys Arg Tyr His Ile Ala Lys Val Tyr Arg Arg Asp Asn
145                      150                      155                      160

Pro Ala Met Thr Arg Gly Arg Tyr Arg Glu Phe Tyr Gln Cys Asp Phe
165                      170                      175

Asp Ile Ala Gly Asn Phe Asp Pro Met Ile Pro Asp Ala Glu Val Leu
180                      185                      190

Lys Ile Met Cys Glu Ile Leu Ser Ser Leu Gln Ile Gly Asp Phe Leu
195                      200                      205

Val Lys Val Asn Asp Arg Arg Ile Leu Asp Gly Met Phe Ala Ile Cys
210                      215                      220

Gly Val Ser Asp Ser Lys Phe Arg Thr Ile Cys Ser Ser Val Asp Lys
225                      230                      235                      240

Leu Asp Lys Val Ser Trp Glu Glu Val Lys Asn Glu Met Val Gly Glu
245                      250                      255

Lys Gly Leu Ala Pro Glu Val Ala Asp Arg Ile Gly Asp Tyr Val Gln
260                      265                      270

Gln His Gly Gly Val Ser Leu Val Glu Gln Leu Leu Gln Asp Pro Lys
275                      280                      285

Leu Ser Gln Asn Lys Gln Ala Leu Glu Gly Leu Gly Asp Leu Lys Leu
290                      295                      300

Leu Phe Glu Tyr Leu Thr Leu Phe Gly Ile Asp Asp Lys Ile Ser Phe
305                      310                      315                      320

Asp Leu Ser Leu Ala Arg Gly Leu Asp Tyr Tyr Thr Gly Val Ile Tyr
325                      330                      335

Glu Ala Val Leu Leu Gln Thr Pro Ala Gln Ala Gly Glu Glu Pro Leu
340                      345                      350

Gly Val Gly Ser Val Ala Ala Gly Gly Arg Tyr Asp Gly Leu Val Gly
355                      360                      365

Met Phe Asp Pro Lys Gly Arg Lys Val Pro Cys Val Gly Leu Ser Ile
370                      375                      380

Gly Val Glu Arg Ile Phe Ser Ile Val Glu Gln Arg Leu Glu Ala Leu
385                      390                      395                      400

Glu Glu Lys Ile Arg Thr Thr Glu Thr Gln Val Leu Val Ala Ser Ala
405                      410                      415

Gln Lys Lys Leu Leu Glu Glu Arg Leu Lys Leu Val Ser Glu Leu Trp
420                      425                      430

Asp Ala Gly Ile Lys Ala Glu Leu Leu Tyr Lys Lys Asn Pro Lys Leu
435                      440                      445

Leu Asn Gln Leu Gln Tyr Cys Glu Glu Ala Gly Ile Pro Leu Val Ala
450                      455                      460

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Ile Ile Gly Glu Gln Glu Leu Lys Asp Gly Val Ile Lys Leu Arg Ser  
465 470 475 480

Val Thr Ser Arg Glu Glu Val Asp Val Arg Arg Glu Asp Leu Val Glu  
485 490 495

Glu Ile Lys Arg Arg Thr Gly Gln Pro Leu  
500 505

<210> 190

<211> 506

<212> PRT

<213> Artificial Sequence

<220>

<223> Cysteine mutated HRS polypeptide

<400> 190

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp  
50 55 60

Tyr Ser Pro Arg Gln Met Ala Val Arg Glu Lys Val Phe Asp Val Ile  
65 70 75 80

Ile Arg Cys Phe Lys Arg His Gly Ala Glu Val Ile Asp Thr Pro Val  
85 90 95

Phe Glu Leu Lys Glu Thr Leu Met Gly Lys Tyr Gly Glu Asp Ser Lys  
100 105 110

Leu Ile Tyr Asp Leu Lys Asp Gln Gly Gly Glu Leu Leu Ser Leu Arg  
115 120 125

Tyr Asp Leu Thr Val Pro Phe Ala Arg Tyr Leu Ala Met Asn Lys Leu  
130 135 140

Thr Asn Ile Lys Arg Tyr His Ile Ala Lys Val Tyr Arg Arg Asp Asn  
145 150 155 160

Pro Ala Met Thr Arg Gly Arg Tyr Arg Glu Phe Tyr Gln Cys Asp Phe  
165 170 175

Asp Ile Ala Gly Asn Phe Asp Pro Met Ile Pro Asp Ala Glu Cys Leu  
180 185 190

Lys Ile Met Cys Glu Ile Leu Ser Ser Leu Gln Ile Gly Asp Phe Leu  
195 200 205

Val Lys Val Asn Asp Arg Arg Ile Leu Asp Gly Met Phe Ala Ile Ser  
210 215 220

Gly Val Ser Asp Ser Lys Phe Arg Thr Ile Cys Ser Ser Val Asp Lys  
225 230 235 240



Leu Asp Lys Val Ser Trp Glu Glu Val Lys Asn Glu Met Val Gly Glu  
245 250 255

Lys Gly Leu Ala Pro Glu Val Ala Asp Arg Ile Gly Asp Tyr Val Gln  
260 265 270

Gln His Gly Gly Val Ser Leu Val Glu Gln Leu Leu Gln Asp Pro Lys  
275 280 285

Leu Ser Gln Asn Lys Gln Ala Leu Glu Gly Leu Gly Asp Leu Lys Leu  
290 295 300

Leu Phe Glu Tyr Leu Thr Leu Phe Gly Ile Asp Asp Lys Ile Ser Phe  
305 310 315 320

Asp Leu Ser Leu Ala Arg Gly Leu Asp Tyr Tyr Thr Gly Val Ile Tyr  
325 330 335

Glu Ala Val Leu Leu Gln Thr Pro Ala Gln Ala Gly Glu Glu Pro Leu  
340 345 350

Gly Val Gly Ser Val Ala Ala Gly Gly Arg Tyr Asp Gly Leu Val Gly  
355 360 365

Met Phe Asp Pro Lys Gly Arg Lys Val Pro Cys Val Gly Leu Ser Ile  
370 375 380

Gly Val Glu Arg Ile Phe Ser Ile Val Glu Gln Arg Leu Glu Ala Leu  
385 390 395 400

Glu Glu Lys Ile Arg Thr Thr Glu Thr Gln Val Leu Val Ala Ser Ala  
405 410 415

Gln Lys Lys Leu Leu Glu Glu Arg Leu Lys Leu Val Ser Glu Leu Trp  
420 425 430

Asp Ala Gly Ile Lys Ala Glu Leu Leu Tyr Lys Lys Asn Pro Lys Leu  
435 440 445

Leu Asn Gln Leu Gln Tyr Cys Glu Glu Ala Gly Ile Pro Leu Val Ala  
450 455 460

Ile Ile Gly Glu Gln Glu Leu Lys Asp Gly Val Ile Lys Leu Arg Ser  
465 470 475 480

Val Thr Ser Arg Glu Glu Val Asp Val Arg Arg Glu Asp Leu Val Glu  
485 490 495

Glu Ile Lys Arg Arg Thr Gly Gln Pro Leu  
500 505

<210> 191

<211> 506

<212> PRT

<213> Artificial Sequence

<220>

<223> Cysteine mutated HRS polypeptide

<400> 191

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15



Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp  
50 55 60

Tyr Ser Pro Arg Gln Met Ala Val Arg Glu Lys Val Phe Asp Val Ile  
65 70 75 80

Ile Arg Cys Phe Lys Arg His Gly Ala Glu Val Ile Asp Thr Pro Val  
85 90 95

Phe Glu Leu Lys Glu Thr Leu Met Gly Lys Tyr Gly Glu Asp Ser Lys  
100 105 110

Leu Ile Tyr Asp Leu Lys Asp Gln Gly Gly Glu Leu Leu Ser Leu Arg  
115 120 125

Tyr Asp Leu Thr Val Pro Phe Ala Arg Tyr Leu Ala Met Asn Lys Leu  
130 135 140

Thr Asn Ile Lys Arg Tyr His Ile Ala Lys Val Tyr Arg Arg Asp Asn  
145 150 155 160

Pro Ala Met Thr Arg Gly Arg Tyr Arg Glu Phe Tyr Gln Cys Asp Phe  
165 170 175

Asp Ile Ala Gly Asn Phe Asp Pro Met Ile Pro Asp Ala Glu Cys Leu  
180 185 190

Lys Ile Met Cys Glu Ile Leu Ser Ser Leu Gln Ile Gly Asp Phe Leu  
195 200 205

Val Lys Val Asn Asp Arg Arg Ile Leu Asp Gly Met Phe Ala Ile Cys  
210 215 220

Gly Val Ser Asp Ser Lys Phe Arg Thr Ile Ser Ser Ser Val Asp Lys  
225 230 235 240

Leu Asp Lys Val Ser Trp Glu Glu Val Lys Asn Glu Met Val Gly Glu  
245 250 255

Lys Gly Leu Ala Pro Glu Val Ala Asp Arg Ile Gly Asp Tyr Val Gln  
260 265 270

Gln His Gly Gly Val Ser Leu Val Glu Gln Leu Leu Gln Asp Pro Lys  
275 280 285

Leu Ser Gln Asn Lys Gln Ala Leu Glu Gly Leu Gly Asp Leu Lys Leu  
290 295 300

Leu Phe Glu Tyr Leu Thr Leu Phe Gly Ile Asp Asp Lys Ile Ser Phe  
305 310 315 320

Asp Leu Ser Leu Ala Arg Gly Leu Asp Tyr Tyr Thr Gly Val Ile Tyr  
325 330 335

Glu Ala Val Leu Leu Gln Thr Pro Ala Gln Ala Gly Glu Glu Pro Leu  
340 345 350

Gly Val Gly Ser Val Ala Ala Gly Gly Arg Tyr Asp Gly Leu Val Gly  
355 360 365



Met Phe Asp Pro Lys Gly Arg Lys Val Pro Cys Val Gly Leu Ser Ile  
 370 375 380

Gly Val Glu Arg Ile Phe Ser Ile Val Glu Gln Arg Leu Glu Ala Leu

385 390 395 400

Glu Glu Lys Ile Arg Thr Thr Glu Thr Gln Val Leu Val Ala Ser Ala  
 405 410 415

Gln Lys Lys Leu Leu Glu Glu Arg Leu Lys Leu Val Ser Glu Leu Trp  
 420 425 430

Asp Ala Gly Ile Lys Ala Glu Leu Leu Tyr Lys Lys Asn Pro Lys Leu  
 435 440 445

Leu Asn Gln Leu Gln Tyr Cys Glu Glu Ala Gly Ile Pro Leu Val Ala  
 450 455 460

Ile Ile Gly Glu Gln Glu Leu Lys Asp Gly Val Ile Lys Leu Arg Ser  
 465 470 475 480

Val Thr Ser Arg Glu Glu Val Asp Val Arg Arg Glu Asp Leu Val Glu  
 485 490 495

Glu Ile Lys Arg Arg Thr Gly Gln Pro Leu  
 500 505

<210> 192

<211> 1518

<212> DNA

<213> Artificial Sequence

<220>

<223> Polynucleotide encoding cysteine mutated HRS protein

<400> 192

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| cttaacaac aaaaagcctc tgcggaattg attgaagaag aagttgccaa attactgaaa    | 120 |
| ctgaaagctc aacttggaac cgatgaaagt aaacaaaaat ttgtgttgaa aacgccc aaa  | 180 |
| ggaaccctg attatagtc acgtcaaatg gccgttcgtg aaaaagtgtt cgacgttatt     | 240 |
| attcgtgtt ttaaactgca cgggtgctgaa gtaatcgata ccccggtatt tgaattgaaa   | 300 |
| gagactctga tgggcaaata tgggtgaagat tctaaactga tttatgattt gaaagaccaa  | 360 |
| ggaggtgaac tgctgagcct gcgctacgac ttaactgtgc cttttgcccg ttacttagcc   | 420 |
| atgaataaat taaccaacat caaacgttac catattgcaa aagtatatcg ccgcgacaac   | 480 |
| cctgcaatga ctcgtggacg ctatcgcgaa ttctatcagg ctgattttga tattgccgga   | 540 |
| aatttcgacc cgatgatccc ggatgccgag tgtttgaaaa ttatgtgtga aattctgagt   | 600 |
| tcgttgca ga tcggagactt tcttgtaaaa gttaatgacc gccgtattct ggatggtatg  | 660 |
| tttgctat ttt gcggtgtttc tgattccaaa ttccgtacaa tctgctcaag cgtggacaaa | 720 |
| ttggataaag tgtcttg gga agaagtaaaa aatgaaatgg tgggagaaaa aggcctggct  | 780 |
| ccagaagtag cagaccgtat tgggtgactat gttcaacaac atggcggtgt gtccttagtc  | 840 |
| gaacagttat tacaggatcc taaactgagc caaaataaac aagcacttga aggactggga   | 900 |
| gatctgaaat tactctttga atatctgacc ttatttggga ttgatgataa aattagcttt   | 960 |



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gatctgagct tggcccgcg tcttgattat tataccggcg tgatttacga agctgttctc 1020
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ccactcgtag ctattattgg tgagcaagaa ttaaaagatg gcgtgattaa actgcgttca 1440
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cgcaccggtc aaccgtta 1518

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&lt;210&gt; 193

&lt;211&gt; 1518

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Polynucleotide encoding cysteine mutated HRS protein

&lt;400&gt; 193

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ggaaccctg attatagtc acgtcaaatg gccgttcgtg aaaaagtgtt cgacgttatt 240
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aatttcgacc cgatgatccc ggatgccgag tgtttgaaaa ttatgtgtga aattctgagt 600
tcgttcgaga tcgggagactt tcttgtaaaa gttaatgacc gccgtattct ggatggatatg 660
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ccactcgtag ctattattgg tgagcaagaa ttaaaagatg gcgtgattaa actgcgttca 1440
gtaacaagcc gtgaagaggt agatgtacgt cgcgaagact tagtggaaga aattaaacgc 1500

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cgcacccggtc aaccgtta 1518

<210> 194

<211> 1518

<212> DNA

<213> Artificial Sequence

<220>

<223> Polynucleotide encoding cysteine mutated HRS protein

<400> 194

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| cttaacaac aaaaagcctc tgcggaattg attgaagaag aagttgccaa attactgaaa   | 120  |
| ctgaaagctc aacttggacc cgatgaaagt aaacaaaaat ttgtgttgaa aacgcccaaa  | 180  |
| ggaacccgtg attatagtcc acgtcaaatg gccgttcgtg aaaaagtgtt cgacgttatt  | 240  |
| attcgctgtt ttaaacgtca cgggtgctgaa gtaatcgata ccccgtatt tgaattgaaa  | 300  |
| gagactctga tgggcaaata tgggtgaagat tctaaactga tttatgattt gaaagaccaa | 360  |
| ggaggtgaac tgctgagcct gcgctacgac ttaactgtgc cttttgcccg ttacttagcc  | 420  |
| atgaataaat taaccaacat caaacgttac catattgcaa aagtatatcg ccgcgacaac  | 480  |
| cctgcaatga ctcggtggacg ctatcgcgaa ttctatcagt gtgattttga tattgccgga | 540  |
| aatttcgacc cgatgatccc ggatgccgag gctttgaaaa ttatgtgtga aattctgagt  | 600  |
| tcggttcaga tcggagactt tcttgtaaaa gttaatgacc gccgtattct ggatggtatg  | 660  |
| tttgctattt gcggtgtttc tgattccaaa ttccgtacaa tctgctcaag cgtggacaaa  | 720  |
| ttggataaag tgtcttggga agaagtaaaa aatgaaatgg tgggagaaaa aggcctggct  | 780  |
| ccagaagtag cagaccgtat tggtgactat gttcaacaac atggcggtgt gtccttagtc  | 840  |
| gaacagttat tacaggatcc taaactgagc caaaataaac aagcacttga aggactggga  | 900  |
| gatctgaaat tactctttga atatctgacc ttatttggga ttgatgataa aattagcttt  | 960  |
| gatctgagct tggcccgcgg tcttgattat tataccggcg tgatttacga agctgttctc  | 1020 |
| ttgcaaacc cagcccaggc gggcgaagag cttttgggag tcggcagtgt ggcagccggt   | 1080 |
| ggtcgttatg atggttttgt aggaatgttt gaccctaaag gccgtaaagt accatgtgtg  | 1140 |
| gggctttcta tcggtgtcga acgtatcttt tctattgttg aacaacgtct tgaagctttg  | 1200 |
| gaggaaaaga tccgtaccac ggaaacccaa gtcttagttg caagtgccca aaaaaactg   | 1260 |
| ttagaagaac gcctgaaact cgtatcagaa ctttgggacg ccggcatcaa ggccgaactg  | 1320 |
| ctgtataaaa agaacccgaa attgttaaac caactccagt attgtgaaga agctgggac   | 1380 |
| ccactcgtag ctattattgg tgagcaagaa ttaaaagatg gcgtgattaa actgcgttca  | 1440 |
| gtaacaagcc gtgaagaggt agatgtacgt cgcgaaagact tagtggaaga aattaaacgc | 1500 |
| cgcacccggtc aaccgtta                                               | 1518 |

<210> 195

<211> 1518

<212> DNA

<213> Artificial Sequence

<220>

<223> Polynucleotide encoding cysteine mutated HRS protein

<400> 195

|                                                                   |     |
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| cttaacaac aaaaagcctc tgcggaattg attgaagaag aagttgccaa attactgaaa  | 120 |



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aatttcgacc cgatgatccc ggatgccgag agtttgaaaa ttatgtgtga aattctgagt 600
tcgttgcaga tcggagactt tcttgtaaaa gttaatgacc gccgtattct ggatggtatg 660
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ttggataaag tgtcttggga agaagtaaaa aatgaaatgg tgggagaaaa aggcctggct 780

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cgcaccggtc aaccgtta 1518

```

&lt;210&gt; 196

&lt;211&gt; 1518

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Polynucleotide encoding cysteine mutated HRS protein

&lt;400&gt; 196

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atggcggaac gtgccgcact ggaagaattg gttaaattac agggagaacg cgtacgtggt 60
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ctgaaagctc aacttggacc cgatgaaagt aaacaaaaat ttgtgttgaa aacgccc aaa 180
ggaacccgtg attatagtcc acgtcaaatg gccgttcgtg aaaaagtgtt cgacgttatt 240
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cctgcaatga ctcgtggacg ctatcgcgaa ttctatcagt gtgattttga tattgccgga 540
aatttcgacc cgatgatccc ggatgccgag gttttgaaaa ttatgtgtga aattctgagt 600
tcgttgcaga tcggagactt tcttgtaaaa gttaatgacc gccgtattct ggatggtatg 660

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gtaacaagcc gtgaagaggt agatgtacgt cgcgaagact tagtggaaga aattaaacgc 1500
cgcaaccggtc aaccgtta 1518

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&lt;210&gt; 197

&lt;211&gt; 1518

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Polynucleotide encoding cysteine mutated HRS protein

&lt;400&gt; 197

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ggaaccctg attatagtcc acgtcaaatg gccgttcgtg aaaaagtgtt cgacgttatt 240
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 ccactcgtag ctattattgg tgagcaagaa ttaaaagatg gcgtgattaa actgcgttca 1440  
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<210> 198

<211> 1518

<212> DNA

<213> Artificial Sequence

<220>

<223> Polynucleotide encoding cysteine mutated HRS protein

<400> 198

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 ccactcgtag ctattattgg tgagcaagaa ttaaaagatg gcgtgattaa actgcgttca 1440  
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<210> 199

<211> 10

<212> DNA



<213> Artificial Sequence

<220>

<223> Kozak consensus sequence

<400> 199

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<210> 200

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Exemplary peptide linker sequence

<400> 200

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<210> 201

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Exemplary peptide linker sequence

<400> 201

Gly Gly Ser Gly  
1

<210> 202

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Exemplary peptide linker sequence

<400> 202

Gly Gly Gly Ser  
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<210> 203

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Exemplary peptide linker sequence

<400> 203

Gly Gly Gly Gly Ser  
1 5

<210> 204

<211> 4

<212> PRT



<213> Artificial Sequence

<220>

<223> Exemplary peptide linker sequence

<400> 204

Gly Asn Gly Asn  
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<210> 205

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Exemplary peptide linker sequence

<400> 205

Gly Gly Asn Gly  
1

<210> 206

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Exemplary peptide linker sequence

<400> 206

Gly Gly Gly Asn  
1

<210> 207

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Exemplary peptide linker sequence

<400> 207

Gly Gly Gly Gly Asn  
1 5

<210> 208

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Exemplary peptide linker sequence

<400> 208

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
1 5 10 15

<210> 209

<211> 22

<212> PRT



<213> Artificial Sequence

<220>

<223> Exemplary peptide linker sequence

<400> 209

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1 5 10 15  
  
Ser Gly Gly Gly Gly Ser  
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<210> 210

<211> 30

<212> PRT

<213> Artificial Sequence

<220>

<223> Exemplary peptide linker sequence

<400> 210

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly  
1 5 10 15  
  
Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
20 25 30

<210> 211

<211> 25

<212> PRT

<213> Artificial Sequence

<220>

<223> Exemplary peptide linker sequence

<400> 211

Asp Ala Ala Ala Lys Glu Ala Ala Ala Lys Asp Ala Ala Ala Arg Glu  
1 5 10 15  
  
Ala Ala Ala Arg Asp Ala Ala Ala Lys  
20 25

<210> 212

<211> 14

<212> PRT

<213> Artificial Sequence

<220>

<223> Exemplary peptide linker sequence

<400> 212

Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg  
1 5 10  
  
<210> 213  
<211> 5  
<212> PRT

<213> Artificial Sequence

<220>

<223> Peptide linker sequence



<400> 213  
Asp Gly Gly Gly Ser  
1 5

<210> 214  
<211> 5  
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<213> Artificial Sequence

<220>  
<223> Peptide linker sequence

<400> 214  
Thr Gly Glu Lys Pro  
1 5

<210> 215  
<211> 4  
<212> PRT  
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<220>  
<223> Peptide linker sequence

<400> 215  
Gly Gly Arg Arg  
1

<210> 216  
<211> 14  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Peptide linker sequence

<400> 216  
Glu Gly Lys Ser Ser Gly Ser Gly Ser Glu Ser Lys Val Asp  
1 5 10

<210> 217  
<211> 18  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Peptide linker sequence

<400> 217  
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1 5 10 15

Leu Asp

<210> 218  
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<212> PRT  
<213> Artificial Sequence



<220>  
<223> Peptide linker sequence

<400> 218  
Gly Gly Arg Arg Gly Gly Gly Ser

1 5

<210> 219  
<211> 9  
<212> PRT  
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<220>  
<223> Peptide linker sequence

<400> 219  
Leu Arg Gln Arg Asp Gly Glu Arg Pro  
1 5

<210> 220  
<211> 12  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Peptide linker sequence

<400> 220  
Leu Arg Gln Lys Asp Gly Gly Gly Ser Glu Arg Pro  
1 5 10

<210> 221  
<211> 16  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Peptide linker sequence

<400> 221  
Leu Arg Gln Lys Asp Gly Gly Gly Ser Gly Gly Gly Ser Glu Arg Pro  
1 5 10 15

<210> 222  
<211> 19  
<212> PRT  
<213> Unknown

<220>  
<223> Naturally occurring 2A or 2A-like self cleaving peptide

<400> 222  
Leu Leu Asn Phe Asp Leu Leu Lys Leu Ala Gly Asp Val Glu Ser Asn  
1 5 10 15

Pro Gly Pro

<210> 223  
<211> 19



<212> PRT  
<213> Unknown

<220>  
<223> Naturally occurring 2A or 2A-like self cleaving peptide

<400> 223  
Thr Leu Asn Phe Asp Leu Leu Lys Leu Ala Gly Asp Val Glu Ser Asn  
1                  5                  10                  15

Pro Gly Pro

<210> 224  
<211> 14  
<212> PRT  
<213> Unknown

<220>  
<223> Naturally occurring 2A or 2A-like self cleaving peptide

<400> 224  
Leu Leu Lys Leu Ala Gly Asp Val Glu Ser Asn Pro Gly Pro  
1                  5                  10

<210> 225  
<211> 17  
<212> PRT  
<213> Unknown

<220>  
<223> Naturally occurring 2A or 2A-like self cleaving peptide

<400> 225  
Asn Phe Asp Leu Leu Lys Leu Ala Gly Asp Val Glu Ser Asn Pro Gly  
1                  5                  10                  15

Pro

<210> 226  
<211> 20  
<212> PRT  
<213> Unknown

<220>  
<223> Naturally occurring 2A or 2A-like self cleaving peptide

<400> 226  
Gln Leu Leu Asn Phe Asp Leu Leu Lys Leu Ala Gly Asp Val Glu Ser  
1                  5                  10                  15

Asn Pro Gly Pro  
                  20

<210> 227  
<211> 24  
<212> PRT  
<213> Unknown

<220>  
<223> Naturally occurring 2A or 2A-like self cleaving peptide



&lt;400&gt; 227

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Pro | Val | Lys | Gln | Thr | Leu | Asn | Phe | Asp | Leu | Leu | Lys | Leu | Ala | Gly |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|
| Asp | Val | Glu | Ser | Asn | Pro | Gly | Pro |
|     |     |     |     | 20  |     |     |     |

&lt;210&gt; 228

&lt;211&gt; 40

&lt;212&gt; PRT

&lt;213&gt; Unknown

&lt;220&gt;

&lt;223&gt; Naturally occurring 2A or 2A-like self cleaving peptide

&lt;400&gt; 228

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Thr | Glu | Leu | Leu | Tyr | Arg | Met | Lys | Arg | Ala | Glu | Thr | Tyr | Cys | Pro |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Pro | Leu | Leu | Ala | Ile | His | Pro | Thr | Glu | Ala | Arg | His | Lys | Gln | Lys |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|
| Ile | Val | Ala | Pro | Val | Lys | Gln | Thr |
|     |     | 35  |     |     |     | 40  |     |

&lt;210&gt; 229

&lt;211&gt; 18

&lt;212&gt; PRT

&lt;213&gt; Unknown

&lt;220&gt;

&lt;223&gt; Naturally occurring 2A or 2A-like self cleaving peptide

&lt;400&gt; 229

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Asn | Phe | Asp | Leu | Leu | Lys | Leu | Ala | Gly | Asp | Val | Glu | Ser | Asn | Pro |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |
|-----|-----|
| Gly | Pro |
|-----|-----|

&lt;210&gt; 230

&lt;211&gt; 40

&lt;212&gt; PRT

&lt;213&gt; Unknown

&lt;220&gt;

&lt;223&gt; Naturally occurring 2A or 2A-like self cleaving peptide

&lt;400&gt; 230

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Leu | Ala | Ile | His | Pro | Thr | Glu | Ala | Arg | His | Lys | Gln | Lys | Ile | Val |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Pro | Val | Lys | Gln | Thr | Leu | Asn | Phe | Asp | Leu | Leu | Lys | Leu | Ala | Gly |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|
| Asp | Val | Glu | Ser | Asn | Pro | Gly | Pro |
|     |     | 35  |     |     |     | 40  |     |

&lt;210&gt; 231

&lt;211&gt; 33

&lt;212&gt; PRT



<213> Unknown

<220>

<223> Naturally occurring 2A or 2A-like self cleaving peptide

<400> 231

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Asn Phe Asp Leu Leu Lys Leu Ala Gly Asp Val Glu Ser Asn Pro Gly  
20 25 30

Pro

<210> 232

<211> 7

<212> PRT

<213> Tobacco etch virus

<220>

<221> VARIANT

<222> (2)..(2)

<223> Xaa = any amino acid

<220>

<221> VARIANT

<222> (3)..(3)

<223> Xaa = any amino acid

<220>

<221> VARIANT

<222> (5)..(5)

<223> Xaa = any amino acid

<220>

<221> VARIANT

<222> (7)..(7)

<223> Xaa is either Gly or Ser

<400> 232

Glu Xaa Xaa Tyr Xaa Gln Xaa  
1 5

<210> 233

<211> 7

<212> PRT

<213> Tobacco etch virus

<400> 233

Glu Asn Leu Tyr Phe Gln Gly  
1 5

<210> 234

<211> 7

<212> PRT

<213> Tabacco etch virus

<400> 234

Glu Asn Leu Tyr Phe Gln Ser



15

<210> 235<br><211> 4<br><212> PRT<br><213> Artificial Sequence&ltbr><220><br><223> Thrombin cleavable linker&ltbr><400> 235<br>Gly Arg Gly Asp<br>1&ltbr><210> 236<br><211> 6<br><212> PRT<br><213> Artificial Sequence&ltbr><220><br><223> Thrombin cleavable linker&ltbr><400> 236<br>Gly Arg Gly Asp Asn Pro<br>15&ltbr><210> 237<br><211> 5<br><212> PRT<br><213> Artificial Sequence&ltbr><220><br><223> Thrombin cleavable linker&ltbr><400> 237<br>Gly Arg Gly Asp Ser<br>15&ltbr><210> 238<br><211> 7<br><212> PRT<br><213> Artificial Sequence&ltbr><220><br><223> Thrombin cleavable linker&ltbr><400> 238<br>Gly Arg Gly Asp Ser Pro Lys<br>15&ltbr><210> 239<br><211> 4<br><212> PRT<br><213> Artificial Sequence&ltbr><220><br><223> Elastase cleavable linker&ltbr><400> 239<br>Ala Ala Pro Val<br>.



1

<210> 240  
<211> 4  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Elastase cleavable linker

<400> 240  
Ala Ala Pro Leu  
1

<210> 241  
<211> 4  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Elastase cleavable linker

<400> 241  
Ala Ala Pro Phe  
1

<210> 242  
<211> 4  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Elastase cleavable linker

<400> 242  
Ala Ala Pro Ala  
1

<210> 243  
<211> 4  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Elastase cleavable linker

<400> 243  
Ala Tyr Leu Val  
1

<210> 244  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Matrix metalloproteinase cleavable linker

<220>  
<221> VARIANT



<222> (3)..(3)

<223> Xaa = Any amino acid

<220>

<221> VARIANT

<222> (6)..(6)

<223> Xaa = Any amino acid

<400> 244

Gly Pro Xaa Gly Pro Xaa  
1 5

<210> 245

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Matrix metalloproteinase cleavable linker

<220>

<221> VARIANT

<222> (4)..(4)

<223> Xaa = Any amino acid

<400> 245

Leu Gly Pro Xaa  
1

<210> 246

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Matrix metalloproteinase cleavable linker

<220>

<221> VARIANT

<222> (6)..(6)

<223> Xaa = Any amino acid

<400> 246

Gly Pro Ile Gly Pro Xaa  
1 5

<210> 247

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Matrix metalloproteinase cleavable linker

<220>

<221> VARIANT

<222> (5)..(5)

<223> Xaa = Any amino acid



<400> 247

Ala Pro Gly Leu Xaa  
1 5

<210> 248

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Collagenase cleavable linker

<220>

<221> VARIANT

<222> (7)..(7)

<223> Xaa = Any amino acid

<400> 248

Pro Leu Gly Pro Asp Arg Xaa  
1 5

<210> 249

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Collagenase cleavable linker

<220>

<221> VARIANT

<222> (7)..(7)

<223> Xaa = Any amino acid

<400> 249

Pro Leu Gly Leu Leu Gly Xaa  
1 5

<210> 250

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Collagenase cleavable linker

<400> 250

Pro Gln Gly Ile Ala Gly Trp  
1 5

<210> 251

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Collagenase cleavable linker



<400> 251

Pro Leu Gly Cys His  
1 5

<210> 252

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Collagenase cleavable linker

<400> 252

Pro Leu Gly Leu Tyr Ala  
1 5

<210> 253

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Collagenase cleavable linker

<400> 253

Pro Leu Ala Leu Trp Ala Arg  
1 5

<210> 254

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Collagenase cleavable linker

<400> 254

Pro Leu Ala Tyr Trp Ala Arg  
1 5

<210> 255

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Stromelysin cleavable linker

<400> 255

Pro Tyr Ala Tyr Tyr Met Arg  
1 5

<210> 256

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Gelatinase cleavable linker



<400> 256

Pro Leu Gly Met Tyr Ser Arg  
1 5

<210> 257

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Angiotensin converting enzyme cleavable linker

<400> 257

Gly Asp Lys Pro  
1

<210> 258

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Angiotensin converting enzyme cleavable linker

<400> 258

Gly Ser Asp Lys Pro  
1 5

<210> 259

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Cathepsin B cleavable linker

<400> 259

Ala Leu Ala Leu  
1

<210> 260

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Cathepsin B cleavable linker

<400> 260

Gly Phe Leu Gly  
1

<210> 261

<211> 198

<212> DNA

<213> Artificial Sequence

<220>

<223> Codon optimized DNA sequence for HRS polypeptide with 6xHis tag



<400> 261  
atggcagaac gtgcggcatt ggaagaattg gttaaactgc aaggtgaacg tgttcgtggt 60  
ctgaagcagc agaaggctag cgcggagctg atcgaagaag aggtggccaa actgctgaag 120  
ctgaaggcgc agctgggccc ggacgagagc aaacaaaagt tcgtcctgaa aaccccgaaa 180  
caccaccatc accatcac 198

<210> 262  
<211> 66  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> HRS polypeptide with 6xHis tag

<400> 262  
Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15  
  
Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30  
  
Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45  
  
Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys His His His His  
50 55 60  
  
His His  
65

<210> 263  
<211> 201  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Codon optimized DNA sequence for HRS polypeptide with 6xHis tag

<400> 263  
atgtgtgcag aaagagccgc cctggaagag ttagttaagt tgcaagggtga acgtgtccgt 60  
ggctctgaagc agcagaaggc tagcgcggag ctgatcgaag aagaggtggc caaactgctg 120  
aagctgaagg cgcagctggg cccggacgag agcaaacaaa agttcgtcct gaaaaccccg 180  
aaacaccacc atcaccatca c 201

<210> 264  
<211> 67  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> HRS polypeptide with 6xHis tag

<400> 264  
Met Cys Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly  
1 5 10 15  
  
Glu Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile  
20 25 30



Glu Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro  
35 40 45

Asp Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys His His His  
50 55 60

His His His  
65

<210> 265  
<211> 198  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Codon optimized DNA sequence for HRS polypeptide with 6xHis tag

<400> 265  
atggcagaac gtgcggcatt ggaagaattg gttaaactgc aaggtgaacg tgttcgtggt 60  
ctgaagcagc agaagtgcag cgcggagctg atcgaagaag aggtggccaa actgctgaag 120  
ctgaaggcgc agctgggccc ggacgagagc aaacaaaagt tcgtcctgaa aaccccgaaa 180  
caccaccatc accatcac 198

<210> 266  
<211> 66  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> HRS polypeptide with 6xHis tag

<400> 266  
Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15  
Arg Val Arg Gly Leu Lys Gln Gln Lys Cys Ser Ala Glu Leu Ile Glu  
20 25 30  
Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45  
Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys His His His His  
50 55 60  
His His  
65

<210> 267  
<211> 201  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Codon optimized DNA sequence for HRS polypeptide with 6xHis tag

<400> 267  
atggcagaac gtgcggcatt ggaagaattg gttaaactgc aaggtgaacg tgttcgtggt 60  
ctgaagcagc agaaggctag cgcggagctg atcgaagaag aggtggccaa actgctgaag 120  
ctgaaggcgc agctgggccc ggacgagagc aaacaaaagt tcgtcctgaa aaccccgaaa 180



tgccaccacc atcaccatca c 201

<210> 268  
<211> 67  
<212> PRT  
<213> Artificial Sequence  
  
<220>  
<223> HRS polypeptide with 6xHis tag

<400> 268  
Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15  
  
Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30  
  
Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45  
  
Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Cys His His His  
50 55 60  
  
His His His  
65

<210> 269  
<211> 1563  
<212> DNA  
<213> Artificial Sequence  
  
<220>  
<223> Codon optimized DNA for HRS full length protein with 6xHis tag

<400> 269  
atggcggaac gtgccgcact ggaagaattg gttaaattac agggagaacg cgtacgtggt 60  
cttaaacaac aaaaagcctc tgcggaattg attgaagaag aagttgccaa attactgaaa 120  
ctgaaagctc aacttggacc cgatgaaagt aaacaaaaat ttgtgttgaa aacgccc aaa 180  
ggaacccgtg attatagtcc acgtcaaatg gccgttcgtg aaaaagtgtt cgacgttatt 240  
attcgctggt ttaaacgtca cgggtgctgaa gtaatcgata cccccgtatt tgaattgaaa 300  
gagactctga tgggcaaata tgggtgaagat tctaaactga tttatgattt gaaagaccaa 360  
ggaggtgaac tgctgagcct gcgctacgac ttaactgtgc cttttgcccg ttacttagcc 420  
atgaataaat taaccaacat caaacgttac catattgcaa aagtatatcg ccgcgacaac 480  
cctgcaatga ctcggtggacg ctatcgcgaa ttctatcagt gtgattttga tattgccgga 540  
aatttcgacc cgatgatccc ggatgccgag tgtttgaaaa ttatgtgtga aattctgagt 600  
tcgttcgaga tcgggagactt tcttgtaaaa gttaatgacc gccgtattct ggatggtatg 660  
tttgctatatt gcggtgtttc tgattccaaa ttccgtacaa tctgctcaag cgtggacaaa 720  
ttggataaag tgtcttggga agaagtaaaa aatgaaatgg tgggagaaaa aggctggct 780  
ccagaagtag cagaccgtat tggtgactat gttcaacaac atggcggtgt gtccttagtc 840  
gaacagttat tacaggatcc taaactgagc caaaataaac aagcacttga aggactggga 900  
gatctgaaat tactctttga atatctgacc ttatttggga ttgatgataa aattagcttt 960  
gatctgagct tggcccgcgg tcttgattat tataccggcg tgatttacga agctgttctc 1020  
ttgcaaacc cagcccaggc gggcgaagag cctttgggag tcggcagtgt ggcagccggt 1080



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ggtcgttatg atggttttgt aggaatgttt gaccctaaag gccgtaaagt accatgtgtg      1140
gggcttttcta tcggtgtcga acgtatcttt tctattgttg aacaacgtct tgaagctttg      1200
gaggaaaaga tccgtaccac ggaaacccaa gtcttagttg caagtgccca aaaaaaactg      1260
ttagaagaac gcctgaaact cgtatcagaa ctttgggacg ccggcatcaa ggccgaactg      1320
ctgtataaaa agaacccgaa attgttaaac caactccagt attgtgaaga agctgggatc      1380
ccactcgtag ctattattgg tgagcaagaa ttaaaagatg gcgtgattaa actgcgttca      1440
gtaacaagcc gtgaagaggt agatgtacgt cgcgaagact tagtggaaga aattaaacgc      1500
cgcaccgggc aaccgttatg tatttgcgcg gccgcactcg agcaccacca ccaccaccac      1560

tga                                                                           1563

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&lt;210&gt; 270

&lt;211&gt; 520

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; HRS full length protein with 6xHis tag

&lt;400&gt; 270

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Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu
1           5           10           15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu
20          25          30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp
35          40          45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp
50          55          60

Tyr Ser Pro Arg Gln Met Ala Val Arg Glu Lys Val Phe Asp Val Ile
65          70          75          80

Ile Arg Cys Phe Lys Arg His Gly Ala Glu Val Ile Asp Thr Pro Val
85          90          95

Phe Glu Leu Lys Glu Thr Leu Met Gly Lys Tyr Gly Glu Asp Ser Lys
100         105         110

Leu Ile Tyr Asp Leu Lys Asp Gln Gly Gly Glu Leu Leu Ser Leu Arg
115         120         125

Tyr Asp Leu Thr Val Pro Phe Ala Arg Tyr Leu Ala Met Asn Lys Leu
130         135         140

Thr Asn Ile Lys Arg Tyr His Ile Ala Lys Val Tyr Arg Arg Asp Asn
145         150         155         160

Pro Ala Met Thr Arg Gly Arg Tyr Arg Glu Phe Tyr Gln Cys Asp Phe
165         170         175

Asp Ile Ala Gly Asn Phe Asp Pro Met Ile Pro Asp Ala Glu Cys Leu
180         185         190

Lys Ile Met Cys Glu Ile Leu Ser Ser Leu Gln Ile Gly Asp Phe Leu
195         200         205

Val Lys Val Asn Asp Arg Arg Ile Leu Asp Glu Met Phe Ala Ile Cys

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210                215                220                225
Gly Val Ser Asp Ser Lys Phe Arg Thr Ile Cys Ser Ser Val Asp Lys
225                230                235                240

Leu Asp Lys Val Ser Trp Glu Glu Val Lys Asn Glu Met Val Gly Glu
245                250                255

Lys Gly Leu Ala Pro Glu Val Ala Asp Arg Ile Gly Asp Tyr Val Gln
260                265                270

Gln His Gly Gly Val Ser Leu Val Glu Gln Leu Leu Gln Asp Pro Lys
275                280                285

Leu Ser Gln Asn Lys Gln Ala Leu Glu Gly Leu Gly Asp Leu Lys Leu
290                295                300

Leu Phe Glu Tyr Leu Thr Leu Phe Gly Ile Asp Asp Lys Ile Ser Phe
305                310                315                320

Asp Leu Ser Leu Ala Arg Gly Leu Asp Tyr Tyr Thr Gly Val Ile Tyr
325                330                335

Glu Ala Val Leu Leu Gln Thr Pro Ala Gln Ala Gly Glu Glu Pro Leu
340                345                350

Gly Val Gly Ser Val Ala Ala Gly Gly Arg Tyr Asp Gly Leu Val Gly
355                360                365

Met Phe Asp Pro Lys Gly Arg Lys Val Pro Cys Val Gly Leu Ser Ile
370                375                380

Gly Val Glu Arg Ile Phe Ser Ile Val Glu Gln Arg Leu Glu Ala Leu
385                390                395                400

Glu Glu Lys Ile Arg Thr Thr Glu Thr Gln Val Leu Val Ala Ser Ala
405                410                415

Gln Lys Lys Leu Leu Glu Glu Arg Leu Lys Leu Val Ser Glu Leu Trp
420                425                430

Asp Ala Gly Ile Lys Ala Glu Leu Leu Tyr Lys Lys Asn Pro Lys Leu
435                440                445

Leu Asn Gln Leu Gln Tyr Cys Glu Glu Ala Gly Ile Pro Leu Val Ala
450                455                460

Ile Ile Gly Glu Gln Glu Leu Lys Asp Gly Val Ile Lys Leu Arg Ser
465                470                475                480

Val Thr Ser Arg Glu Glu Val Asp Val Arg Arg Glu Asp Leu Val Glu
485                490                495

Glu Ile Lys Arg Arg Thr Gly Gln Pro Leu Cys Ile Cys Ala Ala Ala
500                505                510

Leu Glu His His His His His His
515                520

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&lt;210&gt; 271

&lt;211&gt; 10

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence



<220>  
<223> Cysteine modified HARS peptide fragment

<400> 271  
Val Phe Asp Val Ile Ile Arg Cys Phe Lys  
1 5 10

<210> 272  
<211> 39  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Cysteine modified HARS peptide fragment

<400> 272  
Val Tyr Arg Arg Asp Asn Pro Ala Met Thr Arg Gly Arg Tyr Arg Glu  
1 5 10 15  
  
Phe Tyr Gln Cys Asp Phe Asp Ile Ala Gly Asn Phe Asp Pro Met Ile  
20 25 30  
  
Pro Asp Ala Glu Cys Leu Lys  
35

<210> 273  
<211> 17  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Cysteine modified HARS peptide fragment

<400> 273  
Ile Met Cys Glu Ile Leu Ser Ser Leu Gln Ile Gly Asp Phe Leu Val  
1 5 10 15  
  
Lys

<210> 274  
<211> 20  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Cysteine modified HARS peptide fragment

<400> 274  
Val Asn Asp Arg Arg Ile Leu Asp Gly Met Phe Ala Ile Cys Gly Val  
1 5 10 15  
  
Ser Asp Ser Lys  
20

<210> 275  
<211> 10  
<212> PRT  
<213> Artificial Sequence



<220>  
<223> Cysteine modified HARS peptide fragment

<400> 275  
Phe Arg Thr Ile Cys Ser Ser Val Asp Lys  
1                  5                  10

<210> 276  
<211> 13  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Cysteine modified HARS peptide fragment

<400> 276  
Phe Arg Thr Ile Cys Ser Ser Val Asp Lys Leu Asp Lys  
1                  5                  10

<210> 277  
<211> 27  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Cysteine modified HARS peptide fragment

<400> 277  
Val Pro Cys Val Gly Leu Ser Ile Gly Val Glu Arg Ile Phe Ser Ile  
1                  5                  10                  15

Val Glu Gln Arg Leu Glu Ala Leu Glu Glu Lys  
                  20                  25

<210> 278  
<211> 25  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Cysteine modified HARS peptide fragment

<400> 278  
Leu Leu Asn Gln Leu Gln Tyr Cys Glu Glu Ala Gly Ile Pro Leu Val  
1                  5                  10                  15

Ala Ile Ile Gly Glu Gln Glu Leu Lys  
                  20                  25

<210> 279  
<211> 10  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Cysteine modified HARS peptide fragment

<400> 279  
Arg Arg Thr Gly Gln Pro Leu Cys Ile Cys  
1                  5                  10



<210> 280

<211> 41

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer to selectively mutate cysteine residue

<400> 280

gtttgacgta atcatccggt gcttcaagcg ccacggtgca g 41

<210> 281

<211> 41

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer to selectively mutate cysteine residue

<400> 281

ctgcaccgtg gcgctgaag caacggatga ttacgtcaaa c 41

<210> 282

<211> 44

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer to selectively mutate cysteine residue

<400> 282

gccgataccg ggaattctac cagtgtgatt ttgacattgc tggg 44

<210> 283

<211> 44

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer to selectively mutate cysteine residue

<400> 283

cccagcaatg tcaaaatcac actggtagaa ttcccggtat cggc 44

<210> 284

<211> 41

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer to selectively mutate cysteine residue

<400> 284

ccatgatccc tgatgcagag tgctgaaga tcatgtgcga g 41

<210> 285



<211> 41

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer to selectively mutate cysteine residue

<400> 285

ctcgcacatg atcttcaggc actctgcatc agggatcatg g 41

<210> 286

<211> 43

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer to selectively mutate cysteine residue

<400> 286

gcagagtgcc tgaagatcat gtgcgagatc ctgagttcac ttc 43

<210> 287

<211> 43

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer to selectively mutate cysteine residue

<400> 287

gaagtgaact caggatctcg cacatgatct tcaggcactc tgc 43

<210> 288

<211> 45

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer to selectively mutate cysteine residue

<400> 288

ctagatggga tgttgctat ctgtggtgtt tctgacagca agttc 45

<210> 289

<211> 45

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer to selectively mutate cysteine residue

<400> 289

gaacttgctg tcagaaacac cacagatagc aaacatccca tctag 45

<210> 290

<211> 41



<212> DNA

<213> Artificial Sequence

<220>

<223> Primer to selectively mutate cysteine residue

<400> 290

cagcaagttc cgtaccatct gctcctcagt agacaagctg g 41

<210> 291

<211> 41

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer to selectively mutate cysteine residue

<400> 291

ccagcttgct tactgaggag cagatggtac ggaacttgct g 41

<210> 292

<211> 37

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer to selectively mutate cysteine residue

<400> 292

gggcgcaagg tgccatgtgt ggggctcagc attgggg 37

<210> 293

<211> 37

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer to selectively mutate cysteine residue

<400> 293

ccccaatgct gagccccaca catggcacct tgcgccc 37

<210> 294

<211> 38

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer to selectively mutate cysteine residue

<400> 294

ctgaaccagt tacagtactg tgaggaggca ggcatccc 38

<210> 295

<211> 38

<212> DNA



<213> Artificial Sequence

<220>

<223> Primer to selectively mutate cysteine residue

<400> 295

gggatgcctg cctcctcaca gtactgtaac tgggtcag 38

<210> 296

<211> 39

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer to selectively mutate cysteine residue

<400> 296

gagaacaggc cagcccctct gcatctgcta gaaccacgc 39

<210> 297

<211> 39

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer to selectively mutate cysteine residue

<400> 297

gctgggttct agcagatgca gaggggctgg cctgttctc 39

<210> 298

<211> 37

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer to selectively mutate cysteine residue

<400> 298

ccagcccctc tgcattgct agaaccacgc ttcttg 37

<210> 299

<211> 37

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer to selectively mutate cysteine residue

<400> 299

caagaaagct gggttctagc agatgcagag gggctgg 37

<210> 300

<211> 35

<212> DNA

<213> Artificial Sequence



<220>

<223> Primer to selectively mutate cysteine residue

<400> 300

gaacaggcca gcccctctag aaccagctt tcttg 35

<210> 301

<211> 35

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer to selectively mutate cysteine residue

<400> 301

caagaaagct gggttctaga ggggctggcc tgttc 35

<210> 302

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer to selectively mutate cysteine residue

<400> 302

cccggatgcc gaggcttga aaattatgtg 30

<210> 303

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer to selectively mutate cysteine residue

<400> 303

cacataattt tcaaagcctc ggcacccggg 30

<210> 304

<211> 35

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer to selectively mutate cysteine residue

<400> 304

gatcccggat gccgagagtt tgaaaattat gtgtg 35

<210> 305

<211> 35

<212> DNA

<213> Artificial Sequence

<220>



<223> Primer to selectively mutate cysteine residue

<400> 305

cacacataat ttcaaaactc tcggcatccg ggatc 35

<210> 306

<211> 35

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer to selectively mutate cysteine residue

<400> 306

gatcccggat gccgagggtt tgaaaattat gtgtg 35

<210> 307

<211> 35

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer to selectively mutate cysteine residue

<400> 307

cacacataat ttcaaaacc tcggcatccg ggatc 35

<210> 308

<211> 35

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer to selectively mutate cysteine residue

<400> 308

cgcggaattct atcaggctga tttgatatt gccgg 35

<210> 309

<211> 35

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer to selectively mutate cysteine residue

<400> 309

ccggcaatat caaatcagc ctgatagaat tcgcg 35

<210> 310

<211> 34

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer to selectively mutate cysteine residue



<400> 310  
cgcggaattct atcaggttga tttgatatt gccg 34

<210> 311  
<211> 34  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer to selectively mutate cysteine residue

<400> 311  
cggcaatatc aaaatcaacc tgatagaatt cgcg 34

<210> 312  
<211> 33  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer to selectively mutate cysteine residue

<400> 312  
gggatgtttg ctattccgg tgtttctgat tcc 33

<210> 313  
<211> 33  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer to selectively mutate cysteine residue

<400> 313  
ggaatcagaa acaccgaaa tagcaaacat acc 33

<210> 314  
<211> 39  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer to selectively mutate cysteine residue

<400> 314  
ccaaattccg tacaatctcc tcaagcgtgg acaaattgg 39

<210> 315  
<211> 39  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer to selectively mutate cysteine residue

<400> 315



ccaattgtc cacgctgag gagattgtac ggaatttg 39

<210> 316

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer to selectively mutate cysteine residue

<400> 316

cccggatgcc gaggcttga aaattatgtg 30

<210> 317

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer to selectively mutate cysteine residue

<400> 317

cacataattt tcaaagcctc ggcattccggg 30

<210> 318

<211> 41

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer for tag free HRS polypeptide

<400> 318

cgccgcaccg gtcaaccgtt acaccaccac caccaccact g 41

<210> 319

<211> 41

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer for tag free HRS polypeptide

<400> 319

cagtgggtgt ggtgggtgtg taacggttga ccggtgcggc g 41

<210> 320

<211> 39

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer for tag free HRS polypeptide

<400> 320

cgccgcaccg gtcaaccgtt atgagatccg gctgctaac 39



<210> 321  
 <211> 39  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Primer for tag free HRS polypeptide  
  
 <400> 321  
 gtttagcagcc ggatctcata acggttgacc ggtgcggcg 39

<210> 322  
 <211> 38  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> HRS WHEP consensus sequence

<220>  
 <221> MISC\_FEATURE  
 <222> (2)..(6)  
 <223> Xaa = Any Amino Acid

<220>  
 <221> MISC\_FEATURE  
 <222> (7)..(8)  
 <223> Xaa = Any Amino Acid or absent

<220>  
 <221> MISC\_FEATURE  
 <222> (11)..(12)  
 <223> Xaa = Any Amino Acid

<220>  
 <221> MISC\_FEATURE  
 <222> (15)..(15)  
 <223> Xaa = Any Amino Acid

<220>  
 <221> MISC\_FEATURE  
 <222> (18)..(19)  
 <223> Xaa = Any Amino Acid

<220>  
 <221> MISC\_FEATURE  
 <222> (22)..(28)  
 <223> Xaa = Any Amino Acid

<220>  
 <221> MISC\_FEATURE  
 <222> (29)..(30)  
 <223> Xaa = Any Amino Acid or Absent

<220>



<221> MISC\_FEATURE  
<222> (32)..(33)  
<223> Xaa = Any Amino Acid

<220>  
<221> MISC\_FEATURE  
<222> (36)..(36)  
<223> Xaa = Any Amino Acid

<400> 322  
Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Gln Gly Xaa Xaa Val Arg Xaa Leu  
1 5 10 15  
  
Lys Xaa Xaa Lys Ala Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Val Xaa  
20 25 30  
  
Xaa Leu Leu Xaa Leu Lys  
35

<210> 323  
<211> 60  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer used to create the N-terminally fused HRS(1-60) Fc fusion protein

<400> 323  
taattttgtt taactttaag aaggagatat acatatgtct gacaaaactc acacatgccc 60

<210> 324  
<211> 43  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer used to create the N-terminally fused HRS(1-60) Fc fusion protein

<400> 324  
agcctctccc tgtctccggg taaagcagag cgtgcggcgc tgg 43

<210> 325  
<211> 43  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer used to create the N-terminally fused HRS(1-60) Fc fusion protein

<400> 325  
ccagcgcgcg acgctctgct ttaccgggag acagggagag gct 43

<210> 326  
<211> 57  
<212> DNA  
<213> Artificial Sequence



<220>

<223> Primer used to create the N-terminally fused HRS(1-60) Fc fusion protein

<400> 326

tttgtttaa cttaagaag gagatataca tatgtctgac aaaactcaca catgccc 57

<210> 327

<211> 37

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer used to create the N-terminally fused HRS(1-60) Fc fusion protein

<400> 327

ctctccctgt ctccgggtaa agcagagcgt gcggcgc 37

<210> 328

<211> 37

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer used to create the N-terminally fused HRS(1-60) Fc fusion protein

<400> 328

gcgccgcacg ctctgctta cccggagaca gggagag 37

<210> 329

<211> 58

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer used to create the C-terminally fused HRS(1-60) Fc fusion protein

<400> 329

caaacagaaa tttgtgctca aaacccccaa gtctgacaaa actcacacat gcccaccg 58

<210> 330

<211> 49

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer used to create the C-terminally fused HRS(1-60) Fc fusion protein

<400> 330

agcctctccc tgtctccggg taaatgagat ccggctgcta acaaagccc 49

<210> 331

<211> 49

<212> DNA

<213> Artificial Sequence

<220>



<223> Primer used to create the C-terminally fused HRS(1-60) Fc fusion protein

<400> 331  
gggctttgtt agcagccgga tctcatttac ccggagacag ggagaggct 49

<210> 332  
<211> 50  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer used to create the C-terminally fused HRS(1-60) Fc fusion protein

<400> 332  
cagaaatttg tgctcaaaac cccaagtct gacaaaactc acacatgcc 50

<210> 333  
<211> 43  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer used to create the C-terminally fused HRS(1-60) Fc fusion protein

<400> 333  
ctctccctgt ctccgggtaa atgagatccg gctgctaaca aag 43

<210> 334  
<211> 43  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer used to create the C-terminally fused HRS(1-60) Fc fusion protein

<400> 334  
ctttgttagc agccggatct catttaccog gagacagggga gag 43

<210> 335  
<211> 867  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> DNA sequence of FC-HRS(1-60) (N-terminal Fc fusion)

<400> 335  
atgtctgaca aaactcacac atgcccacog tgcccagcac ctgaactcct ggggggaccg 60  
tcagtcttcc tcttcccccc aaaaccaag gacacctca tgatctcccg gacctctgag 120  
gtcacatgog tgggtggtgga cgtgagccac gaagaccctg aggtcaagtt caactggtac 180  
gtggacggcg tggaggtgca taatgccaag acaaagccgc gggaggagca gtacaacagc 240  
acgtaccgtg tggtcagcgt cctcacctgc ctgcaccagg actggctgaa tggcaaggag 300  
tacaagtgca aggtctccaa caaagccctc ccagccccca tcgagaaaac catctccaaa 360  
gccaaagggc agccccgaga accacaggtg tacacctgc ccccatcccg ggaggagatg 420  
accaagaacc aactcaacct acctacctc atcaaaagct tctatcccaq caacatccc 480



-----  
gtggagtggg agagcaatgg gcagccggag aacaactaca agaccacgcc tcccgtgctg 540  
gactccgacg gctccttctt cctctacagc aagctcaccg tggacaagag caggtggcag 600  
caggggaacg tcttctcatg ctccgtgatg cagcaggctc tgcacaacca ctacacgcag 660  
aagagcctct ccctgtctcc gggtaaagca gagcgtgcgg cgctggagga gctggtgaaa 720  
cttcagggag agcgcgtgcg aggcctcaag cagcagaagg ccagcgccga gctgatcgag 780  
gaggaggtgg cgaaactcct gaaactgaag gcacagctgg gtccctgatga aagcaaacag 840  
aaatttgtgc tcaaaacccc caagtga 867

<210> 336  
<211> 866  
<212> DNA  
<213> Artificial Sequence  
  
<220>  
<223> DNA sequence of HRS(1-60)-Fc (C-terminal Fc fusion)

<400> 336  
atggcagagc gtgcggcgct ggaggagctg gtgaaacttc agggagagcg cgtgcgaggc 60  
ctcaagcagc agaaggccag cgccgagctg atcgaggagg aggtggcgaa actcctgaaa 120  
ctgaaggcac agctgggtcc tgatgaagca aacagaaatt tgtgctcaa accccaagt 180  
ctgacaaaac tcacacatgc ccaccgtgcc cagcacctga actcctgggg ggaccgtcag 240  
tcttcctctt cccccaaaaa cccaaggaca ccctcatgat ctcccgacc cctgaggtca 300  
catgcgtggt ggtggacgtg agccacgaag accctgaggt caagttcaac tggtagctgg 360  
acggcgtgga ggtgcataat gccaaagaaa agccgcggga ggagcagtac aacagcacgt 420  
accgtgtggt cagcgtcttc accgtcctgc accaggactg gctgaatggc aaggagtaca 480  
agtgcaaggt ctccaacaaa gccctcccag ccccatcga gaaaaccatc tccaaagcca 540  
aagggcagcc ccgagaacca caggtgtaca ccctgcccc atcccgagg gagatgacca 600  
agaaccaggt cagcctgacc tgccctggtc aaggcttcta tcccagcgac atcgccgtgg 660  
agtgggagag caatgggcag ccggagaaca actacaagac cagcctccc gtgctggact 720  
ccgacggctc cttcttcctc tacagcaagc tcaccgtgga caagagcagg tggcagcagg 780  
ggaacgtctt ctcatgctcc gtgatgcacg aggctctgca caaccactac acgcagaaga 840  
gcctctccct gtctccgggt aaatga 866

<210> 337  
<211> 288  
<212> PRT  
<213> Artificial Sequence  
  
<220>  
<223> Protein sequence of Fc-HRS(1-60) (N-terminal Fc fusion)

<400> 337  
Met Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu  
1 5 10 15  
  
Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr  
20 25 30  
  
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val  
35 40 45



Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val  
50 55 60

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser  
65 70 75 80

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu  
85 90 95

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala  
100 105 110

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro  
115 120 125

Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln  
130 135 140

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala  
145 150 155 160

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr  
165 170 175

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu  
180 185 190

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser  
195 200 205

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser  
210 215 220

Leu Ser Pro Gly Lys Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys  
225 230 235 240

Leu Gln Gly Glu Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala  
245 250 255

Glu Leu Ile Glu Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln  
260 265 270

Leu Gly Pro Asp Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys  
275 280 285

<210> 338

<211> 288

<212> PRT

<213> Artificial Sequence

<220>

<223> Protein sequence of HRS(1-60)-Fc (C-terminal Fc fusion)

<400> 338

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45



Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Ser Asp Lys Thr  
50 55 60

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser  
65 70 75 80

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg  
85 90 95

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro  
100 105 110

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala  
115 120 125

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val  
130 135 140

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr  
145 150 155 160

Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr  
165 170 175

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu  
180 185 190

Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys  
195 200 205

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser  
210 215 220

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp  
225 230 235 240

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser  
245 250 255

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala  
260 265 270

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys  
275 280 285

<210> 339

<211> 229

<212> PRT

<213> Homo sapiens

<400> 339

Met Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu  
1 5 10 15

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr  
20 25 30

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val  
35 40 45

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val  
50 55 60



Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser  
65 70 75 80

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu  
85 90 95

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala  
100 105 110

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro  
115 120 125

Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln  
130 135 140

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala  
145 150 155 160

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr  
165 170 175

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu  
180 185 190

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser  
195 200 205

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser  
210 215 220

Leu Ser Pro Gly Lys  
225

<210> 340

<211> 228

<212> PRT

<213> Homo sapiens

<400> 340

Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu  
1 5 10 15

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu  
20 25 30

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser  
35 40 45

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu  
50 55 60

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr  
65 70 75 80

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn  
85 90 95

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro  
100 105 110

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln  
115 120 125



Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val  
130 135 140

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val  
145 150 155 160

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro  
165 170 175

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr  
180 185 190

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val  
195 200 205

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu  
210 215 220

Ser Pro Gly Lys  
225

<210> 341

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> modified human IgG1 hinge region

<400> 341

Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro  
1 5 10

<210> 342

<211> 229

<212> PRT

<213> Homo sapiens

<400> 342

Met Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu  
1 5 10 15

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr  
20 25 30

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val  
35 40 45

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val  
50 55 60

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser  
65 70 75 80

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu  
85 90 95

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala  
100 105 110

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro  
115 120 125



113

120

123

Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln

130135140

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala

145150155160

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr

165170175

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu

180185190

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser

195200205

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser

210215220

Leu Ser Pro Gly Lys

225

<210> 343

<211> 34

<212> DNA

<213> Artificial Sequence

<220>

<223> primer to amplify Fc-HRS (2-60)

<400> 343

gatatacata tgtctgacaa aactcacaca tgcc 34

<210> 344

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> primer to amplify Fc-HRS (2-60)

<400> 344

gatacctcgag tcacttgggg gttttg 26

<210> 345

<211> 25

<212> DNA

<213> Artificial Sequence

<220>

<223> primer to amplify HRS (1-60)-Fc

<400> 345

gatatacata tggcagagcg tgcgg 25

<210> 346

<211> 26

<212> DNA



<213> Artificial Sequence

<220>

<223> primer to amplify HRS (1-60)-Fc

<400> 346

gatcctcgag tcatttacct ggagac 26

<210> 347

<211> 867

<212> DNA

<213> Artificial Sequence

<220>

<223> DNA sequence of Fc-HRS(2-60)

<400> 347

|                                                                    |     |
|--------------------------------------------------------------------|-----|
| atgtctgaca aaactcacac atgcccaccg tgcccagcac ctgaactcct ggggggaccg  | 60  |
| tcagtcttcc tcttcccccc aaaacccaag gacaccctca tgatctcccg gaccctgag   | 120 |
| gtcacatgag tgggtggtgga cgtgagccac gaagaccctg aggtcaagtt caactggtac | 180 |
| gtggacggcg tggaggtgca taatgccaag acaaagccgc gggaggagca gtacaacagc  | 240 |
| acgtaccgtg tggtcagcgt cctcacctgc ctgcaccagg actggctgaa tggcaaggag  | 300 |
| tacaagtgca aggtctccaa caaagccctc ccagccccca tcgagaaaac catctccaaa  | 360 |
| gccaaagggc agccccgaga accacaggtg tacaccctgc ccccatcccg ggaggagatg  | 420 |
| accaagaacc aggtcagcct gacctgcctg gtcaaaggct tctatcccag cgacatcgcc  | 480 |
| gtggagtggg agagcaatgg gcagccggag aacaactaca agaccacgcc tcccgtgctg  | 540 |
| gactccgacg gctccttctt cctctacagc aagctcaccg tggacaagag caggtggcag  | 600 |
| caggggaaag tcttctcatg ctccgtgatg cagcaggctc tgcacaacca ctacacgcag  | 660 |
| aagagcctct ccctgtctcc gggtaaagca gagcgtgcgg cgctggagga gctggtgaaa  | 720 |
| cttcaggag agcgcgtgag aggcctcaag cagcagaagg ccagcggcga gctgatcgag   | 780 |
| gaggaggtgg cgaaactcct gaaactgaag gcacagctgg gtccctgatga aagcaaacag | 840 |
| aaatttgtgc tcaaaacccc caagtga                                      | 867 |

<210> 348

<211> 867

<212> DNA

<213> Artificial Sequence

<220>

<223> DNA sequence of HRS(1-60)-Fc (C-terminal Fc fusion)

<400> 348

|                                                                   |     |
|-------------------------------------------------------------------|-----|
| atggcagagc gtgcggcgct ggaggagctg gtgaaacttc agggagagcg cgtgcgaggc | 60  |
| ctcaagcagc agaaggccag cgccgagctg atcgaggagg aggtggcgaa actcctgaaa | 120 |
| ctgaaggcac agctgggtcc tgatgaaagc aaacagaaat ttgtgctcaa aacccccaa  | 180 |
| tctgacaaaa ctcacacatg cccaccgtgc ccagcacctg aactcctggg gggaccgtca | 240 |
| gtcttctctt tccccccaaa acccaaggac accctcatga tctccgggac ccctgaggtc | 300 |
| acatgcgtgg tgggtggcgt gagccacgaa gaccctgagg tcaagttcaa ctggtacgtg | 360 |
| gacggcgtgg aggtgcataa tgccaagaca aagccgcggg aggagcagta caacagcacg | 420 |
| taccgtgtgg tcagcgtcct caccgtcctg caccaggact ggctgaatgg caaggagtac | 480 |



```

aagtgcagg tctccaacaa agccctccca gcccccatcg agaaaacat ctccaaagcc 540
aaagggcagc cccgagaacc acaggtgtac accctgcccc catcccggga ggagatgacc 600
aagaaccagg tcagcctgac ctgcctggtc aaaggcttct atcccagcga catcgccgtg 660
gagtgggaga gcaatgggca gccggagAAC aactacaaga ccacgcctcc cgtgctggac 720
tccgaagggt ccttcttctt ctacagcaag ctcaccgtgg acaagagcag gtggcagcag 780
gggaacgtct tctcatgctc cgtgatgcac gaggtctctg acaaccacta cagcagaag 840
agcctctccc tgtctccggg taaatga 867

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&lt;210&gt; 349

&lt;211&gt; 288

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Protein sequence of Fc-HRS(2-60) (N-terminal Fc fusion)

&lt;400&gt; 349

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Met Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
1          5          10          15

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
20          25          30

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
35          40          45

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
50          55          60

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
65          70          75          80

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
85          90          95

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
100         105         110

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
115         120         125

Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
130         135         140

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
145         150         155         160

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
165         170         175

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
180         185         190

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
195         200         205

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
210         215         220

Leu Ser Pro Gly Lys Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys
225         230         235         240

```



Leu Gln Gly Glu Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala  
245 250 255

Glu Leu Ile Glu Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln  
260 265 270

Leu Gly Pro Asp Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys  
275 280 285

<210> 350

<211> 288

<212> PRT

<213> Artificial Sequence

<220>

<223> Protein sequence of HRS(1-60)-Fc (C-terminal Fc fusion)

<400> 350

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp  
35 40 45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Ser Asp Lys Thr  
50 55 60

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser  
65 70 75 80

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg  
85 90 95

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro  
100 105 110

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala  
115 120 125

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val  
130 135 140

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr  
145 150 155 160

Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr  
165 170 175

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu  
180 185 190

Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys  
195 200 205

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser  
210 215 220

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp  
225 230 235 240

Asn Ser Glu Ser Phe Phe Tyr Thr Ser Tyr Tyr Thr Val Ser Tyr Ser



Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser  
245 250 255

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala  
260 265 270

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys  
275 280 285

- <210> 351
- <211> 39
- <212> DNA
- <213> Artificial Sequence
- <220>
- <223> primer to amplify Fc-HRS polypeptides
- <400> 351
- ggtggcgaaa ctctgaaat gactcgagga tccggctgc 39
- <210> 352
- <211> 39
- <212> DNA
- <213> Artificial Sequence
- <220>
- <223> primer to amplify Fc-HRS polypeptides
- <400> 352
- gcagccgga cctcgagtca ttcaggagt ttcgccacc 39
- <210> 353
- <211> 35
- <212> DNA
- <213> Artificial Sequence
- <220>
- <223> primer to amplify Fc-HRS polypeptides
- <400> 353
- ctgaaggcac agctgtgact cgaggatccg gctgc 35
- <210> 354
- <211> 35
- <212> DNA
- <213> Artificial Sequence
- <220>
- <223> primer to amplify Fc-HRS polypeptides
- <400> 354
- gcagccgga cctcgagtca cagctgtgcc ttcag 35
- <210> 355
- <211> 36
- <212> DNA
- <213> Artificial Sequence



<220>

<223> primer to amplify Fc-HRS polypeptides

<400> 355

gggtcctgat gaaagctgac tcgaggatcc ggctgc 36

<210> 356

<211> 36

<212> DNA

<213> Artificial Sequence

<220>

<223> primer to amplify Fc-HRS polypeptides

<400> 356

gcagccggat cctcgagtca gctttcatca ggaccc 36

<210> 357

<211> 37

<212> DNA

<213> Artificial Sequence

<220>

<223> primer to amplify Fc-HRS polypeptides

<400> 357

gcaaacagaa atttgtgtga ctcgaggatc cggctgc 37

<210> 358

<211> 37

<212> DNA

<213> Artificial Sequence

<220>

<223> primer to amplify Fc-HRS polypeptides

<400> 358

gcagccggat cctcgagtca cacaaatttc tgtttgc 37

<210> 359

<211> 50

<212> DNA

<213> Artificial Sequence

<220>

<223> primer to amplify Fc-HRS polypeptides

<400> 359

gctcaaaacc cccaagggaa cccgtgatta tagttgactc gaggatccgg 50

<210> 360

<211> 50

<212> DNA

<213> Artificial Sequence

<220>



<223> primer to amplify Fc-HRS polypeptides

<400> 360

cgggacctc gagtcaacta taatcacggg ttccttggg ggtttgagc 50

<210> 361

<211> 40

<212> DNA

<213> Artificial Sequence

<220>

<223> primer to amplify HRS-Fc polypeptides

<400> 361

ggtggcgaaa ctctgaaat ctgacaaaac tcacacatgc 40

<210> 362

<211> 40

<212> DNA

<213> Artificial Sequence

<220>

<223> primer to amplify HRS-Fc polypeptides

<400> 362

gcatgtgtga gttttgtcag atttcaggag ttgcgccacc 40

<210> 363

<211> 42

<212> DNA

<213> Artificial Sequence

<220>

<223> primer to amplify HRS-Fc polypeptides

<400> 363

ctgaaactga aggcacagct gtctgacaaa actcacacat gc 42

<210> 364

<211> 42

<212> DNA

<213> Artificial Sequence

<220>

<223> primer to amplify HRS-Fc polypeptides

<400> 364

gcatgtgtga gttttgtcag acagctgtgc cttcagtttc ag 42

<210> 365

<211> 40

<212> DNA

<213> Artificial Sequence

<220>

<223> primer to amplify HRS-Fc polypeptides



<400> 365  
gctgggtcct gatgaaagct ctgacaaaac tcacacatgc 40

<210> 366  
<211> 40  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> primer to amplify HRS-Fc polypeptides

<400> 366  
gcatgtgtga gttttgtcag agctttcatc aggaccagc 40

<210> 367  
<211> 42  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> primer to amplify HRS-Fc polypeptides

<400> 367  
gaaagcaaac agaaattgt gtctgacaaa actcacacat gc 42

<210> 368  
<211> 42  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> primer to amplify HRS-Fc polypeptides

<400> 368  
gcatgtgtga gttttgtcag acacaaattt ctgttgctt tc 42

<210> 369  
<211> 49  
<212> DNA  
<213> Artificial Sequenc

<220>  
<223> primer to amplify HRS-Fc polypeptides

<400> 369  
gctcaaaacc cccaaggga cccgtgatta tagttctgac aaaactcac 49

<210> 370  
<211> 49  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> primer to amplify HRS-Fc polypeptides

<400> 370



gtgagttttg tcagaactat aatcacgggt tccctgggg gttttgagc 49

<210> 371

<211> 807

<212> DNA

<213> Artificial Sequence

<220>

<223> DNA sequence of Fc-HRS (2-40) (N-terminal Fc fusion)

<400> 371

|                                                                    |     |
|--------------------------------------------------------------------|-----|
| atgtctgaca aaactcacac atgcccaccg tgcccagcac ctgaactcct ggggggaccg  | 60  |
| tcagtcttcc tcttcccccc aaaacccaag gacaccctca tgatctcccg gaccctgag   | 120 |
| gtcacatgcg tgggtggtgga cgtgagccac gaagaccctg aggtcaagtt caactggtac | 180 |
| gtggacggcg tggaggtgca taatgccaaag acaaagccgc gggaggagca gtacaacagc | 240 |
| acgtaccgtg tggtcagcgt cctcacccgc ctgcaccagg actggctgaa tggcaaggag  | 300 |
| tacaagtgca aggtctccaa caaagccctc ccagccccc tcgagaaaac catctccaaa   | 360 |
| gccaaagggc agccccgaga accacaggtg tacaccctgc ccccatcccg ggaggagatg  | 420 |
| accaagaacc aggtcagcct gacctgctg gtcaaaggct tctatcccag cgacatcgcc   | 480 |
| gtggagtggg agagcaatgg gcagccggag aacaactaca agaccacgcc tcccgctgctg | 540 |
| gactccgacg gctccttctt cctctacagc aagctcaccg tggacaagag caggtggcag  | 600 |
| caggggaacg tcttctcatg ctccgtgatg cagcaggctc tgcacaacca ctacacgcag  | 660 |
| aagagcctct ccctgtctcc gggtaaagca gagcgtgcgg cgctggagga gctggtgaaa  | 720 |
| cttcagggag agcgcgtgcg aggcctcaag cagcagaagg ccagcgccga gctgatcgag  | 780 |
| gaggaggtgg cgaaactcct gaaatga                                      | 807 |

<210> 372

<211> 822

<212> DNA

<213> Artificial Sequence

<220>

<223> DNA sequence of Fc-HRS (2-45) (N-terminal Fc fusion)

<400> 372

|                                                                    |     |
|--------------------------------------------------------------------|-----|
| atgtctgaca aaactcacac atgcccaccg tgcccagcac ctgaactcct ggggggaccg  | 60  |
| tcagtcttcc tcttcccccc aaaacccaag gacaccctca tgatctcccg gaccctgag   | 120 |
| gtcacatgcg tgggtggtgga cgtgagccac gaagaccctg aggtcaagtt caactggtac | 180 |
| gtggacggcg tggaggtgca taatgccaaag acaaagccgc gggaggagca gtacaacagc | 240 |
| acgtaccgtg tggtcagcgt cctcacccgc ctgcaccagg actggctgaa tggcaaggag  | 300 |
| tacaagtgca aggtctccaa caaagccctc ccagccccc tcgagaaaac catctccaaa   | 360 |
| gccaaagggc agccccgaga accacaggtg tacaccctgc ccccatcccg ggaggagatg  | 420 |
| accaagaacc aggtcagcct gacctgctg gtcaaaggct tctatcccag cgacatcgcc   | 480 |
| gtggagtggg agagcaatgg gcagccggag aacaactaca agaccacgcc tcccgctgctg | 540 |
| gactccgacg gctccttctt cctctacagc aagctcaccg tggacaagag caggtggcag  | 600 |
| caggggaacg tcttctcatg ctccgtgatg cagcaggctc tgcacaacca ctacacgcag  | 660 |
| aagagcctct ccctgtctcc gggtaaagca gagcgtgcgg cgctggagga gctggtgaaa  | 720 |
| cttcagggag agcgcgtgcg aggcctcaag cagcagaagg ccagcgccga gctgatcgag  | 780 |
| gaggaggtgg cgaaactcct gaaatga                                      | 807 |



gaggaggtgg cgaaactcct gaaactgaag gcacagctgt ga

822

<210> 373

<211> 837

<212> DNA

<213> Artificial Sequence

<220>

<223> DNA sequence of Fc-HRS (2-50) (N-terminal Fc fusion)

<400> 373

|                                                                    |     |
|--------------------------------------------------------------------|-----|
| atgtctgaca aaactcacac atgcccaccg tgcccagcac ctgaactcct ggggggaccg  | 60  |
| tcagtcttcc tcttcccccc aaaacccaag gacaccctca tgatctcccg gaccctgag   | 120 |
| gtcacatgcg tggtggtgga cgtgagccac gaagaccctg aggtcaagtt caactggtac  | 180 |
| gtggacggcg tggaggtgca taatgccaaag acaaagccgc gggaggagca gtacaacagc | 240 |
| acgtaccgtg tggtcagcgt cctcaccgtc ctgcaccagg actggctgaa tggcaaggag  | 300 |
| tacaagtgca aggtctccaa caaagccctc ccagccccca tcgagaaaac catctccaaa  | 360 |
| gccaaagggc agccccgaga accacaggtg tacaccctgc ccccatcccg ggaggagatg  | 420 |
| accaagaacc aggtcagcct gacctgcctg gtcaaaggct tctatcccag cgacatcgcc  | 480 |
| gtggagtggg agagcaatgg gcagccggag aacaactaca agaccacgcc tcccgctgctg | 540 |
| gactccgacg gctccttctt cctctacagc aagctcaccg tggacaagag caggtggcag  | 600 |
| caggggaacg tcttctcatg ctccgtgatg cagcaggctc tgcacaacca ctacacgcag  | 660 |
| aagagcctct ccctgtctcc gggtaaagca gagcgtgcgg cgctggagga gctggtgaaa  | 720 |
| cttcagggag agcgcgtgcg aggcctcaag cagcagaagg ccagcggcga gctgatcgag  | 780 |
| gaggaggtgg cgaaactcct gaaactgaag gcacagctgg gtccctgatga aagctga    | 837 |

<210> 374

<211> 852

<212> DNA

<213> Artificial Sequence

<220>

<223> DNA sequence of Fc-HRS (2-55) (N-terminal Fc fusion)

<400> 374

|                                                                    |     |
|--------------------------------------------------------------------|-----|
| atgtctgaca aaactcacac atgcccaccg tgcccagcac ctgaactcct ggggggaccg  | 60  |
| tcagtcttcc tcttcccccc aaaacccaag gacaccctca tgatctcccg gaccctgag   | 120 |
| gtcacatgcg tggtggtgga cgtgagccac gaagaccctg aggtcaagtt caactggtac  | 180 |
| gtggacggcg tggaggtgca taatgccaaag acaaagccgc gggaggagca gtacaacagc | 240 |
| acgtaccgtg tggtcagcgt cctcaccgtc ctgcaccagg actggctgaa tggcaaggag  | 300 |
| tacaagtgca aggtctccaa caaagccctc ccagccccca tcgagaaaac catctccaaa  | 360 |
| gccaaagggc agccccgaga accacaggtg tacaccctgc ccccatcccg ggaggagatg  | 420 |
| accaagaacc aggtcagcct gacctgcctg gtcaaaggct tctatcccag cgacatcgcc  | 480 |
| gtggagtggg agagcaatgg gcagccggag aacaactaca agaccacgcc tcccgctgctg | 540 |
| gactccgacg gctccttctt cctctacagc aagctcaccg tggacaagag caggtggcag  | 600 |
| caggggaacg tcttctcatg ctccgtgatg cagcaggctc tgcacaacca ctacacgcag  | 660 |
| aagagcctct ccctgtctcc gggtaaagca gagcgtgcgg cgctggagga gctggtgaaa  | 720 |
| cttcagggag agcgcgtgcg aggcctcaag cagcagaagg ccagcggcga gctgatcgag  | 780 |
| gaggaggtgg cgaaactcct gaaactgaag gcacagctgg gtccctgatga aagcaaacag | 840 |



aaatttgtgt ga 852

<210> 375

<211> 885

<212> DNA

<213> Artificial Sequence

<220>

<223> DNA sequence of Fc-HRS (2-66) (N-terminal Fc fusion)

<400> 375

|                                                                     |     |
|---------------------------------------------------------------------|-----|
| atgtctgaca aaactcacac atgcccaccg tgcccagcac ctgaactcct ggggggaccg   | 60  |
| tcagtctttcc tcttcccccc aaaacccaag gacaccctca tgatctcccg gaccctgag   | 120 |
| gtcacatgcg tggtggtgga cgtgagccac gaagaccctg aggtcaagtt caactggtac   | 180 |
| gtggacggcg tggaggtgca taatgccaaag acaaagccgc gggaggagca gtacaacagc  | 240 |
| acgtaccgtg tggtcagcgt cctcaccgtc ctgcaccagg actgggtgaa tggcaaggag   | 300 |
| tacaagtgca aggtctccaa caaagccctc ccagccccc tggagaaaac catctccaaa    | 360 |
| gccaaagggc agccccgaga accacaggtg tacaccctgc ccccatcccg ggaggagatg   | 420 |
| accaagaacc aggtcagcct gacctgctg gtcaaaggct tctatcccag cgacatcgcc    | 480 |
| gtggagtggt agagcaatgg gcagccggag aacaactaca agaccacgcc tcccgctgtg   | 540 |
| gactccgacg gctccttctt cctctacagc aagctcaccg tggacaagag caggtggcag   | 600 |
| caggggaaacg tcttctcatg ctccgtgatg cagcaggctc tgcaacaacca ctacacgcag | 660 |
| aagagcctct ccctgtctcc gggtaaagca gagcgtgcgg cgctggagga gctggtgaaa   | 720 |
| cttcagggag agcgcgtgcg aggcctcaag cagcagaagg ccagcgccga gctgatcgag   | 780 |
| gaggaggtgg cgaaactcct gaaactgaag gcacagctgg gtcctgatga aagcaaacag   | 840 |
| aaatttgtgc tcaaaacccc caaggaacc cgtgattata gttga                    | 885 |

<210> 376

<211> 807

<212> DNA

<213> Artificial Sequence

<220>

<223> DNA sequence of HRS(1-40)-Fc (C-terminal Fc fusion)

<400> 376

|                                                                   |     |
|-------------------------------------------------------------------|-----|
| atggcagagc gtgcggcgct ggaggagctg gtgaaacttc agggagagcg cgtgcgaggc | 60  |
| ctcaagcagc agaaggccag cgccgagctg atcgaggagg aggtggcgaa actcctgaaa | 120 |
| tctgacaaaa ctcacacatg cccaccgtgc ccagcacctg aactcctggg gggaccgtca | 180 |
| gtcttcctct tcccccaaa acccaaggac accctcatga tctcccggac ccctgaggtc  | 240 |
| acatgcgtgg tggtggaagt gagccacgaa gaccctgagg tcaagttcaa ctggtacgtg | 300 |
| gacggcgtgg aggtgcataa tgccaagaca aagccgcggg aggagcagta caacagcacg | 360 |
| taccgtgtgg tcagcgtcct caccgtcctg caccaggact ggctgaatgg caaggagtac | 420 |
| aagtgcaagg tctccaacaa agccctccca gccccatcg agaaaaccat ctccaaagcc  | 480 |
| aaagggcagc cccgagaacc acaggtgtac accctgcccc catcccggga ggagatgacc | 540 |
| aagaaccagg tcagcctgac ctgcctggtc aaaggcttct atcccagcga catcgccgtg | 600 |
| gagtgggaga gcaatgggca gccggagaac aactacaaga ccacgcctcc cgtgctggac | 660 |
| tccgacggct ccttcttctt ctacagcaag ctaccctggg acaagagcag gtggcagcag | 720 |



gggaacgtct tctcatgctc cgtgatgcac gaggtctctgc acaaccacta cagcgagaag 780  
agcctctccc tgtctccggg taaatga 807

<210> 377

<211> 822

<212> DNA

<213> Artificial Sequence

<220>

<223> DNA sequence of HRS(1-45)-Fc (C-terminal Fc fusion)

<400> 377

atggcagagc gtgcggcgct ggaggagctg gtgaaacttc agggagagcg cgtgcgaggc 60  
ctcaagcagc agaaggccag cgcagagctg atcgaggagg aggtggcgaa actcctgaaa 120  
ctgaaggcac agctgtctga caaaactcac acatgcccac cgtgcccagc acctgaactc 180  
ctgggggggac cgtcagtctt cctcttcccc ccaaaaccca aggacaccct catgatctcc 240  
cggacccctg aggtcacatg cgtggtggtg gacgtgagcc acgaagacc tgaggtcaag 300  
ttcaactggt acgtggacgg cgtggagggtg cataatgcc aagacaaagc gcgggaggag 360  
cagtacaaca gcacgtaccg tgtggtcagc gtctcaccg tctgcacca ggactggctg 420  
aatggcaagg agtacaagtg caaggtctcc aacaaagccc tcccagcccc catcgagaaa 480  
accatctcca aagccaaagg gcagccccga gaaccacagg tgtacaccct gccccatcc 540  
cgggaggaga tgaccaagaa ccaggtcagc ctgacctgcc tggtaaagg cttctatccc 600  
agcgacatcg ccgtggagtg ggagagcaat gggcagccgg agaacaacta caagaccacg 660  
cctcccgtgc tggactccga cggctccttc ttctctaca gcaagctcac cgtggacaag 720  
agcaggtggc agcaggggaa cgtcttctca tgctccgtga tgcacgagc tctgcacaac 780  
cactacacgc agaagagcct ctccctgtct ccgggtaaat ga 822

<210> 378

<211> 837

<212> DNA

<213> Artificial Sequence

<220>

<223> DNA sequence of HRS(1-50)-Fc (C-terminal Fc fusion)

<400> 378

atggcagagc gtgcggcgct ggaggagctg gtgaaacttc agggagagcg cgtgcgaggc 60  
ctcaagcagc agaaggccag cgcagagctg atcgaggagg aggtggcgaa actcctgaaa 120  
ctgaaggcac agctgggtcc tgatgaaagc tctgacaaaa ctcacacatg cccaccgtgc 180  
ccagcacctg aactcctggg gggaccgtca gtcttctct tcccccaaa acccaaggac 240  
accctcatga tctcccgga cctgaggtc acatgcgtgg tggaggacgt gagccacgaa 300  
gaccctgagg tcaagttcaa ctggtacgtg gacggcgtgg aggtgcataa tgccaagaca 360  
aagccgcggg aggagcagta caacagcacg taccgtgtgg tcagcgtcct caccgtcctg 420  
caccaggact ggctgaatgg caaggagtac aagtgcagg tctccaacaa agccctccca 480  
gccccatcg agaaaaccat ctccaaagcc aaagggcagc cccgagaacc acaggtgtac 540  
accctgcccc catcccgga ggagatgacc aagaaccagg tcagcctgac ctgcctggtc 600  
aaaggcttct atcccagcga catcgccgtg gagtgggaga gcaatgggca gccggagaa 660  
aactacaaga ccacgcctcc cgtgctggac tccgacggct ccttcttct ctacagcaag 720  
ctacacatcg aagcagcagc ctggagcagc aggaagctct tctgctgctc gctgctgagc 780



```

ctcaccgctgg acaagagcag ggggcagcag gggaaagctc cctcatgctc cgtgatgcac      780
gaggctctgc acaaccacta caccgagaag agcctctccc tgtctccggg taaatga      837

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&lt;210&gt; 379

&lt;211&gt; 852

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; DNA sequence of HRS(1-55)-Fc (C-terminal Fc fusion)

&lt;400&gt; 379

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atggcagagc gtgcggcgct ggaggagctg gtgaaacttc agggagagcg cgtgcgaggc      60
ctcaagcagc agaaggccag cggcgagctg atcgaggagg aggtggcgaa actcctgaaa      120
ctgaaggcac agctgggtcc tgatgaaagc aaacagaaat ttgtgtctga caaaactcac      180
acatgcccac cgtgcccagc acctgaactc ctgggggggac cgtcagtctt cctcttcccc      240
ccaaaaccca aggacacct catgatctcc cggaccctct aggtcacatg cgtggtggtg      300
gacgtgagcc acgaagacc tgagggtcaag ttcaactggt acgtggacgg cgtggaggtg      360
cataatgcc agacaaagcc gggggaggag cagtacaaca gcacgtaccg tgtggtcagc      420
gtcctcaccg tcttgacca ggactggctg aatggcaagg agtacaagtg caaggtctcc      480
aacaagccc tcccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga      540
gaaccacagg tgtacacct gccccatcc cgggaggaga tgaccaagaa ccaggtcagc      600
ctgacctgcc tgggtcaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat      660
gggcagccgg agaacaacta caagaccacg cctcccgtgc tggactccga cggctccttc      720
ttcctctaca gcaagctcac cgtggacaag agcagggtggc agcaggggaa cgtcttctca      780
tgctccgtga tgcacgaggc tctgcacaac cactacacgc agaagagcct ctccctgtct      840
ccgggtaaat ga      852

```

&lt;210&gt; 380

&lt;211&gt; 885

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; DNA sequence of HRS(1-66)-Fc (C-terminal Fc fusion)

&lt;400&gt; 380

```

atggcagagc gtgcggcgct ggaggagctg gtgaaacttc agggagagcg cgtgcgaggc      60
ctcaagcagc agaaggccag cggcgagctg atcgaggagg aggtggcgaa actcctgaaa      120
ctgaaggcac agctgggtcc tgatgaaagc aaacagaaat ttgtgtctca aacccccaa      180
ggaacccgtg attatagttc tgacaaaact cacacatgcc caccgtgcc agcacctgaa      240
ctcctggggg gaccgtcagt cttcctcttc cccccaaaac ccaaggacac cctcatgac      300
tcccggaccc ctgaggtcac atgcgtggtg gtggacgtga gccacgaaga ccctgaggtc      360
aagttcaact ggtacgtgga cggcgtggag gtgcataatg ccaagacaaa gccgcgggag      420
gagcagtaca acagcacgta ccgtgtggtc agcgtcctca ccgtcctgca ccaggactgg      480
ctgaatggca aggagtacaa gtgcaaggtc tccaacaaag ccctcccagc ccccatcgag      540
aaaaccatct ccaaagccaa agggcagccc cgagaaccac aggtgtacac cctgccccca      600
tcccgggagg agatgaccaa gaaccaggtc agcctgacct gcctggtcaa aggcttctat      660
cccagcgaca tcgcctgga gtgggagagc aatgggcagc cggagaacaa ctacaagacc      720

```



```

acgcctcccg tgctggactc cgacggctcc ttcttctct acagcaagct caccgtggac      780
aagagcaggt ggcagcaggg gaacgtcttc tcatgctccg tgatgcacga ggctctgcac      840
aaccactaca cgcagaagag cctctccctg tctccgggta aatga                      885

```

&lt;210&gt; 381

&lt;211&gt; 268

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Protein sequence of Fc-HRS (2-40) (N-terminal Fc fusion)

&lt;400&gt; 381

```

Met Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
1      5      10      15

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
20      25      30

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
35      40      45

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
50      55      60

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
65      70      75      80

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
85      90      95

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
100     105     110

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
115     120     125

Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
130     135     140

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
145     150     155     160

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
165     170     175

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
180     185     190

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
195     200     205

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
210     215     220

Leu Ser Pro Gly Lys Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys
225     230     235     240

Leu Gln Gly Glu Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala
245     250     255

```



Glu Leu Ile Glu Glu Glu Val Ala Lys Leu Leu Lys  
260 265

<210> 382

<211> 273

<212> PRT

<213> Artificial Sequence

<220>

<223> Protein sequence of Fc-HRS (2-45) (N-terminal Fc fusion)

<400> 382

Met Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu  
1 5 10 15

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr  
20 25 30

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val  
35 40 45

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val  
50 55 60

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser  
65 70 75 80

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu  
85 90 95

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala  
100 105 110

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro  
115 120 125

Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln  
130 135 140

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala  
145 150 155 160

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr  
165 170 175

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu  
180 185 190

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser  
195 200 205

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser  
210 215 220

Leu Ser Pro Gly Lys Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys  
225 230 235 240

Leu Gln Gly Glu Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala  
245 250 255

Glu Leu Ile Glu Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln  
260 265 270



Leu

&lt;210&gt; 383

&lt;211&gt; 278

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Protein sequence of Fc-HRS (2-50) (N-terminal Fc fusion)

&lt;400&gt; 383

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Ser | Asp | Lys | Thr | His | Thr | Cys | Pro | Pro | Cys | Pro | Ala | Pro | Glu | Leu |
| 1   |     |     |     | 5   |     |     |     | 10  |     |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Gly | Gly | Pro | Ser | Val | Phe | Leu | Phe | Pro | Pro | Lys | Pro | Lys | Asp | Thr |
|     |     |     | 20  |     |     |     | 25  |     |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Met | Ile | Ser | Arg | Thr | Pro | Glu | Val | Thr | Cys | Val | Val | Val | Asp | Val |
|     | 35  |     |     |     |     |     | 40  |     |     |     | 45  |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | His | Glu | Asp | Pro | Glu | Val | Lys | Phe | Asn | Trp | Tyr | Val | Asp | Gly | Val |
|     | 50  |     |     |     |     | 55  |     |     |     | 60  |     |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Val | His | Asn | Ala | Lys | Thr | Lys | Pro | Arg | Glu | Glu | Gln | Tyr | Asn | Ser |
| 65  |     |     |     |     | 70  |     |     |     | 75  |     |     |     |     |     | 80  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thr | Tyr | Arg | Val | Val | Ser | Val | Leu | Thr | Val | Leu | His | Gln | Asp | Trp | Leu |
|     |     |     | 85  |     |     |     |     | 90  |     |     |     |     |     | 95  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asn | Gly | Lys | Glu | Tyr | Lys | Cys | Lys | Val | Ser | Asn | Lys | Ala | Leu | Pro | Ala |
|     |     | 100 |     |     |     |     |     | 105 |     |     |     |     | 110 |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Ile | Glu | Lys | Thr | Ile | Ser | Lys | Ala | Lys | Gly | Gln | Pro | Arg | Glu | Pro |
|     | 115 |     |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Val | Tyr | Thr | Leu | Pro | Pro | Ser | Arg | Glu | Glu | Met | Thr | Lys | Asn | Gln |
|     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Ser | Leu | Thr | Cys | Leu | Val | Lys | Gly | Phe | Tyr | Pro | Ser | Asp | Ile | Ala |
| 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Glu | Trp | Glu | Ser | Asn | Gly | Gln | Pro | Glu | Asn | Asn | Tyr | Lys | Thr | Thr |
|     |     |     | 165 |     |     |     |     | 170 |     |     |     |     |     | 175 |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Pro | Val | Leu | Asp | Ser | Asp | Gly | Ser | Phe | Phe | Leu | Tyr | Ser | Lys | Leu |
|     |     | 180 |     |     |     |     |     | 185 |     |     |     |     | 190 |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thr | Val | Asp | Lys | Ser | Arg | Trp | Gln | Gln | Gly | Asn | Val | Phe | Ser | Cys | Ser |
|     |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Met | His | Glu | Ala | Leu | His | Asn | His | Tyr | Thr | Gln | Lys | Ser | Leu | Ser |
|     | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Ser | Pro | Gly | Lys | Ala | Glu | Arg | Ala | Ala | Leu | Glu | Glu | Leu | Val | Lys |
| 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Gln | Gly | Glu | Arg | Val | Arg | Gly | Leu | Lys | Gln | Gln | Lys | Ala | Ser | Ala |
|     |     |     | 245 |     |     |     |     | 250 |     |     |     |     |     | 255 |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Leu | Ile | Glu | Glu | Glu | Val | Ala | Lys | Leu | Leu | Lys | Leu | Lys | Ala | Gln |
|     |     | 260 |     |     |     |     |     | 265 |     |     |     |     | 270 |     |     |

|     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|
| Leu | Gly | Pro | Asp | Glu | Ser |
|     |     | 275 |     |     |     |



&lt;210&gt; 384

&lt;211&gt; 283

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Protein sequence of Fc-HRS (2-55) (N-terminal Fc fusion)

&lt;400&gt; 384

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Ser | Asp | Lys | Thr | His | Thr | Cys | Pro | Pro | Cys | Pro | Ala | Pro | Glu | Leu |
| 1   |     |     |     | 5   |     |     |     | 10  |     |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Gly | Gly | Pro | Ser | Val | Phe | Leu | Phe | Pro | Pro | Lys | Pro | Lys | Asp | Thr |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Met | Ile | Ser | Arg | Thr | Pro | Glu | Val | Thr | Cys | Val | Val | Val | Asp | Val |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | His | Glu | Asp | Pro | Glu | Val | Lys | Phe | Asn | Trp | Tyr | Val | Asp | Gly | Val |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Val | His | Asn | Ala | Lys | Thr | Lys | Pro | Arg | Glu | Glu | Gln | Tyr | Asn | Ser |
| 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thr | Tyr | Arg | Val | Val | Ser | Val | Leu | Thr | Val | Leu | His | Gln | Asp | Trp | Leu |
|     |     |     | 85  |     |     |     |     |     | 90  |     |     |     |     | 95  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asn | Gly | Lys | Glu | Tyr | Lys | Cys | Lys | Val | Ser | Asn | Lys | Ala | Leu | Pro | Ala |
|     |     | 100 |     |     |     |     |     | 105 |     |     |     |     | 110 |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Ile | Glu | Lys | Thr | Ile | Ser | Lys | Ala | Lys | Gly | Gln | Pro | Arg | Glu | Pro |
|     |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Val | Tyr | Thr | Leu | Pro | Pro | Ser | Arg | Glu | Glu | Met | Thr | Lys | Asn | Gln |
|     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Ser | Leu | Thr | Cys | Leu | Val | Lys | Gly | Phe | Tyr | Pro | Ser | Asp | Ile | Ala |
| 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Glu | Trp | Glu | Ser | Asn | Gly | Gln | Pro | Glu | Asn | Asn | Tyr | Lys | Thr | Thr |
|     |     |     | 165 |     |     |     |     |     | 170 |     |     |     |     | 175 |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Pro | Val | Leu | Asp | Ser | Asp | Gly | Ser | Phe | Phe | Leu | Tyr | Ser | Lys | Leu |
|     |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thr | Val | Asp | Lys | Ser | Arg | Trp | Gln | Gln | Gly | Asn | Val | Phe | Ser | Cys | Ser |
|     |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Met | His | Glu | Ala | Leu | His | Asn | His | Tyr | Thr | Gln | Lys | Ser | Leu | Ser |
|     | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Ser | Pro | Gly | Lys | Ala | Glu | Arg | Ala | Ala | Leu | Glu | Glu | Leu | Val | Lys |
| 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Gln | Gly | Glu | Arg | Val | Arg | Gly | Leu | Lys | Gln | Gln | Lys | Ala | Ser | Ala |
|     |     |     | 245 |     |     |     |     |     | 250 |     |     |     |     | 255 |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Leu | Ile | Glu | Glu | Glu | Val | Ala | Lys | Leu | Leu | Lys | Leu | Lys | Ala | Gln |
|     |     | 260 |     |     |     |     |     | 265 |     |     |     |     | 270 |     |     |

|     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Gly | Pro | Asp | Glu | Ser | Lys | Gln | Lys | Phe | Val |
|     |     | 275 |     |     |     |     | 280 |     |     |     |



&lt;210&gt; 385

&lt;211&gt; 294

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Protein sequence of Fc-HRS (2-66) (N-terminal Fc fusion)

&lt;400&gt; 385

```

Met Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
1          5          10          15

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
20          25          30

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
35          40          45

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
50          55          60

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
65          70          75          80

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
85          90          95

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
100         105         110

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
115         120         125

Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
130         135         140

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
145         150         155         160

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
165         170         175

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
180         185         190

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
195         200         205

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
210         215         220

Leu Ser Pro Gly Lys Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys
225         230         235         240

Leu Gln Gly Glu Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala
245         250         255

Glu Leu Ile Glu Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln
260         265         270

Leu Gly Pro Asp Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys
275         280         285

```



Gly Thr Arg Asp Tyr Ser  
290

<210> 386

<211> 268

<212> PRT

<213> Artificial Sequence

<220>

<223> Protein sequence of HRS(1-40)-Fc (C-terminal Fc fusion)

<400> 386

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu  
1 5 10 15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu  
20 25 30

Glu Glu Val Ala Lys Leu Leu Lys Ser Asp Lys Thr His Thr Cys Pro  
35 40 45

Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe  
50 55 60

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val  
65 70 75 80

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe  
85 90 95

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro  
100 105 110

Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr  
115 120 125

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val  
130 135 140

Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala  
145 150 155 160

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg  
165 170 175

Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly  
180 185 190

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro  
195 200 205

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser  
210 215 220

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln  
225 230 235 240

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His  
245 250 255

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys  
260 265



&lt;210&gt; 387

&lt;211&gt; 273

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Protein sequence of HRS(1-45)-Fc (C-terminal Fc fusion)

&lt;400&gt; 387

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Ala | Glu | Arg | Ala | Ala | Leu | Glu | Glu | Leu | Val | Lys | Leu | Gln | Gly | Glu |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Val | Arg | Gly | Leu | Lys | Gln | Gln | Lys | Ala | Ser | Ala | Glu | Leu | Ile | Glu |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Glu | Val | Ala | Lys | Leu | Leu | Lys | Leu | Lys | Ala | Gln | Leu | Ser | Asp | Lys |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thr | His | Thr | Cys | Pro | Pro | Cys | Pro | Ala | Pro | Glu | Leu | Leu | Gly | Gly | Pro |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Val | Phe | Leu | Phe | Pro | Pro | Lys | Pro | Lys | Asp | Thr | Leu | Met | Ile | Ser |
| 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Thr | Pro | Glu | Val | Thr | Cys | Val | Val | Val | Asp | Val | Ser | His | Glu | Asp |
|     |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Glu | Val | Lys | Phe | Asn | Trp | Tyr | Val | Asp | Gly | Val | Glu | Val | His | Asn |
|     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Lys | Thr | Lys | Pro | Arg | Glu | Glu | Gln | Tyr | Asn | Ser | Thr | Tyr | Arg | Val |
|     |     | 115 |     |     |     |     | 120 |     |     |     |     |     | 125 |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Ser | Val | Leu | Thr | Val | Leu | His | Gln | Asp | Trp | Leu | Asn | Gly | Lys | Glu |
|     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Tyr | Lys | Cys | Lys | Val | Ser | Asn | Lys | Ala | Leu | Pro | Ala | Pro | Ile | Glu | Lys |
| 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thr | Ile | Ser | Lys | Ala | Lys | Gly | Gln | Pro | Arg | Glu | Pro | Gln | Val | Tyr | Thr |
|     |     |     | 165 |     |     |     |     |     | 170 |     |     |     |     | 175 |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Pro | Pro | Ser | Arg | Glu | Glu | Met | Thr | Lys | Asn | Gln | Val | Ser | Leu | Thr |
|     |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Cys | Leu | Val | Lys | Gly | Phe | Tyr | Pro | Ser | Asp | Ile | Ala | Val | Glu | Trp | Glu |
|     |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Asn | Gly | Gln | Pro | Glu | Asn | Asn | Tyr | Lys | Thr | Thr | Pro | Pro | Val | Leu |
|     | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asp | Ser | Asp | Gly | Ser | Phe | Phe | Leu | Tyr | Ser | Lys | Leu | Thr | Val | Asp | Lys |
| 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Arg | Trp | Gln | Gln | Gly | Asn | Val | Phe | Ser | Cys | Ser | Val | Met | His | Glu |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|

245

250

255

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Leu | His | Asn | His | Tyr | Thr | Gln | Lys | Ser | Leu | Ser | Leu | Ser | Pro | Gly |
|     |     | 260 |     |     |     |     |     | 265 |     |     |     |     | 270 |     |     |

Lys



&lt;210&gt; 388

&lt;211&gt; 278

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Protein sequence of HRS(1-50)-Fc (C-terminal Fc fusion)

&lt;400&gt; 388

```

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu
 1           5           10           15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu
          20           25           30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp
          35           40           45

Glu Ser Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
 50           55           60

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
65           70           75           80

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
          85           90           95

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
          100          105          110

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
          115          120          125

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
130          135          140

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
145          150          155          160

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
          165          170          175

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn
          180          185          190

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
          195          200          205

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
210          215          220

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
225          230          235          240

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
          245          250          255

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
          260          265          270

Ser Leu Ser Pro Gly Lys
          275

```



&lt;210&gt; 389

&lt;211&gt; 283

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Protein sequence of HRS(1-55)-Fc (C-terminal Fc fusion)

&lt;400&gt; 389

```

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu
 1              5              10              15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu
 20              25              30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp
 35              40              45

Glu Ser Lys Gln Lys Phe Val Ser Asp Lys Thr His Thr Cys Pro Pro
 50              55              60

Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
 65              70              75              80

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
 85              90              95

Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
100              105              110

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
115              120              125

Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
130              135              140

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
145              150              155              160

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
165              170              175

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu
180              185              190

Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
195              200              205

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
210              215              220

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
225              230              235              240

Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
245              250              255

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
260              265              270

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
275              280

```



&lt;210&gt; 390

&lt;211&gt; 294

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Protein sequence of HRS(1-66)-Fc (C-terminal Fc fusion)

&lt;400&gt; 390

```

Met Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu
1          5          10          15

Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu
20          25          30

Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp
35          40          45

Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys Gly Thr Arg Asp
50          55          60

Tyr Ser Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
65          70          75          80

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
85          90          95

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
100         105         110

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
115         120         125

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
130         135         140

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
145         150         155         160

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
165         170         175

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
180         185         190

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn
195         200         205

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
210         215         220

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
225         230         235         240

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
245         250         255

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
260         265         270

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
275         280         285

Ser Leu Ser Pro Gly Lys
290

```



<210> 391  
<211> 24  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> primer used to amplify Fc-HRS (2-60)

<400> 391  
tattctcgag gcagagcgtg cggc 24

<210> 392  
<211> 26  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> primer used to amplify Fc-HRS (2-60)

<400> 392  
gcgcctcgag tcactggggg gttttg 26

<210> 393  
<211> 37  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> primer used to amplify Fc-HRS (2-60)

<400> 393  
gtgctcaaaa cccccaaggc agagcgtgcg gcgctgg 37

<210> 394  
<211> 37  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> primer used to amplify Fc-HRS (2-60)

<400> 394  
ccagcgccgc acgctctgcc ttggggggtt tgagcac 37

<210> 395  
<211> 1044  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> DNA sequence of Fc-HRS(2-60) HRS(2-60) (N-terminal Fc fusion)

<400> 395  
atgtctgaca aaactcacac atgccacccg tgcccagcac ctgaactcct ggggggaccg 60  
tcagtcttcc tcttcccccc aaaacccaag gacaccctca tgatctcccg gaccctgag 120  
ctcacatccg tcttctctga cctgagccac gaagaccctg aggtcaagtt caactggtac 180



```

gtggacggcg tggaggtgca taatgccaag acaaagccgc gggaggagca gtacaacagc 240
acgtaccgtg tggtcagcgt cctcaccgtc ctgcaccagg actggctgaa tggcaaggag 300
tacaagtgca aggtctccaa caaagccctc ccagccccc a tcgagaaaac catctccaaa 360
gccaaagggc agccccgaga accacaggtg tacaccctgc ccccatcccg ggaggagatg 420
accaagaacc aggtcagcct gacctgcctg gtcaaaggct tctatcccag cgacatcgcc 480
gtggagtggg agagcaatgg gcagccggag aacaactaca agaccacgcc tcccgctgctg 540
gactccgacg gctccttctt cctctacagc aagctcaccg tggacaagag caggtggcag 600
caggggaacg tcttctcatg ctccgtgatg cagcaggctc tgcacaacca ctacacgcag 660
aagagcctct ccctgtctcc gggtaaagca gagcgtgcgg cgctggagga gctggtgaaa 720
cttcagggag agcgcgtgcg aggcctcaag cagcagaagg ccagcgccga gctgatcgag 780
gaggaggtgg cgaaactcct gaaactgaag gcacagctgg gtctgatga aagcaaacag 840
aaatttgtgc tcaaaacccc caaggcagag cgtgcggcgc tggaggagct ggtgaaactt 900
cagggagagc gcgtgcgagg cctcaagcag cagaaggcca gcgccgagct gatcgaggag 960
gaggtggcga aactcctgaa actgaaggca cagctgggtc ctgatgaaag caaacagaaa 1020
tttgtgctca aaacccccaa gtga 1044

```

&lt;210&gt; 396

&lt;211&gt; 347

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Protein sequence of Fc-HRS(2-60) HRS(2-60) (N-terminal Fc fusion)

&lt;400&gt; 396

```

Met Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
1          5          10          15

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
20          25          30

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
35          40          45

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
50          55          60

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
65          70          75          80

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
85          90          95

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
100         105         110

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
115         120         125

Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
130         135         140

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
145         150         155         160

```



Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr  
165 170 175

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu  
180 185 190

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser  
195 200 205

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser  
210 215 220

Leu Ser Pro Gly Lys Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys  
225 230 235 240

Leu Gln Gly Glu Arg Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala  
245 250 255

Glu Leu Ile Glu Glu Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln  
260 265 270

Leu Gly Pro Asp Glu Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys  
275 280 285

Ala Glu Arg Ala Ala Leu Glu Glu Leu Val Lys Leu Gln Gly Glu Arg  
290 295 300

Val Arg Gly Leu Lys Gln Gln Lys Ala Ser Ala Glu Leu Ile Glu Glu  
305 310 315 320

Glu Val Ala Lys Leu Leu Lys Leu Lys Ala Gln Leu Gly Pro Asp Glu  
325 330 335

Ser Lys Gln Lys Phe Val Leu Lys Thr Pro Lys  
340 345

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## PATENTKRAV

1. Terapeutisk præparat til anvendelse til reduktion af lungeinflammation, omfattende en farmaceutisk acceptabel bærer eller excipients og et histidyl-tRNA-syntetase-(HRS)-Fc-fusionspolypeptid, som omfatter en aminosyresekvens, der er mindst 98% identisk med SEQ ID NO: 337, hvor HRS-Fc-fusionspolypeptidet har en antiinflammatorisk aktivitet og har ændret farmakokinetik i forhold til et umodificeret HRS-polypeptid med en aminosyresekvens ifølge SEQ ID NO: 6, hvor den ændrede farmakokinetik er forøget serumhalveringstid, forøget biotilgængelighed og/eller nedsat clearance.
2. Terapeutisk præparat til anvendelse ifølge krav 1, hvor lungeinflammationen er associeret med en autoimmun eller inflammatorisk sygdom.
3. Terapeutisk præparat til anvendelse ifølge krav 1, hvor den autoimmune eller inflammatoriske sygdom er valgt blandt sarkoidose, systemisk sklerodermi, respiratorisk distress-syndrom, herunder respiratorisk distress-syndrom hos voksne (ARDS), granulomatose, astma og immunrespons medieret af akut og forsinket overfølsomhed.
4. Terapeutisk præparat til anvendelse ifølge krav 1 eller 2 til behandling af en interstiell lungesygdom (ILD).
5. Terapeutisk præparat til anvendelse ifølge krav 4, hvor ILD er forårsaget af bindevæv eller autoimmun sygdom eller erhvervsmæssig og miljømæssig eksponering.
6. Terapeutisk præparat til anvendelse ifølge krav 5, hvor bindevævet eller den autoimmune sygdom omfatter sklerodermi/progressiv systemisk sklerose, lupus (systemisk lupus erythematosus), reumatoid arthritis eller polymyositis/dermatomyositis.
7. Terapeutisk præparat til anvendelse ifølge krav 5, hvor den erhvervsmæssige og miljømæssige eksponering omfatter eksponering for støv, gasser, gift, kemoterapi eller strålebehandling.
8. Terapeutisk præparat til anvendelse ifølge et hvilket som helst af kravene 4-7, hvor HRS-Fc-fusionspolypeptidet reducerer immuncelleaktivering og invasion i beskadiget



lunge.

9. Terapeutisk præparat til anvendelse ifølge et hvilket som helst af kravene 1-8, hvor Fc-regionen og HRS-polypeptidet er adskilt af en peptidlinker.

5

10. Terapeutisk præparat til anvendelse ifølge krav 9, hvor peptidlinkeren har en længde på 1-10 aminosyrer eller 1-5 aminosyrer.

10 11. Terapeutisk præparat til anvendelse ifølge et hvilket som helst af kravene 1-10, hvor HRS-Fc-fusionspolypeptidet har en halveringstid på mindst 30 timer i mus.

12. Terapeutisk præparat til anvendelse ifølge et hvilket som helst af kravene 1-10, hvor biotilgængeligheden er subkutan biotilgængelighed, der er forøget med mindst 30%.

15

13. Terapeutisk præparat til anvendelse ifølge et hvilket som helst af kravene 1-11, hvor HRS-Fc-fusionspolypeptidet er mindst 95% rent og mindre end 5% aggregeret, og hvor præparatet i det væsentlige er endotoksinfrit.



DRAWINGS

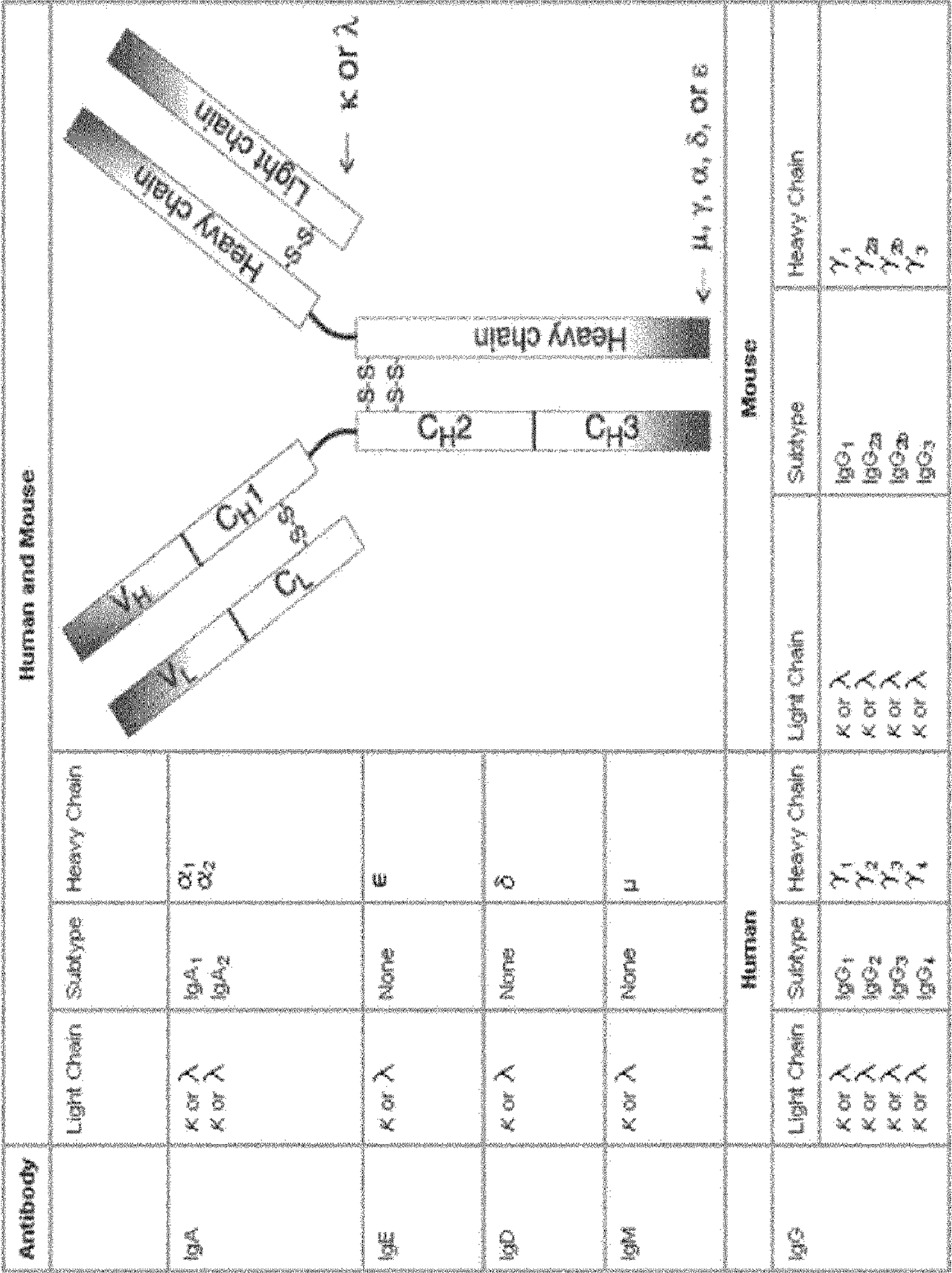


Fig. 1



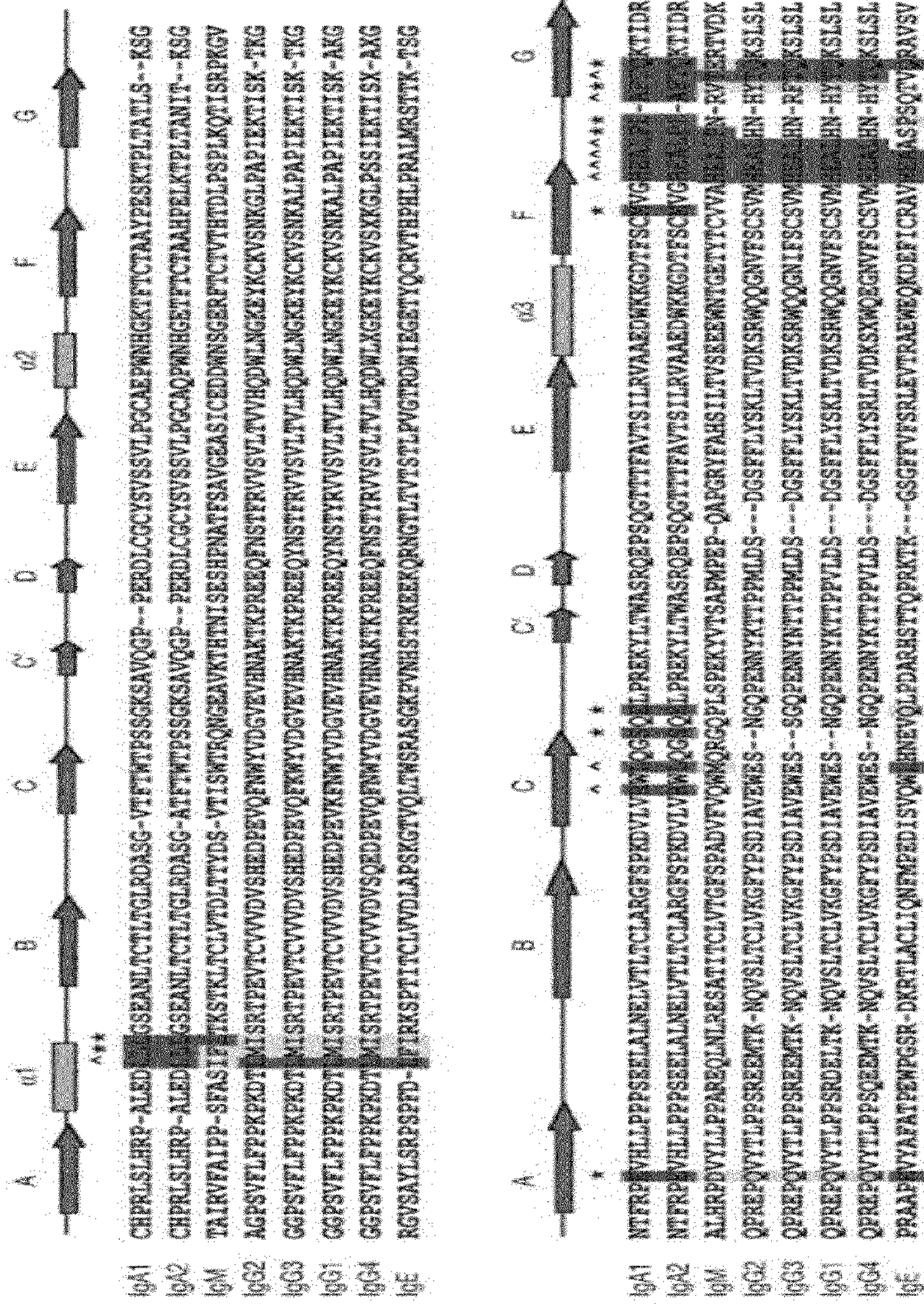


**Fig. 2**



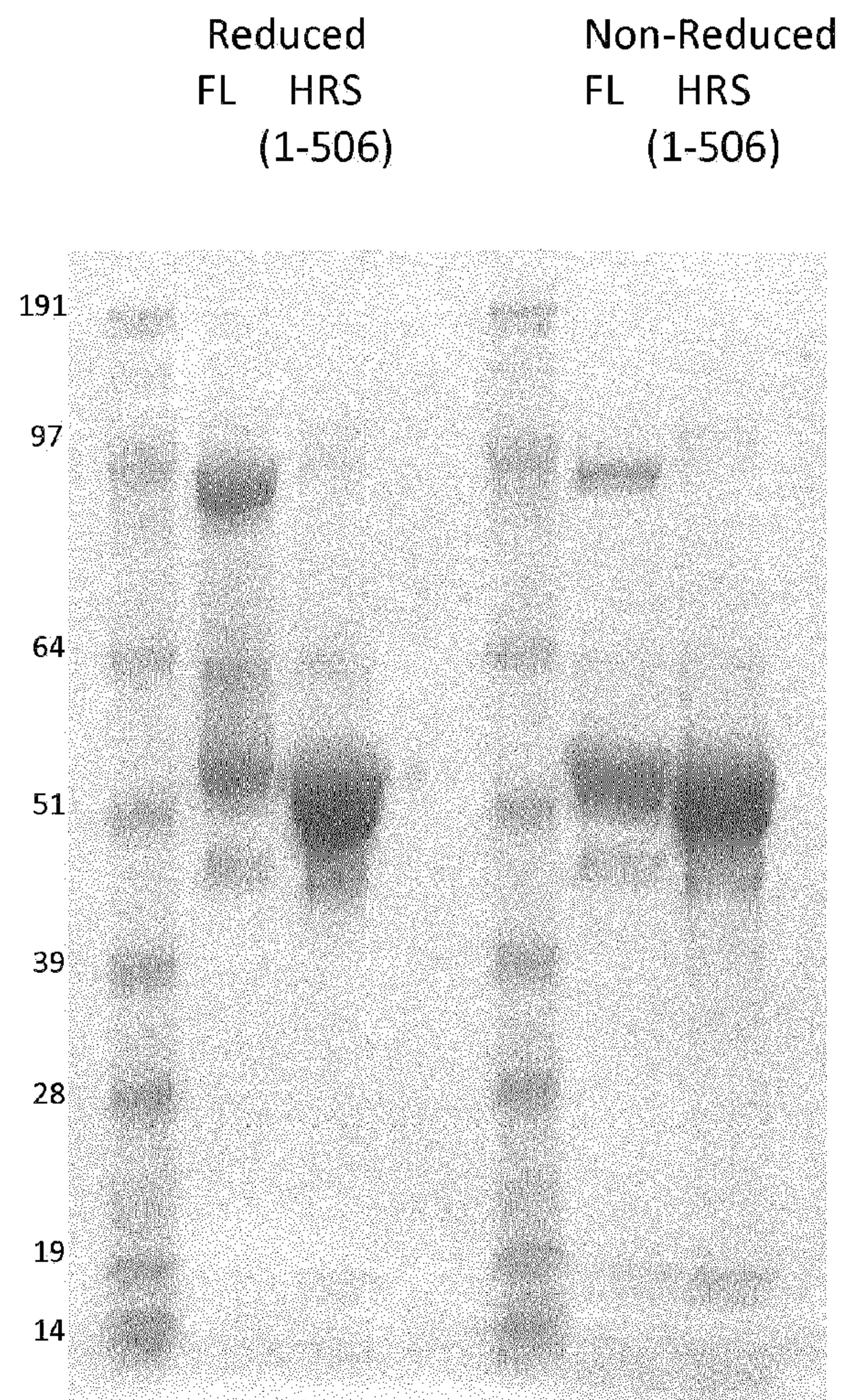


Fig. 3



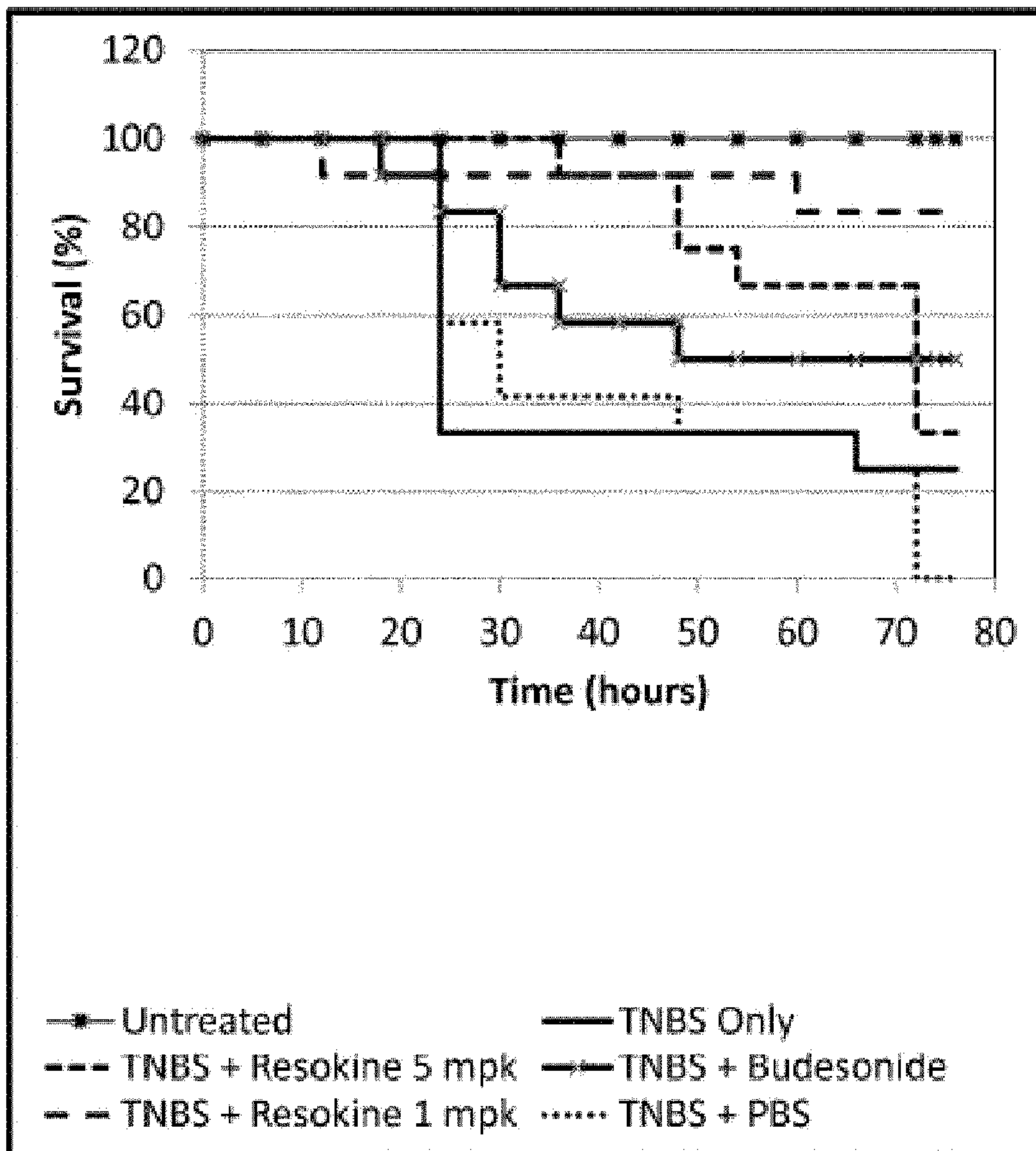


Fig. 4



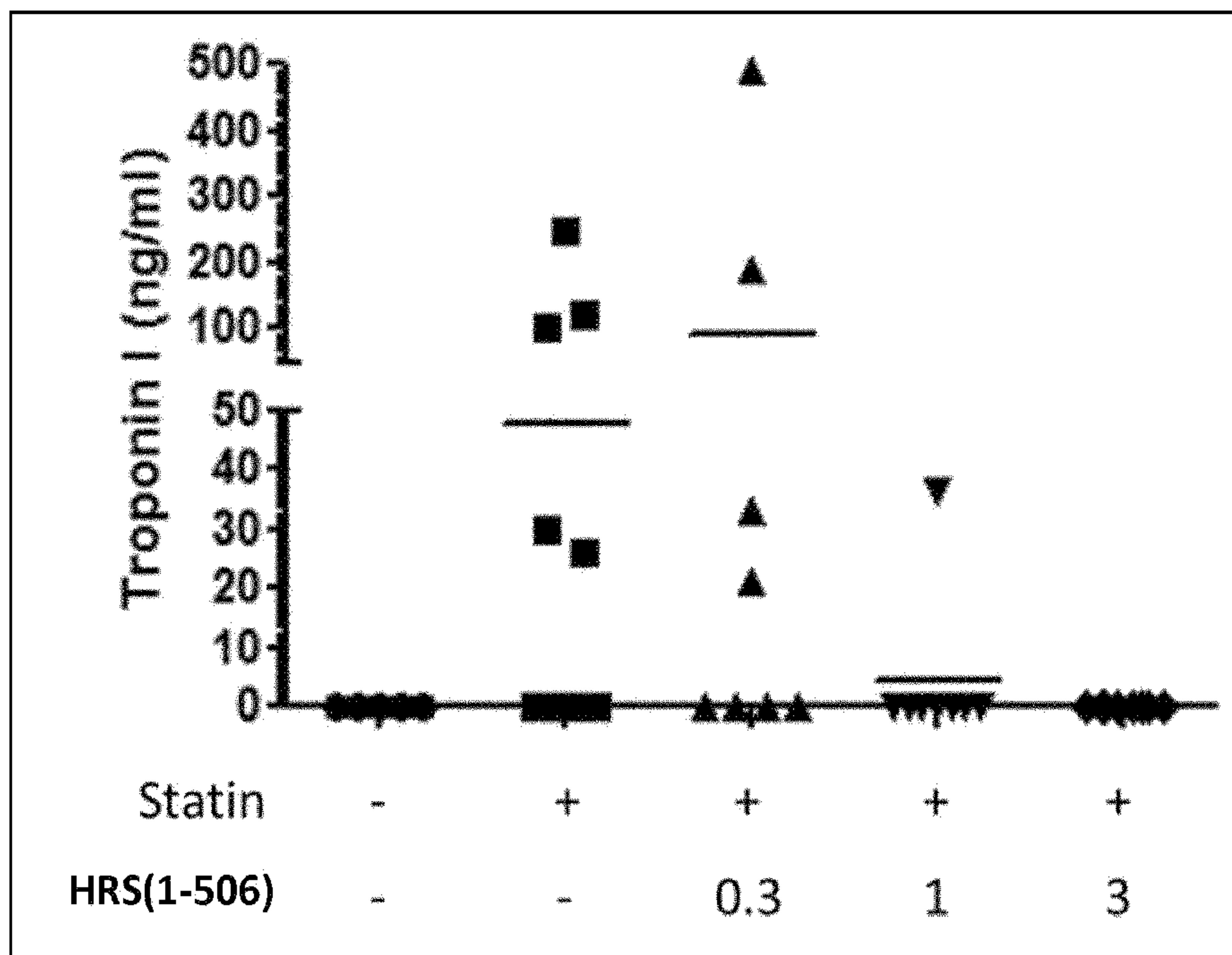
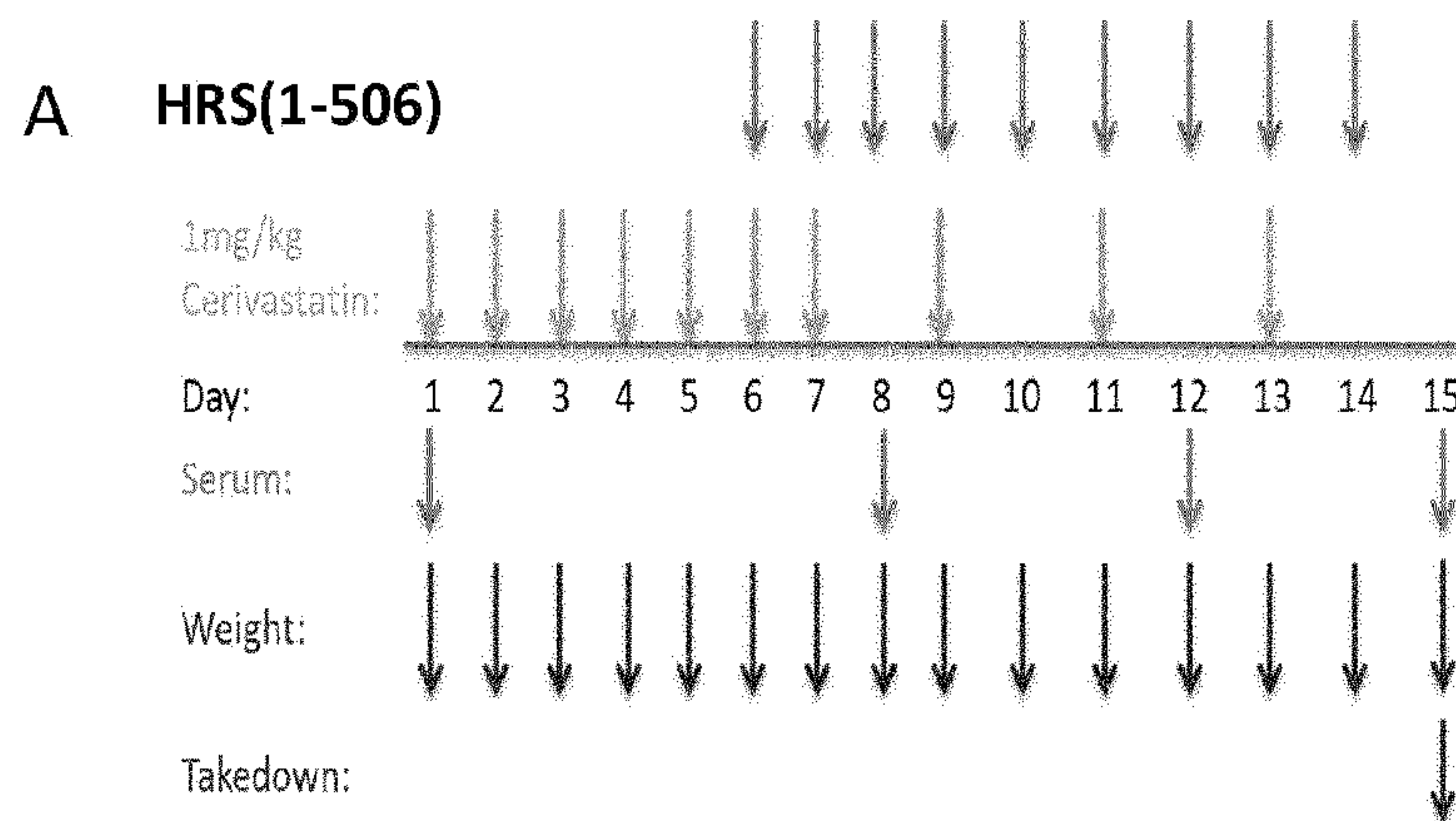


**Fig. 5**



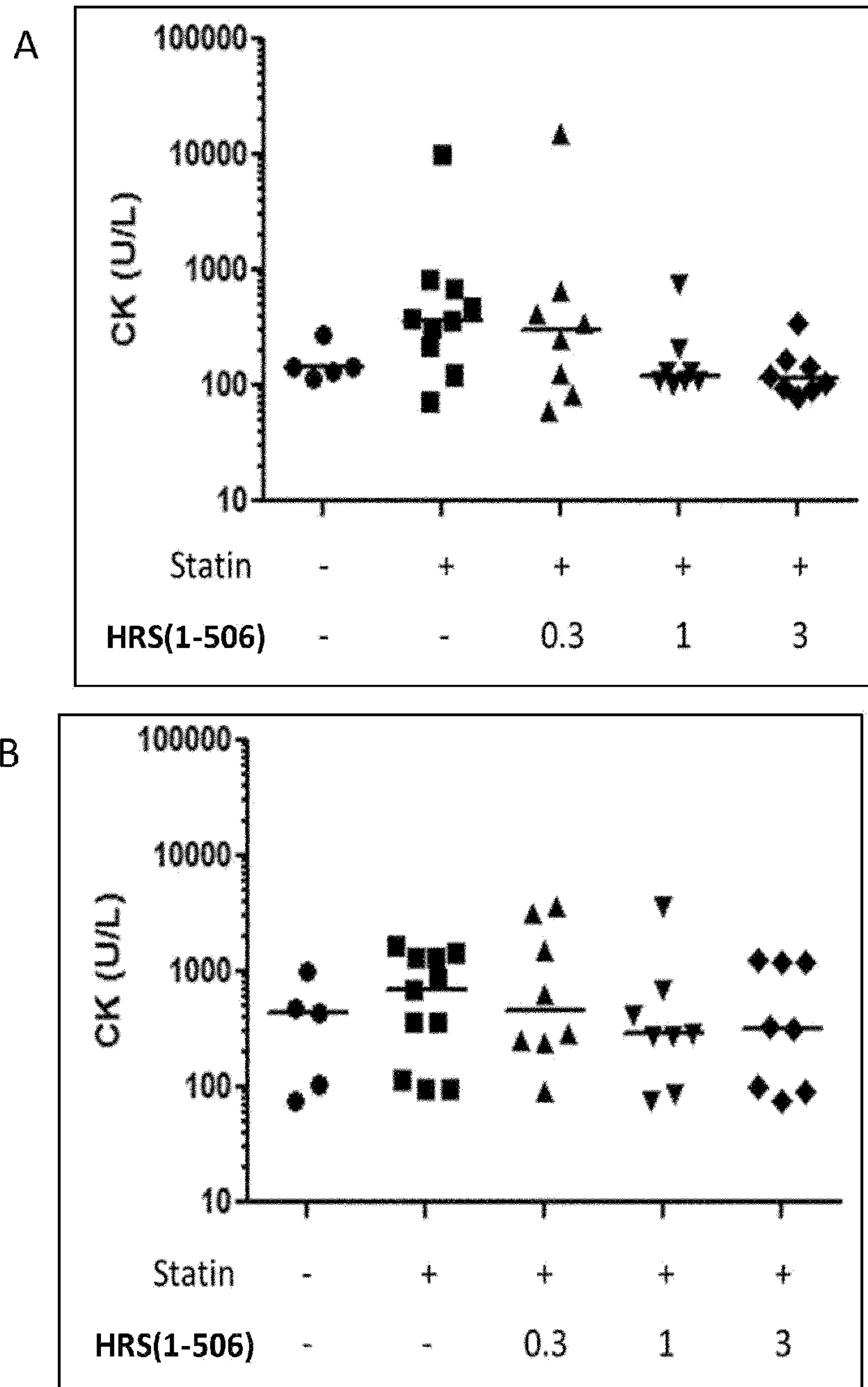


Fig. 6



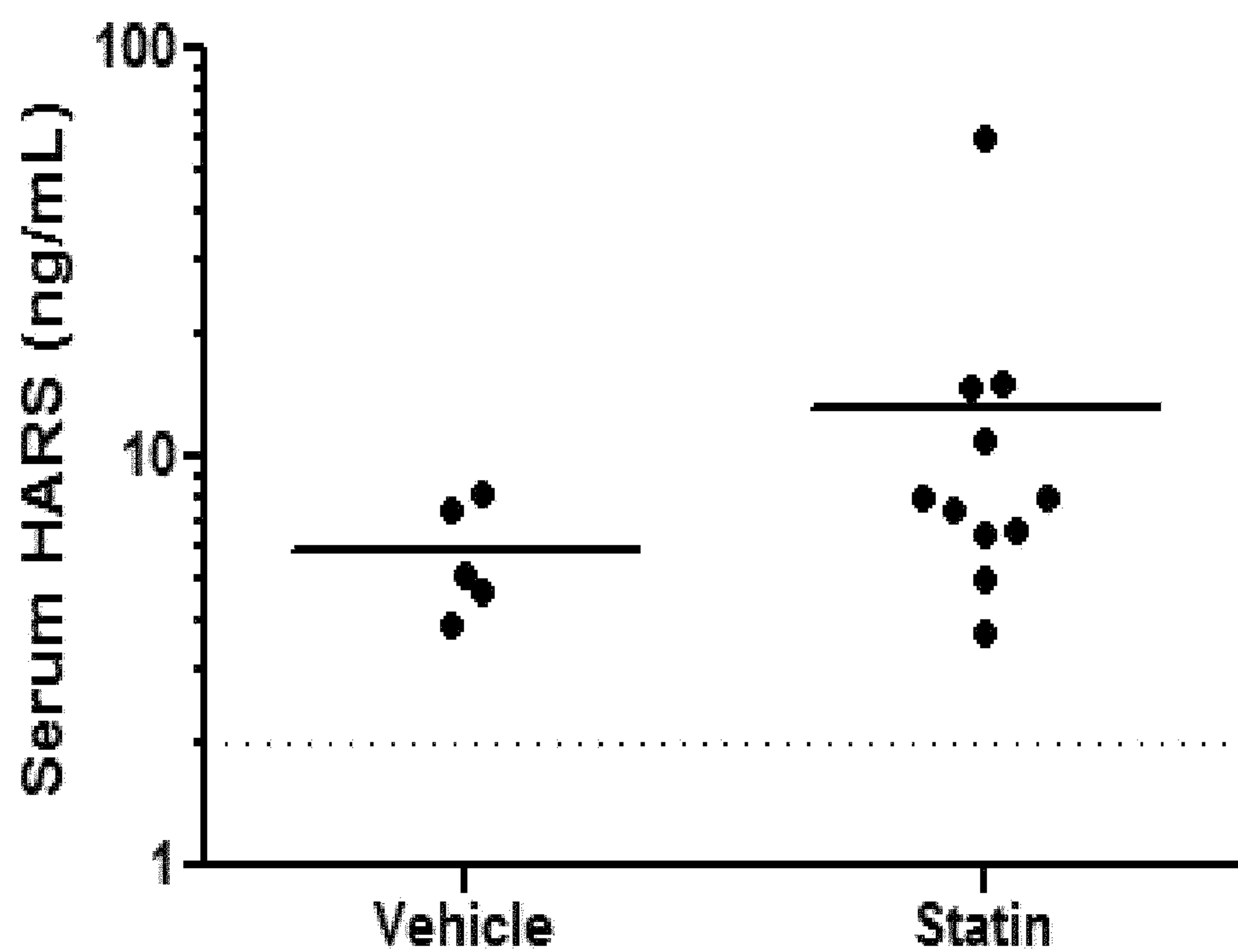
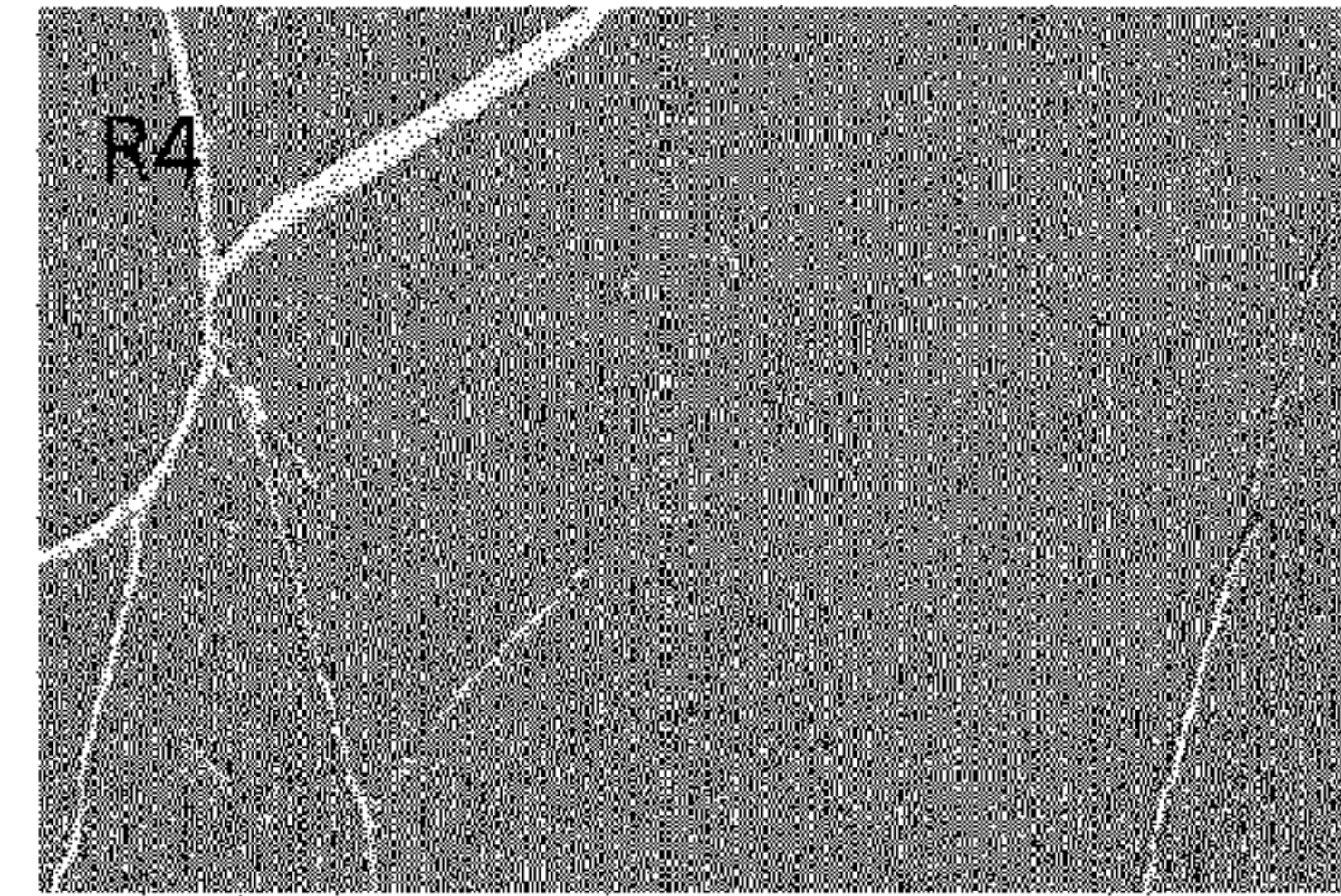
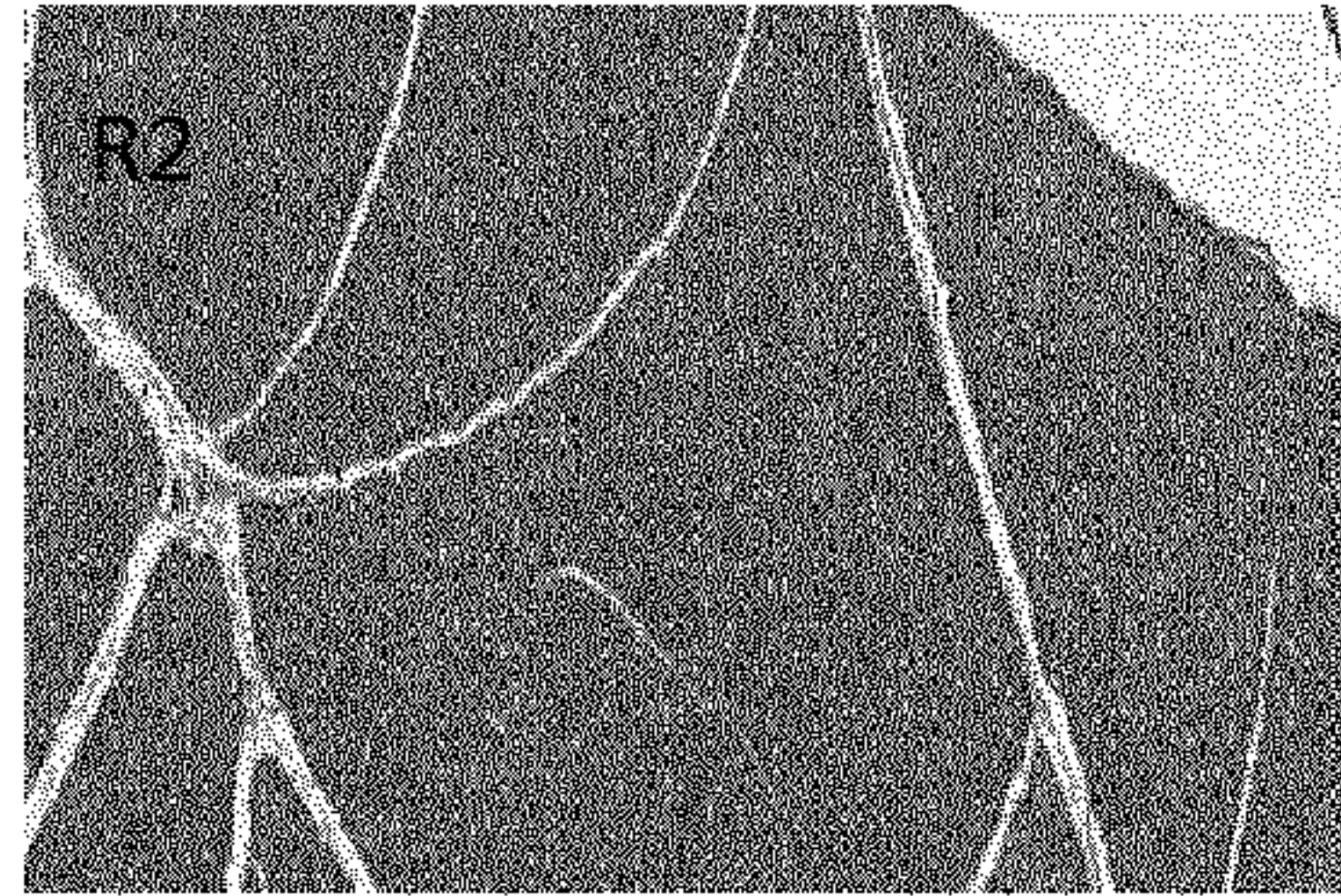


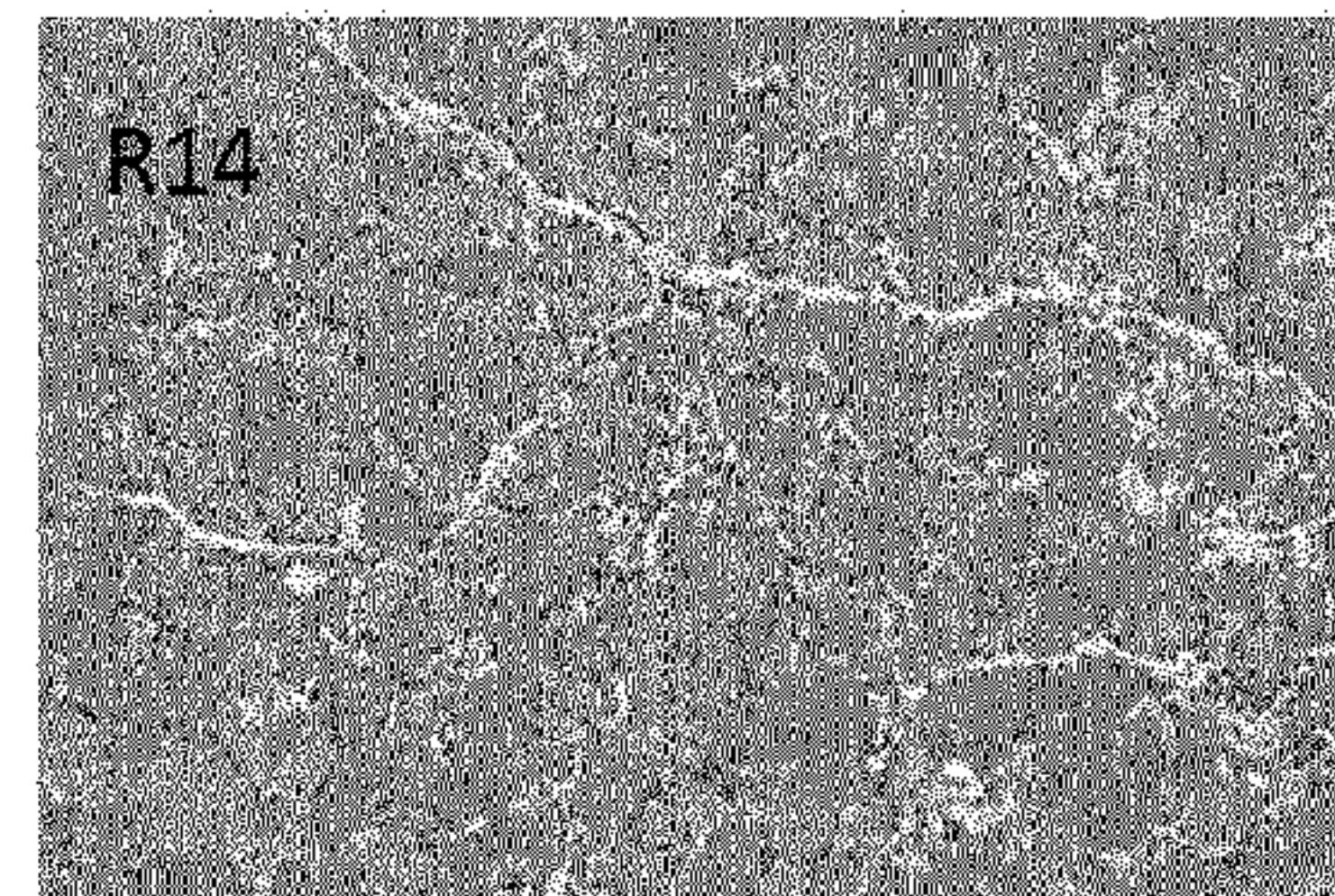
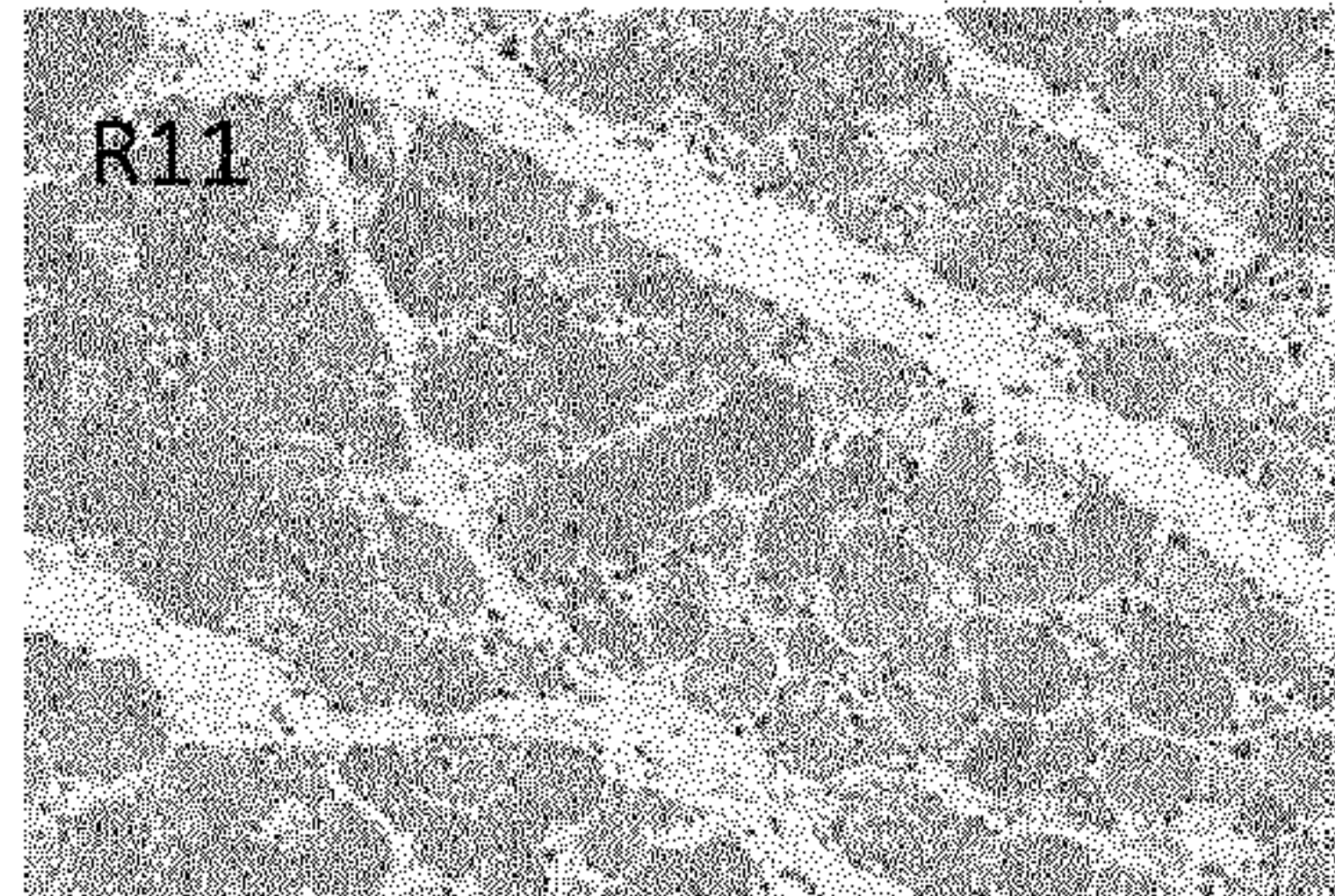
Fig. 7



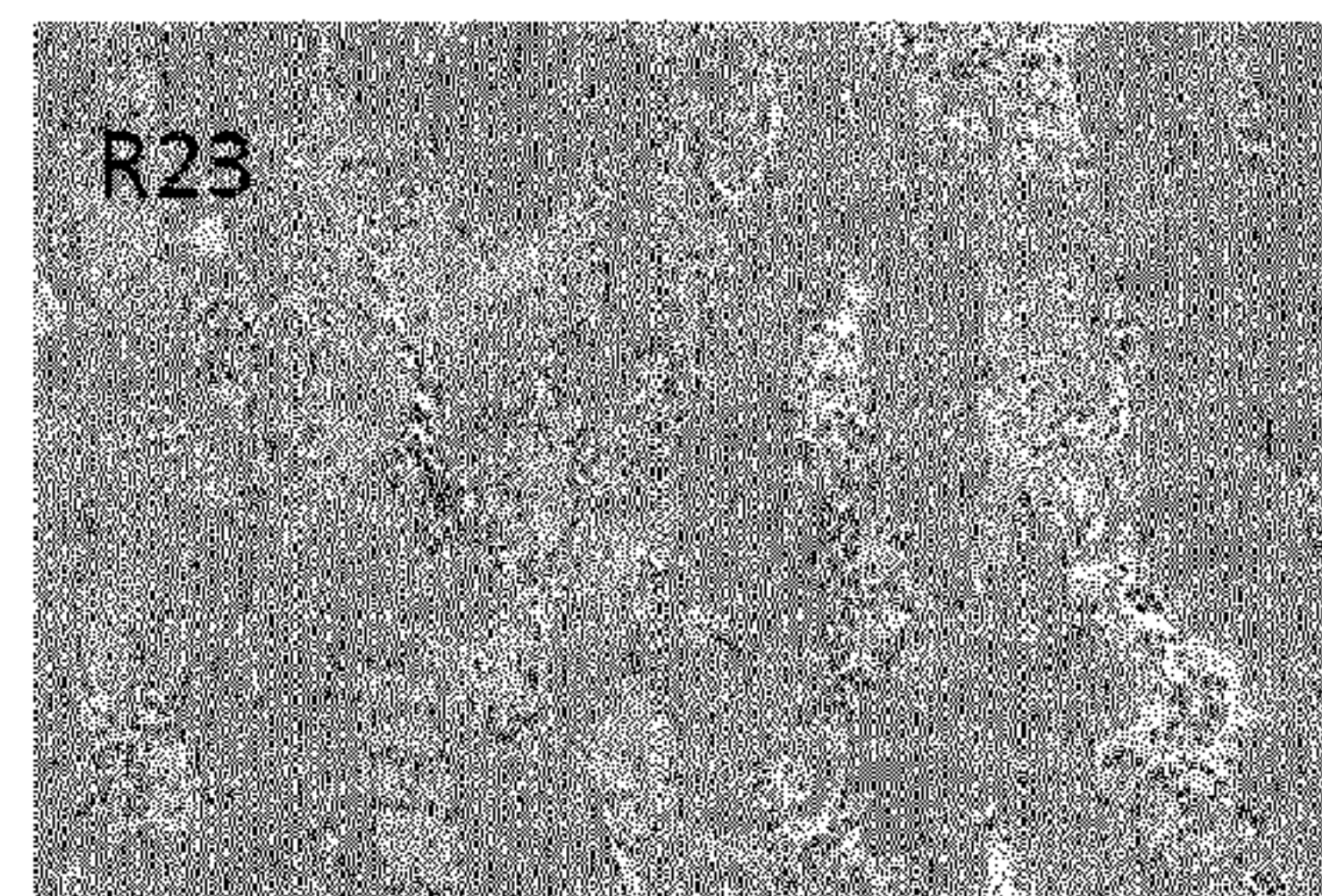
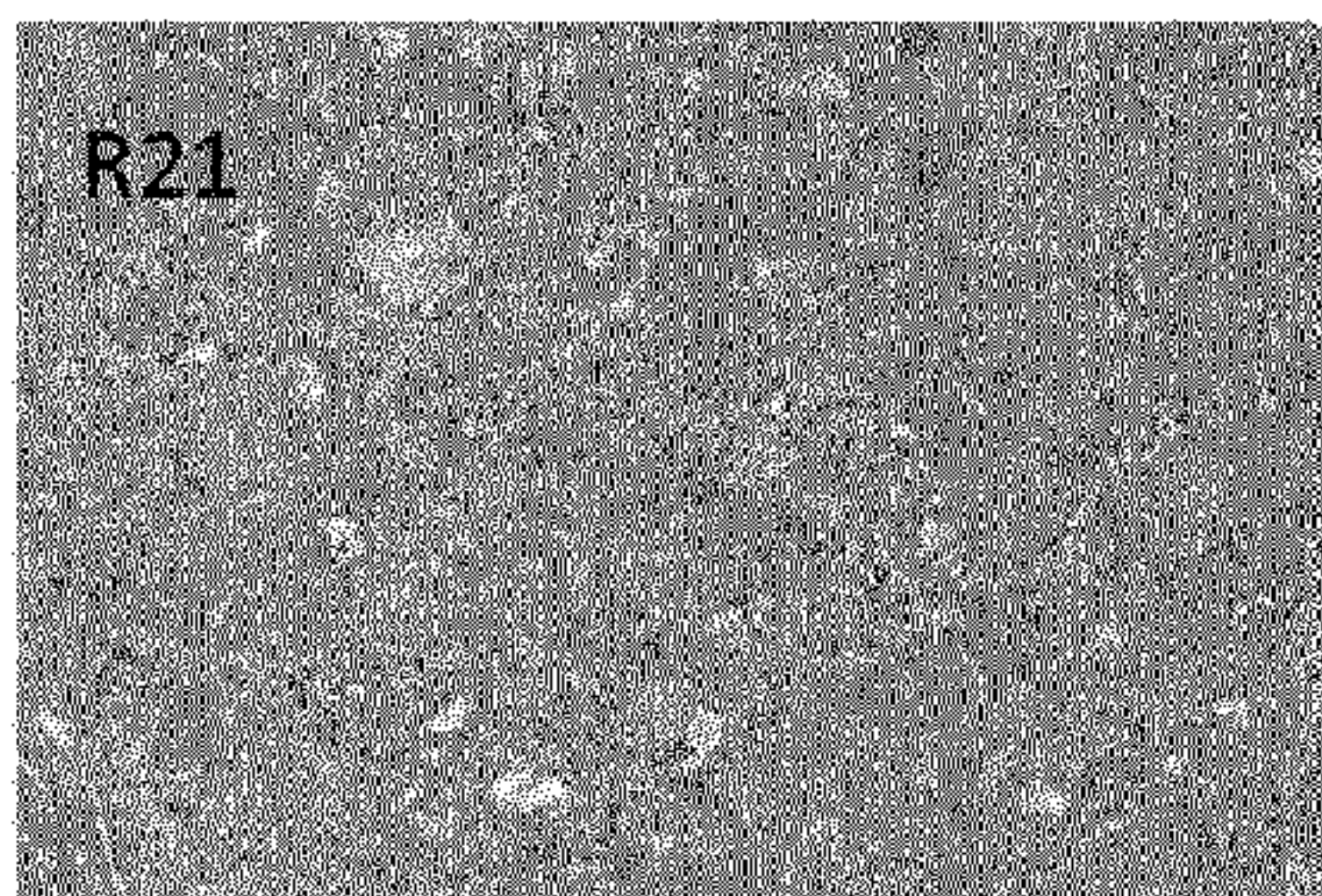
No Statin



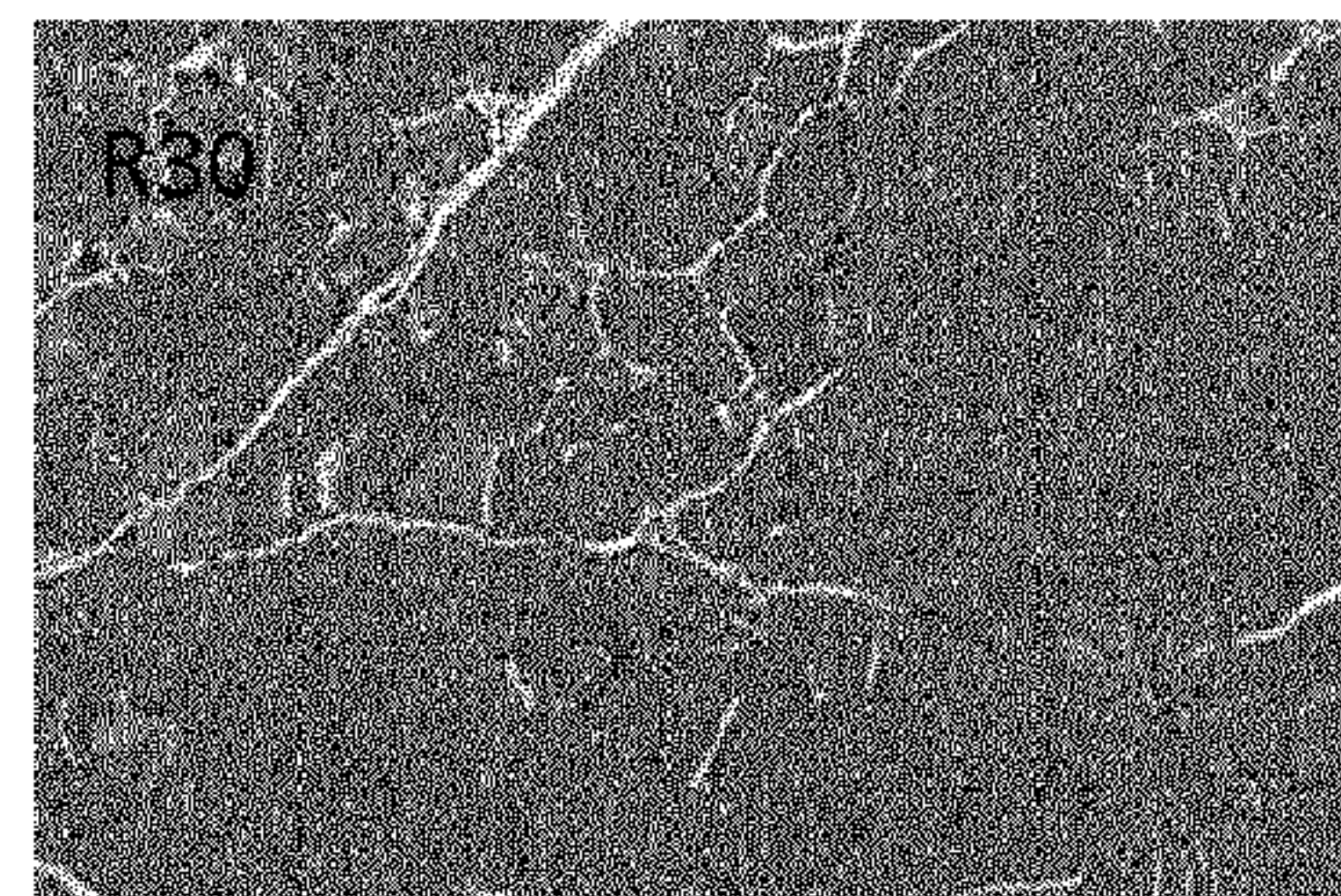
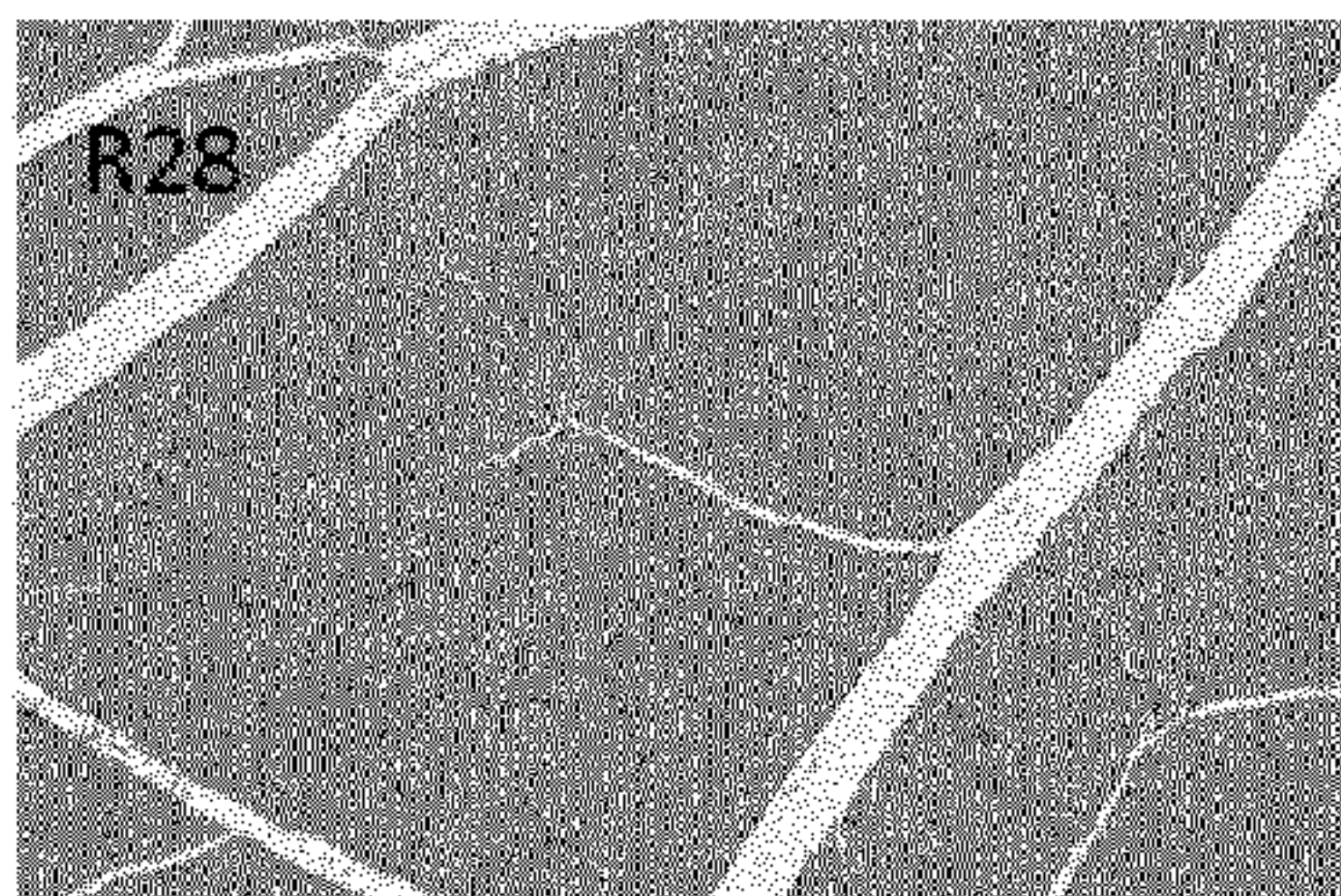
Statin + Vehicle



Statin + 0.3mpk  
HRS(1-506)



Statin + 1.0mpk  
HRS(1-506)



Statin + 3.0mpk  
HRS(1-506)

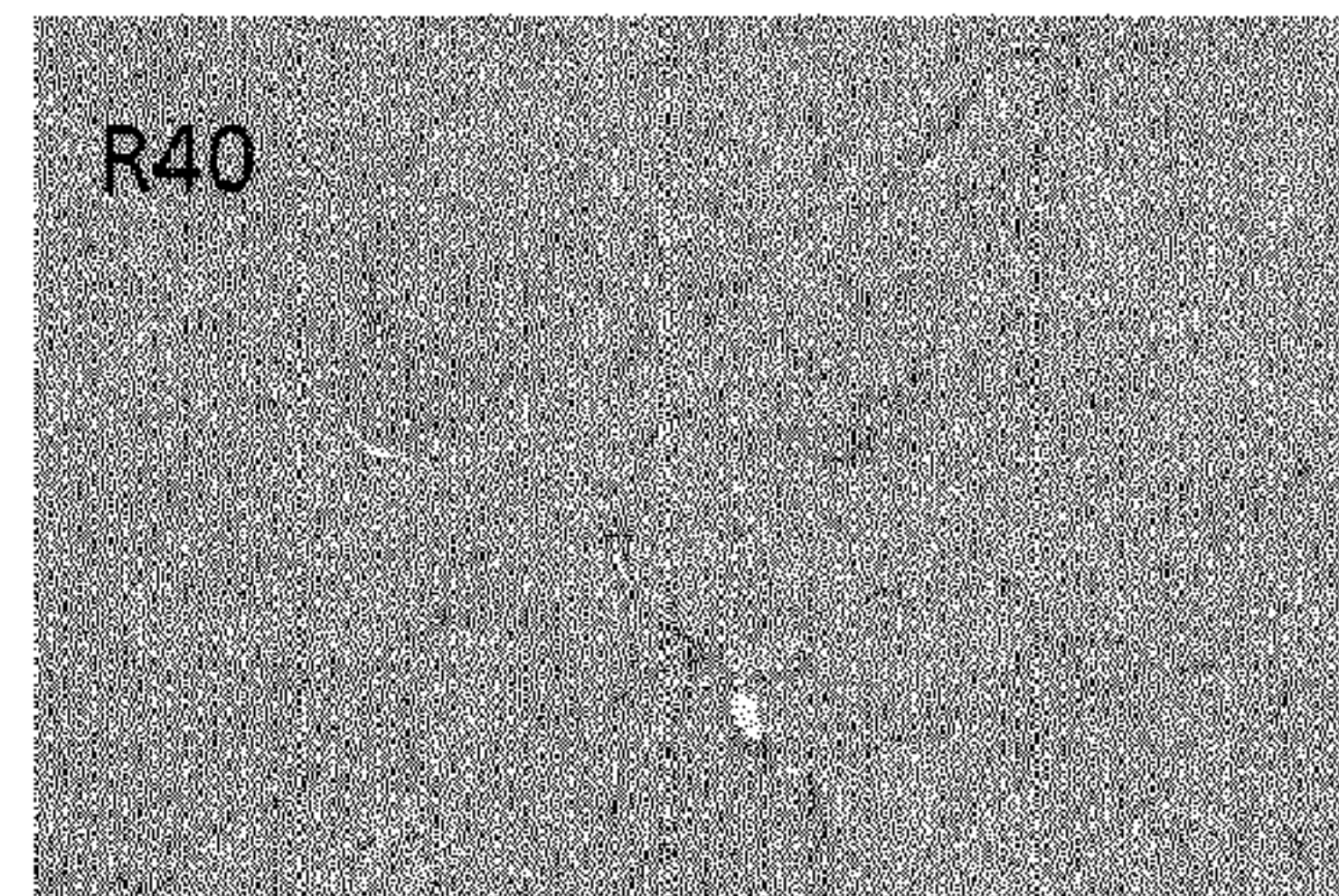
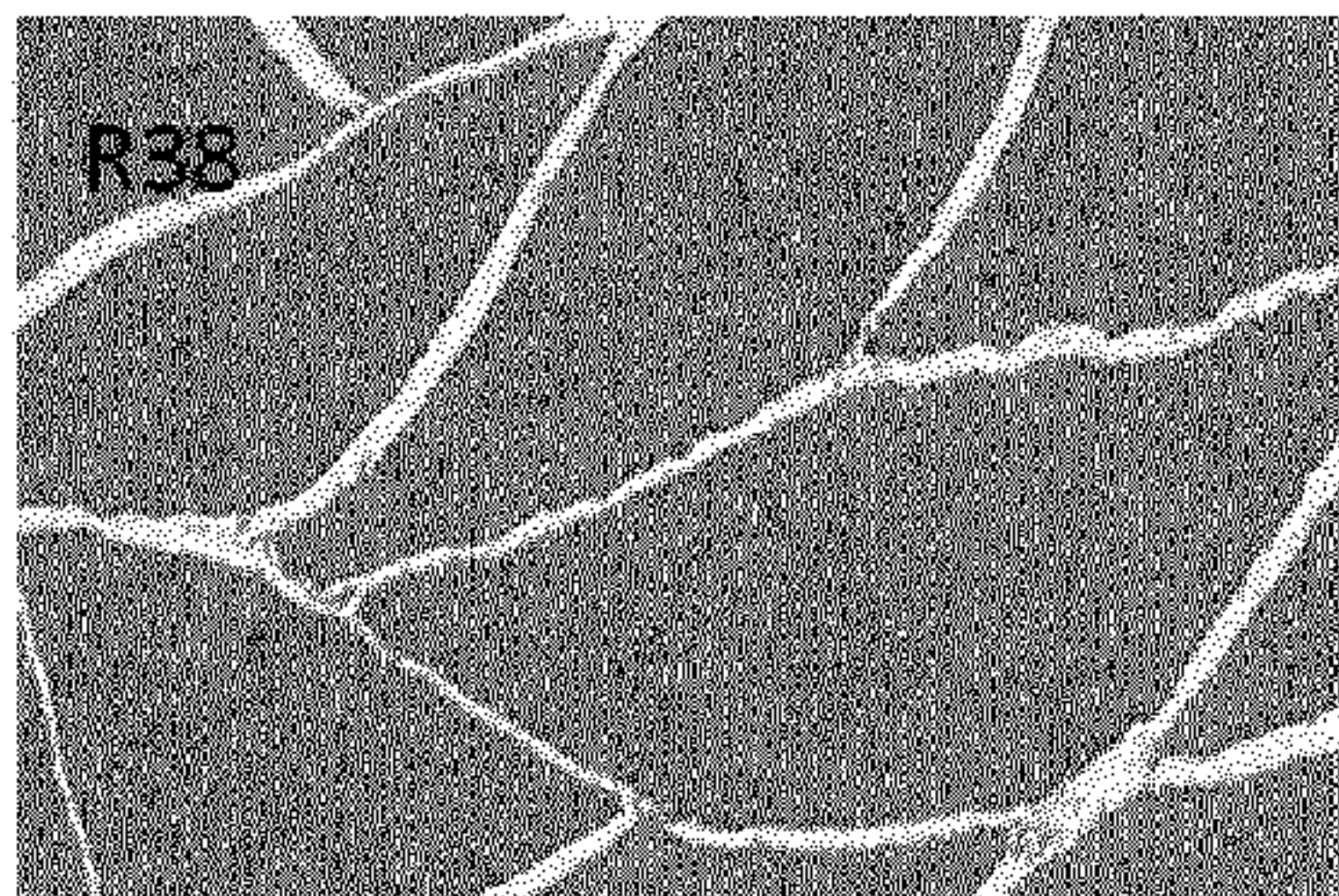


Fig. 8



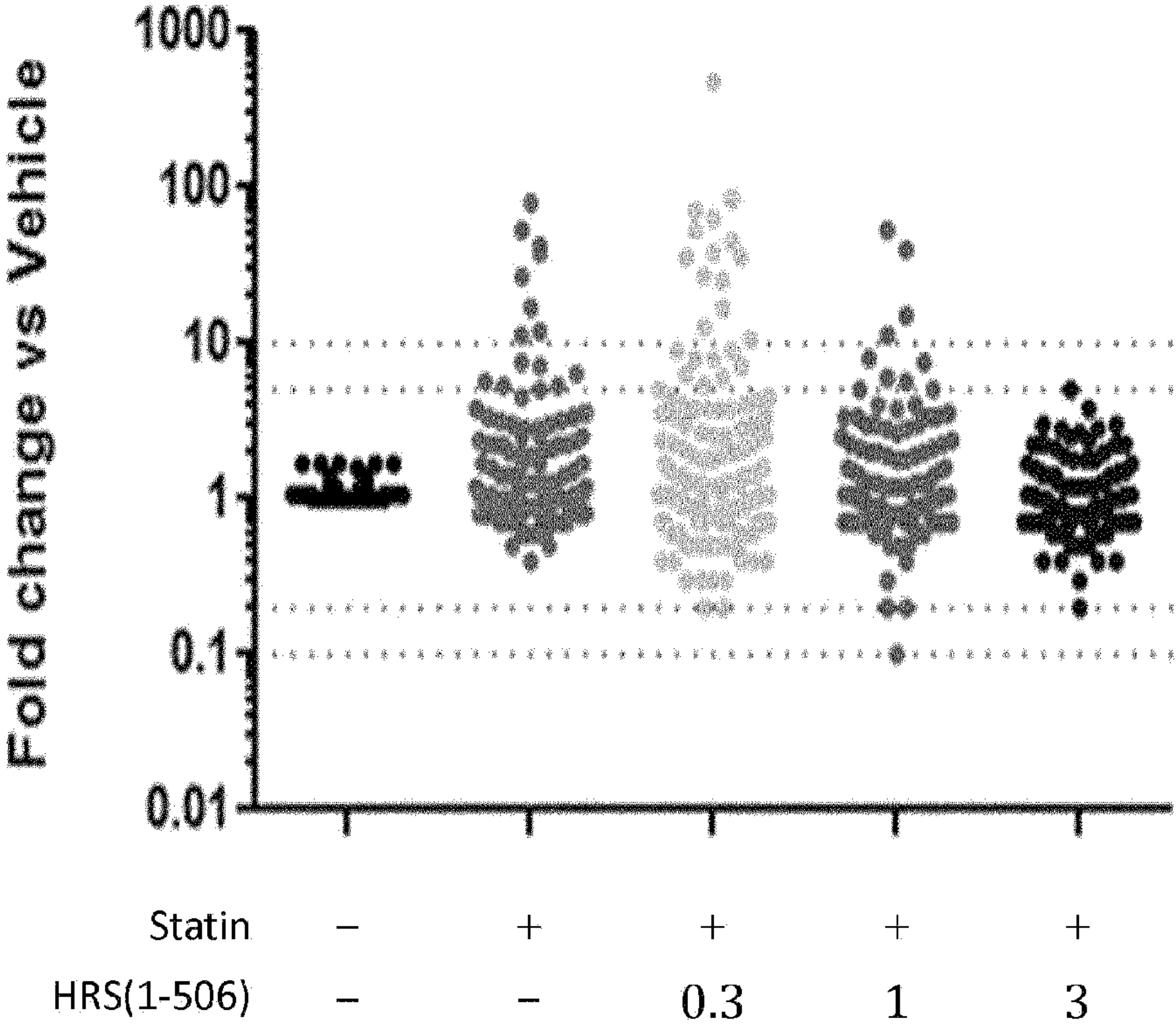
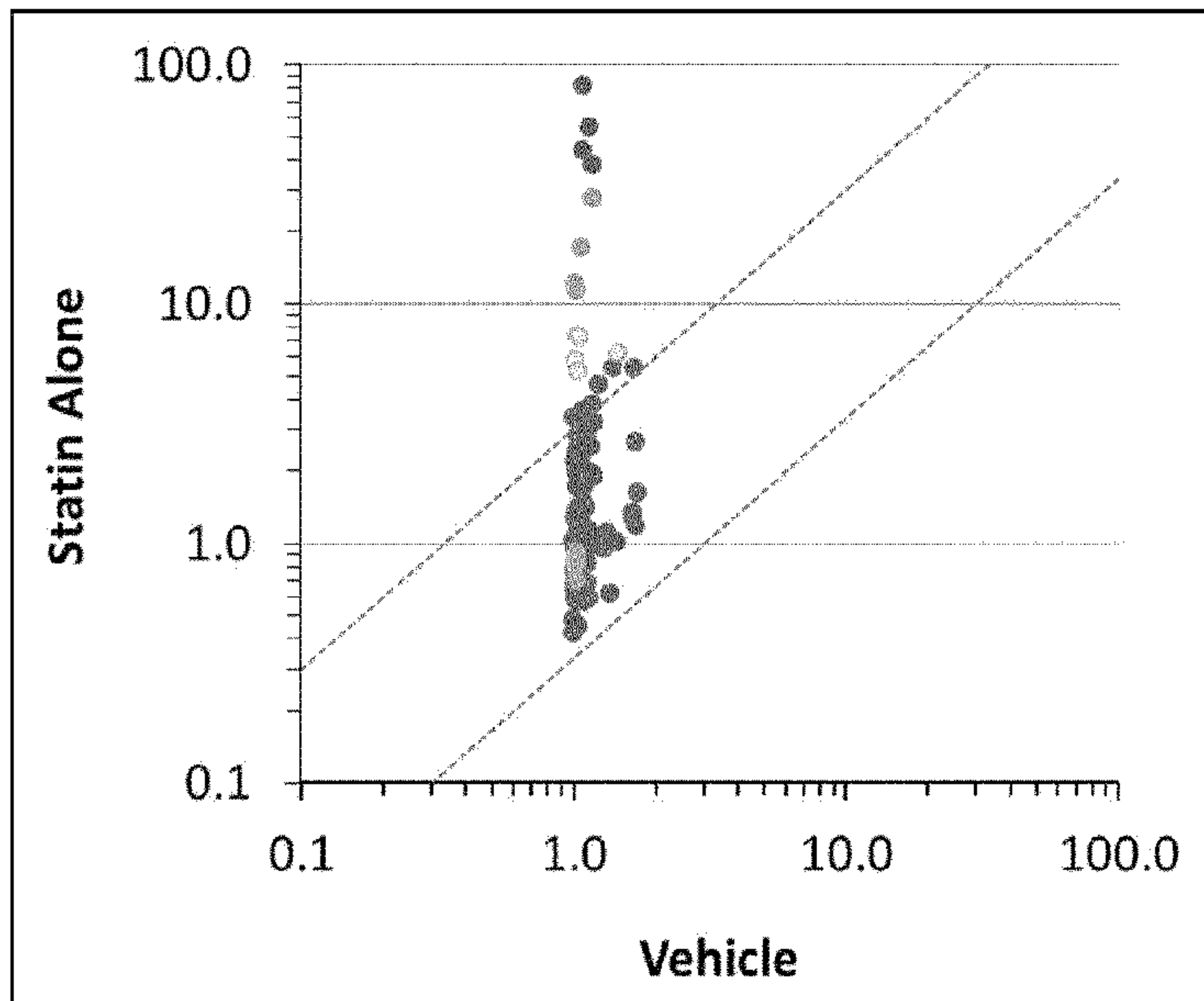


Fig. 9



A



B

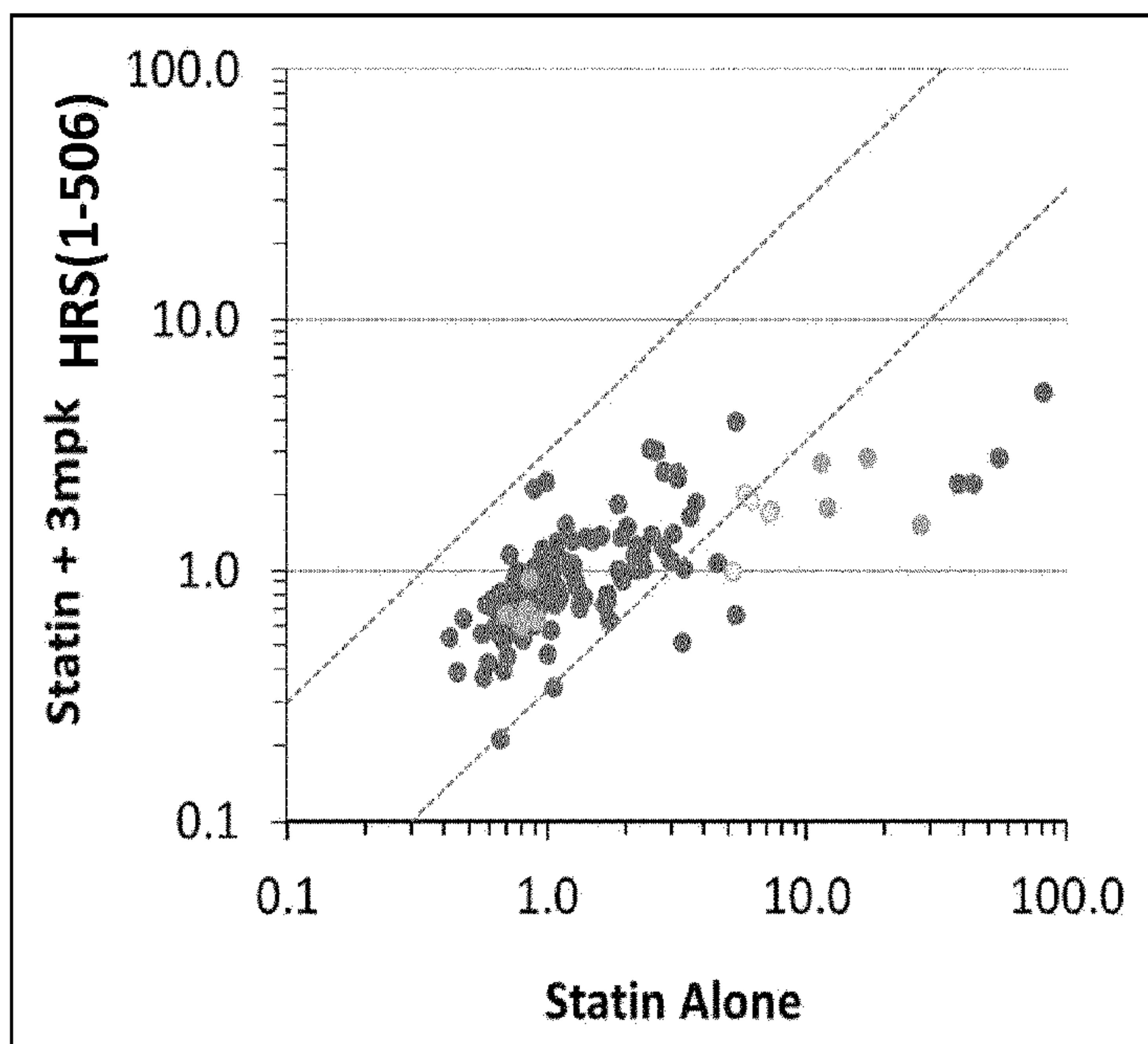


Fig. 10



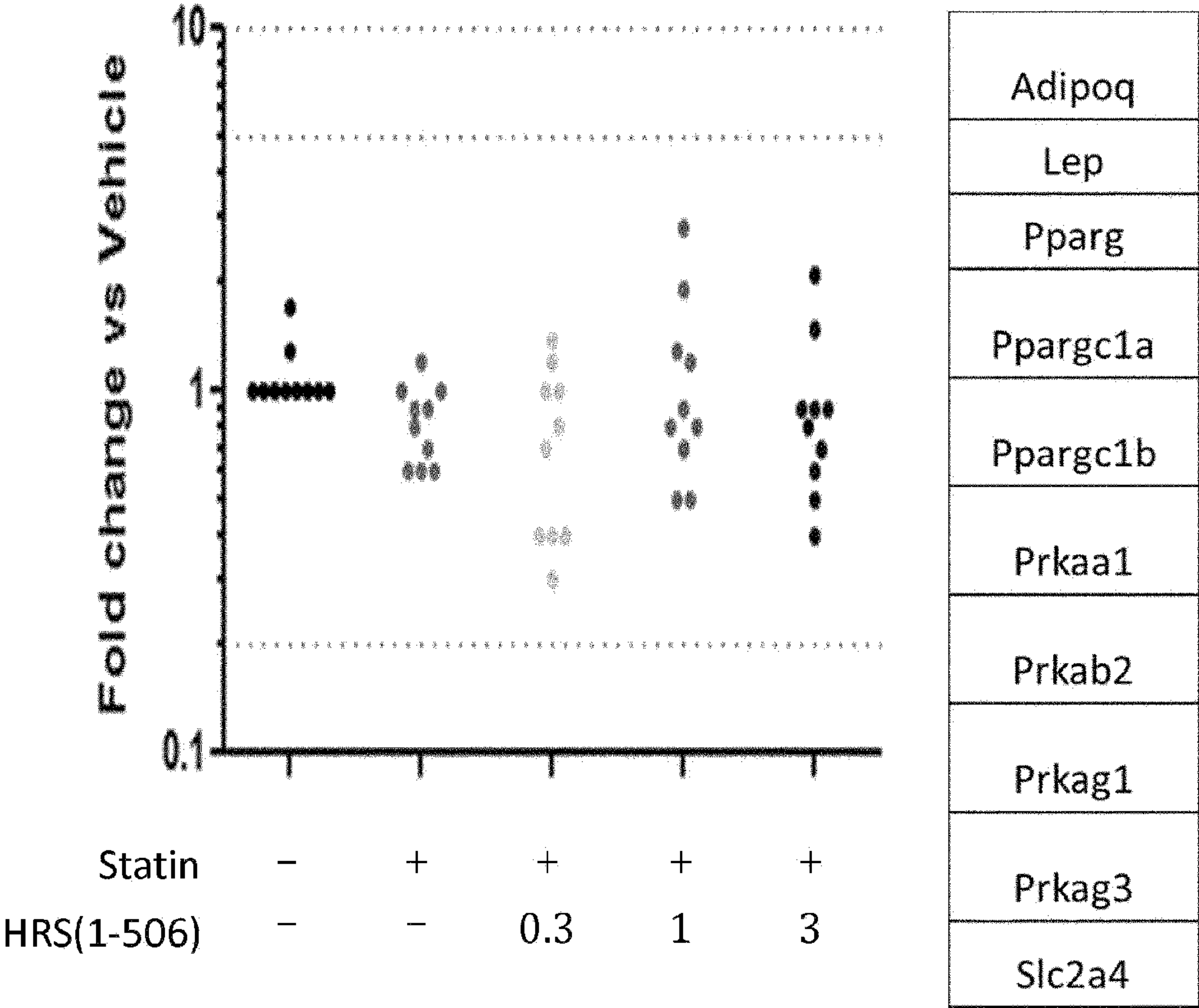


Fig. 11



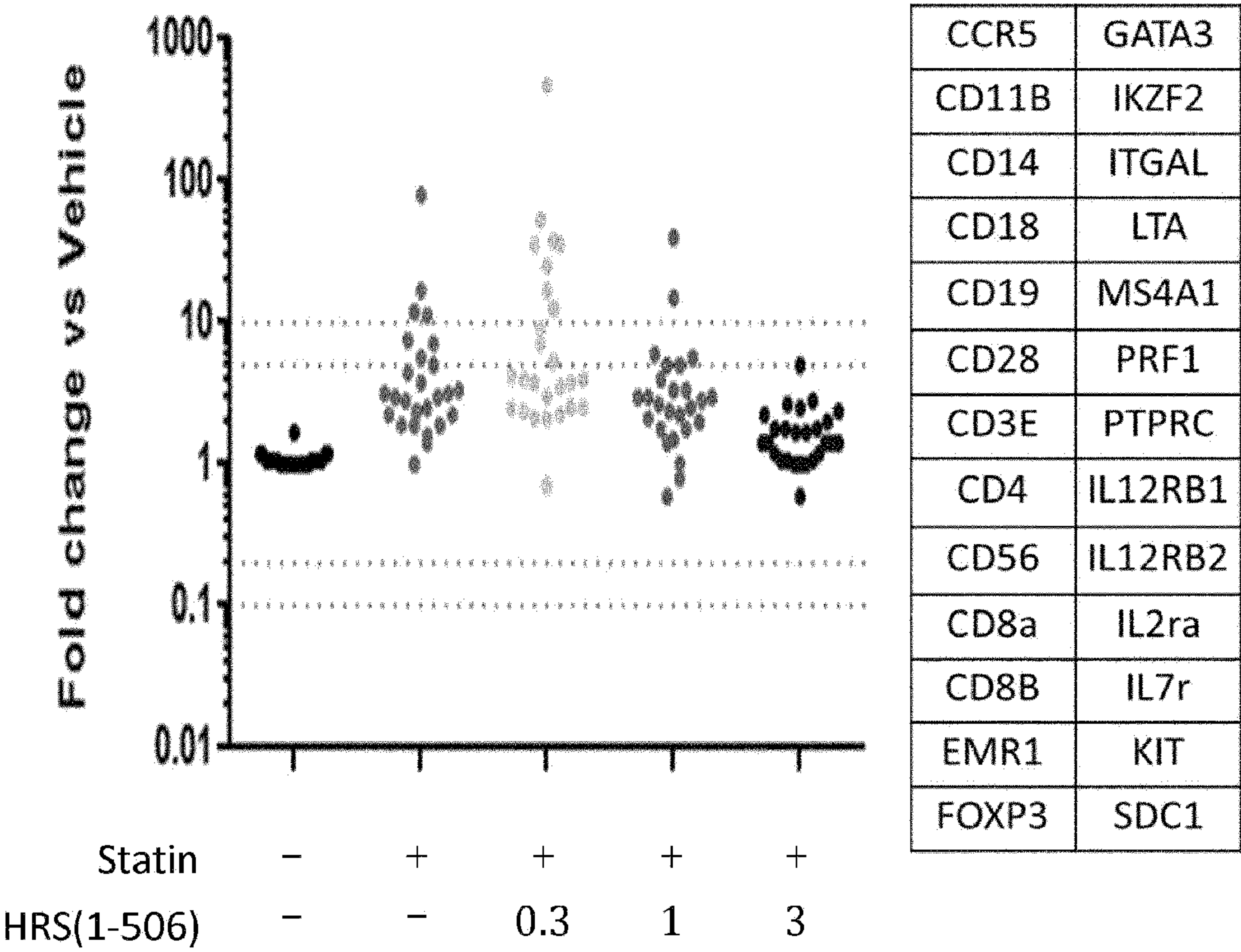


Fig. 12



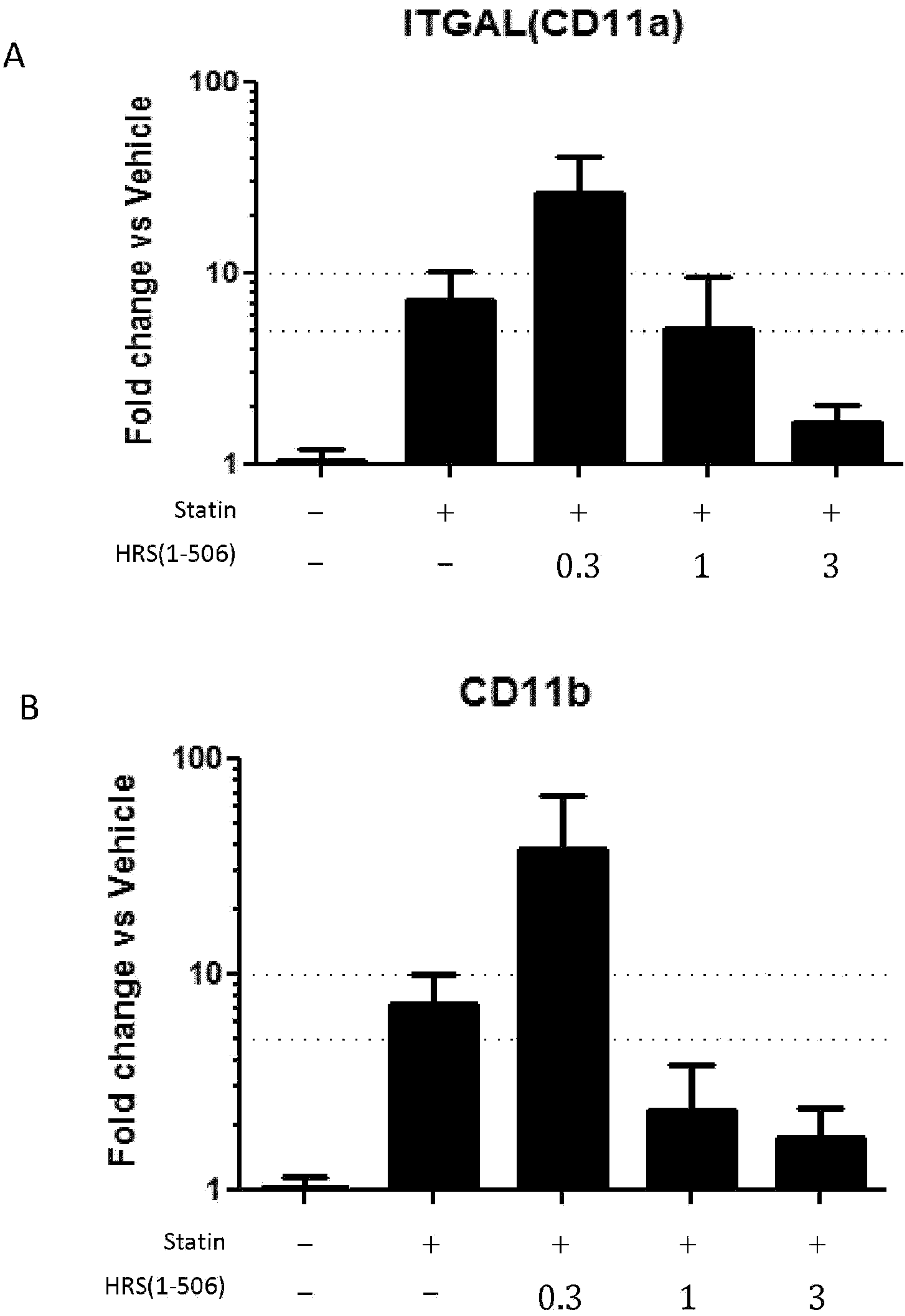
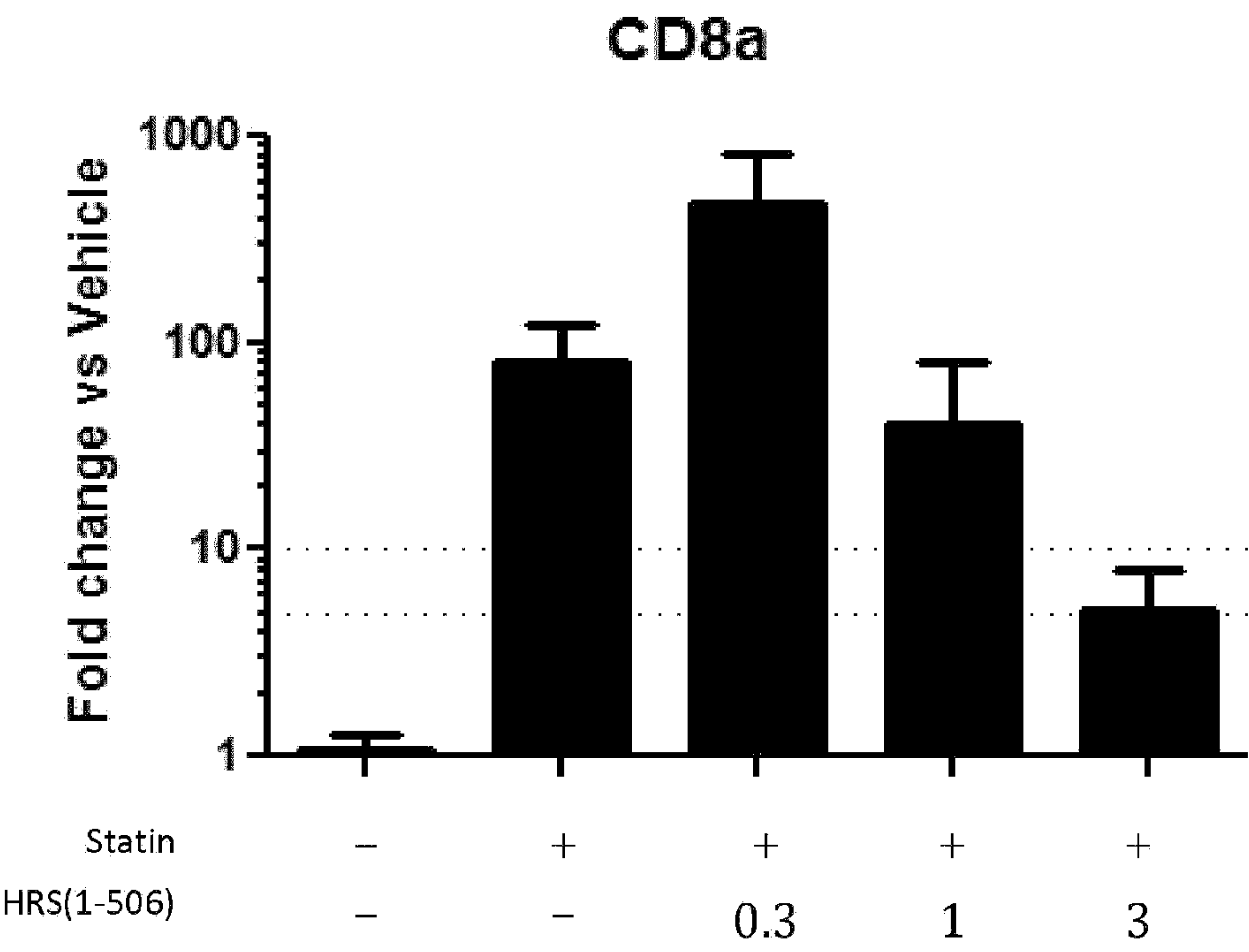


Fig. 13



C



D

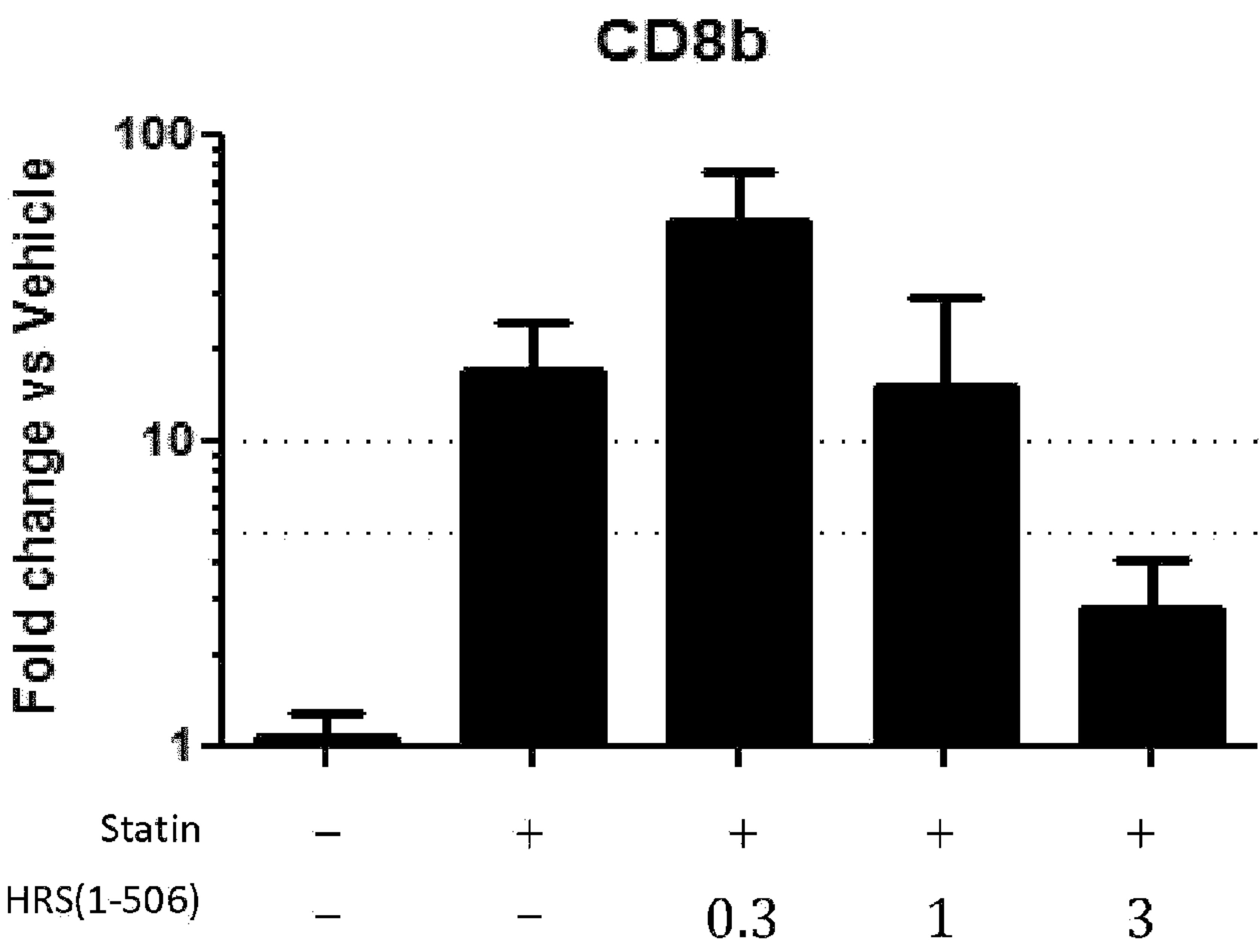


Fig. 13



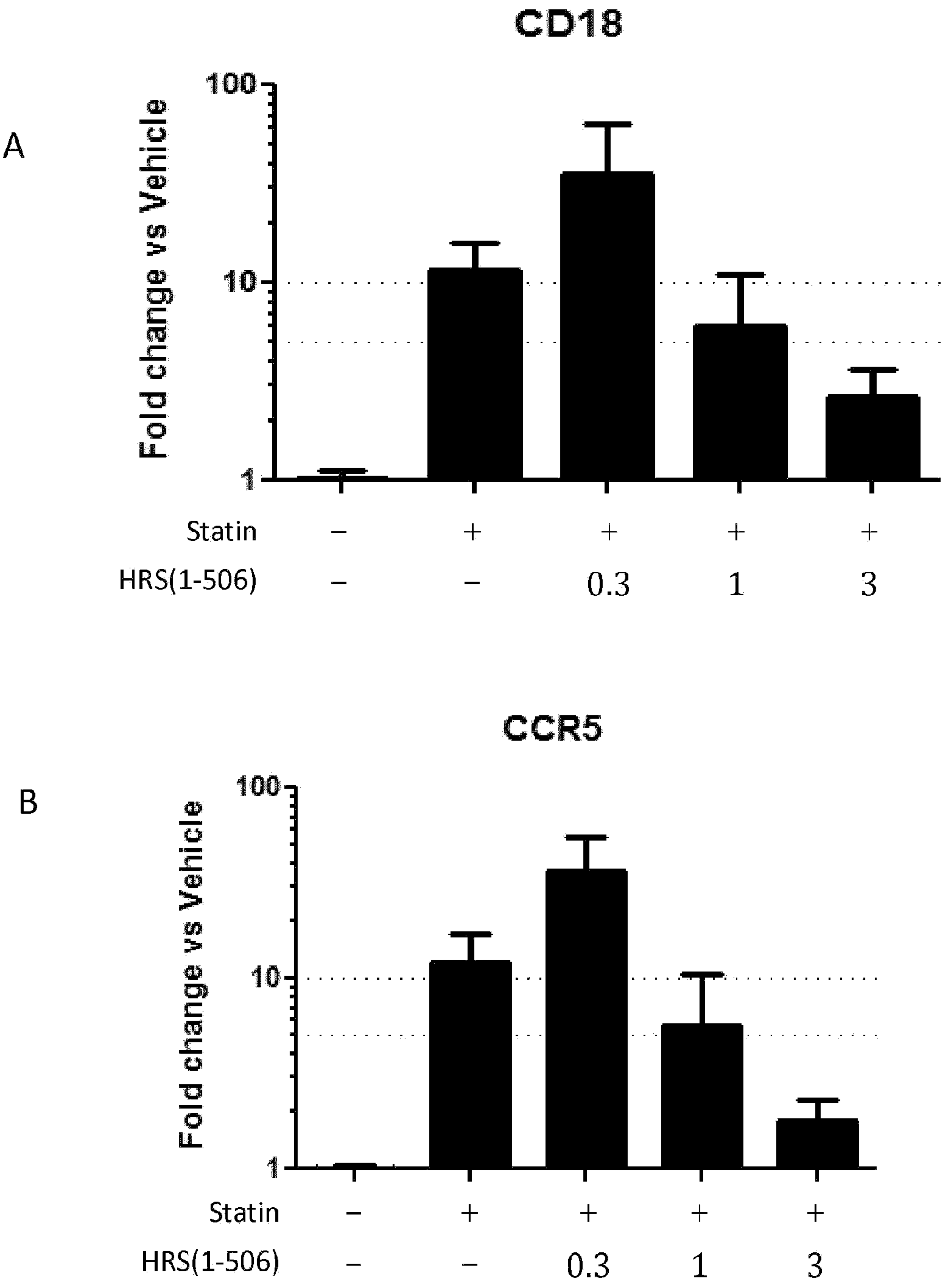


Fig. 14



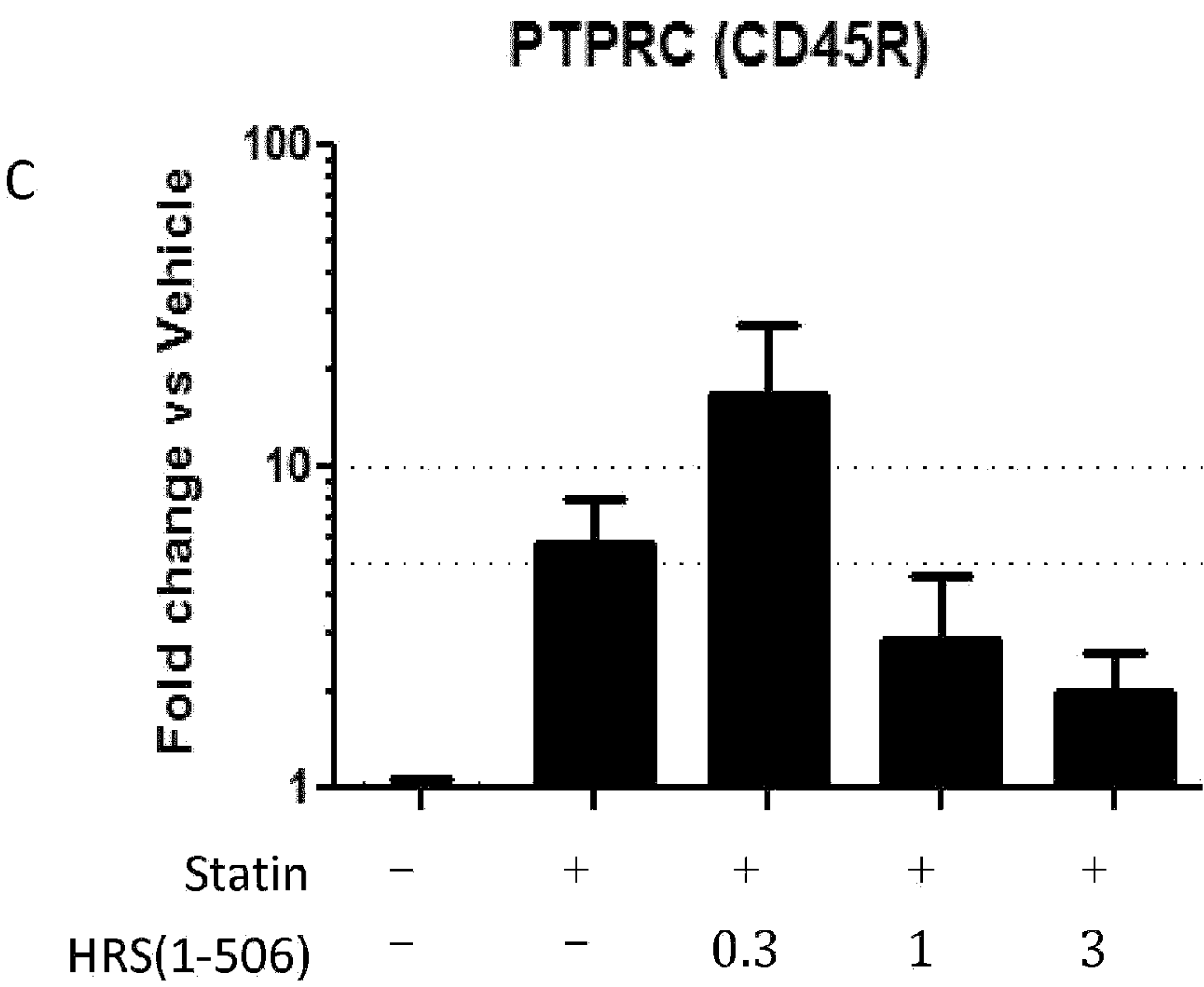


Fig. 14



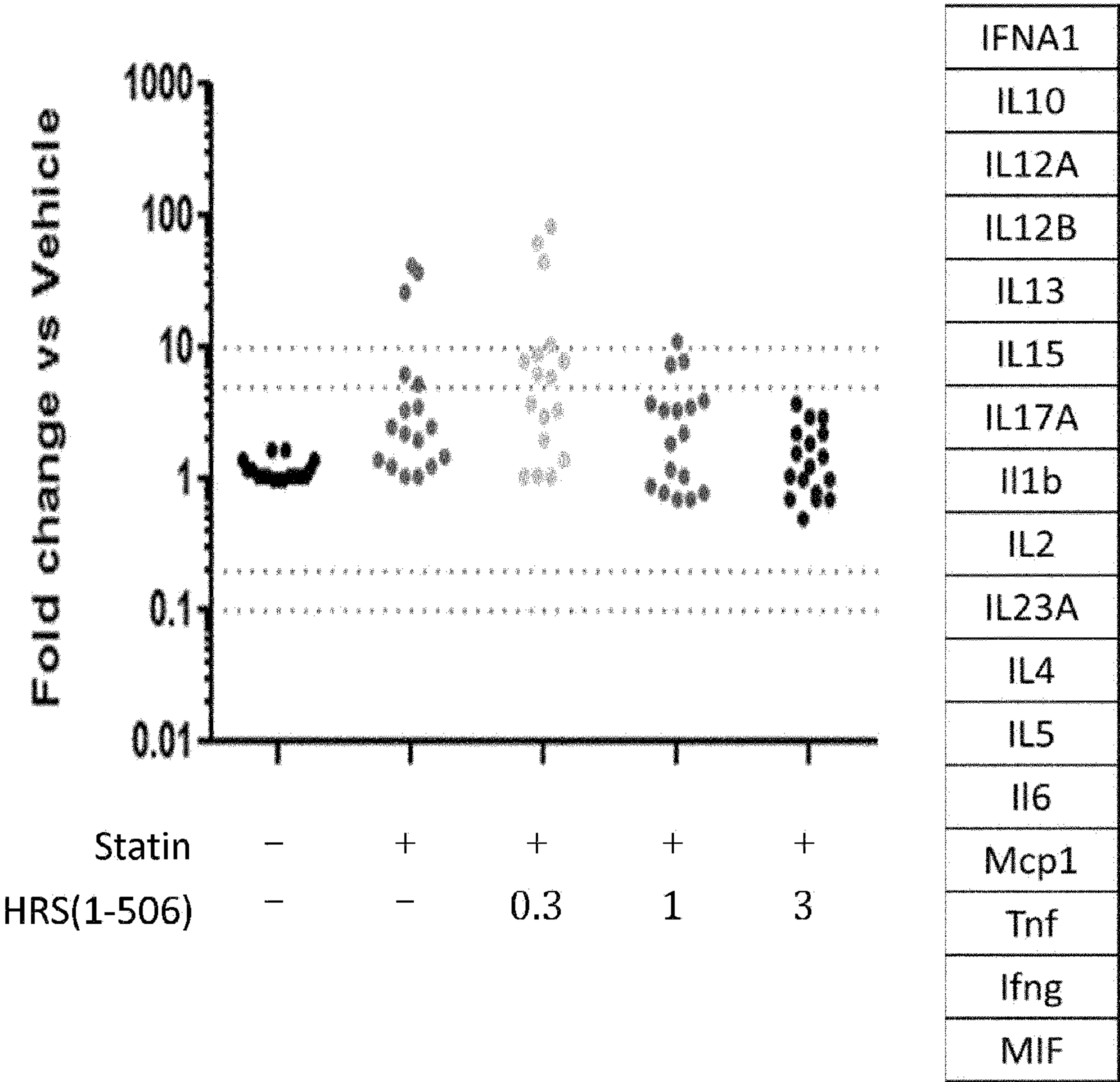


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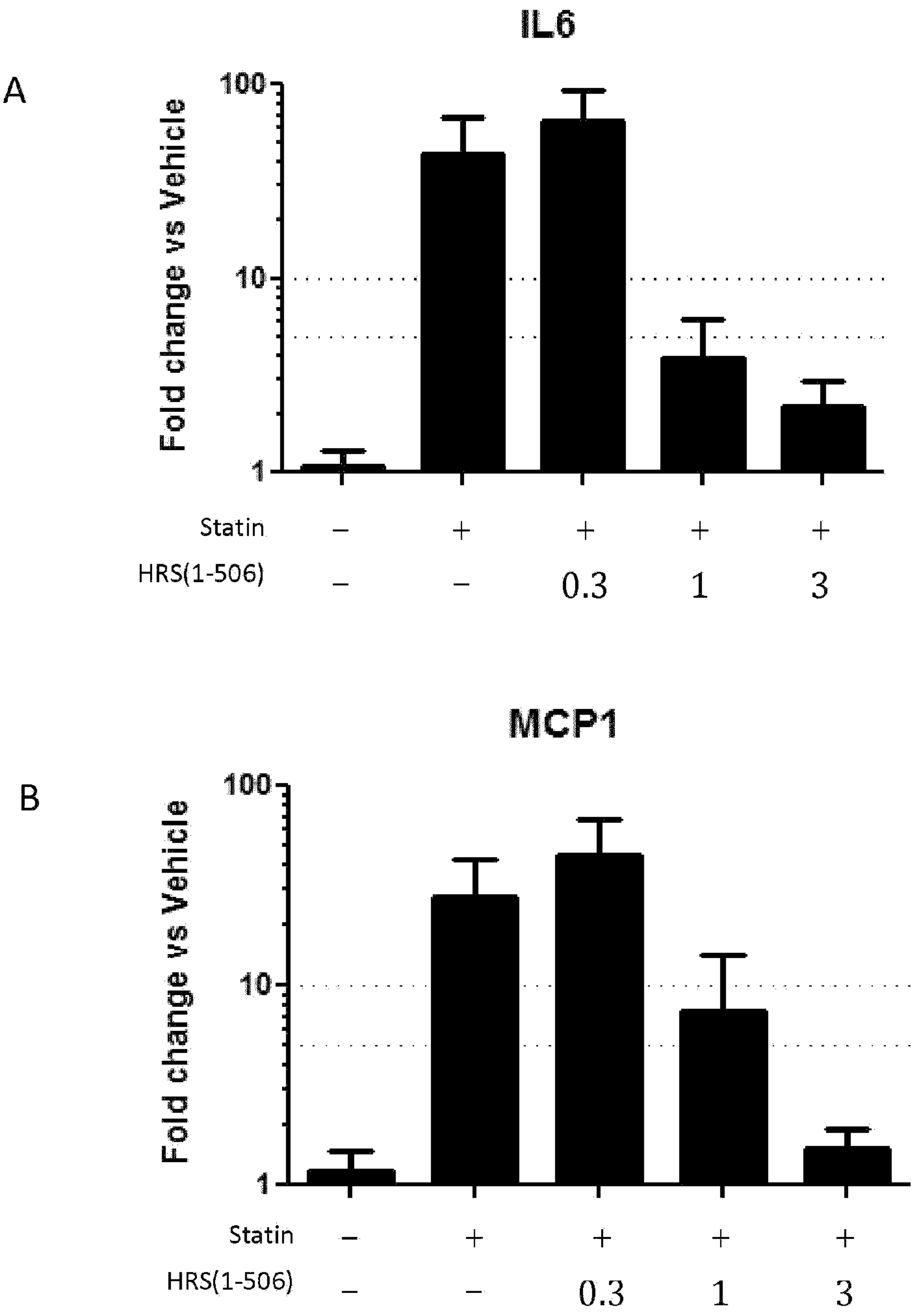


Fig. 16



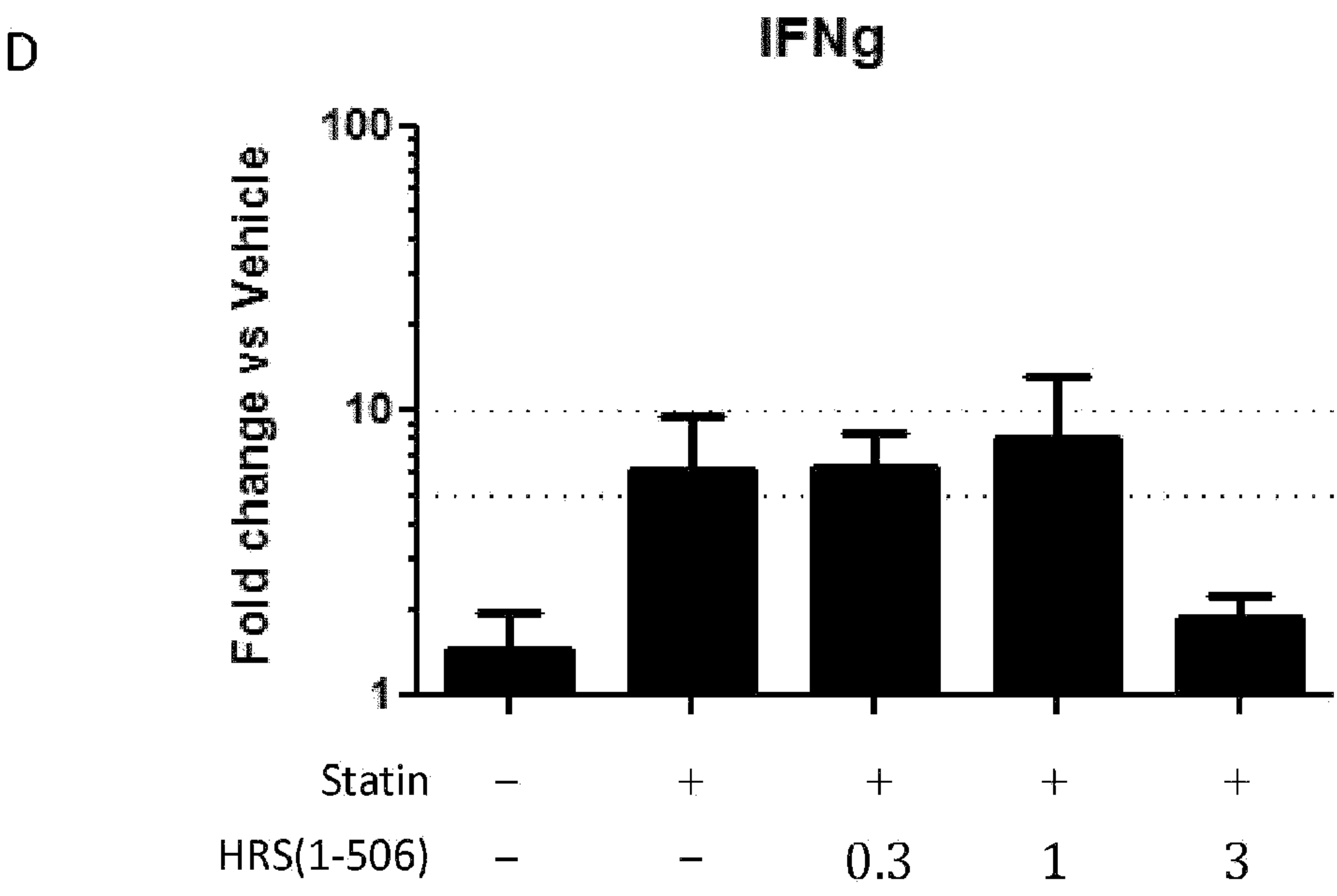
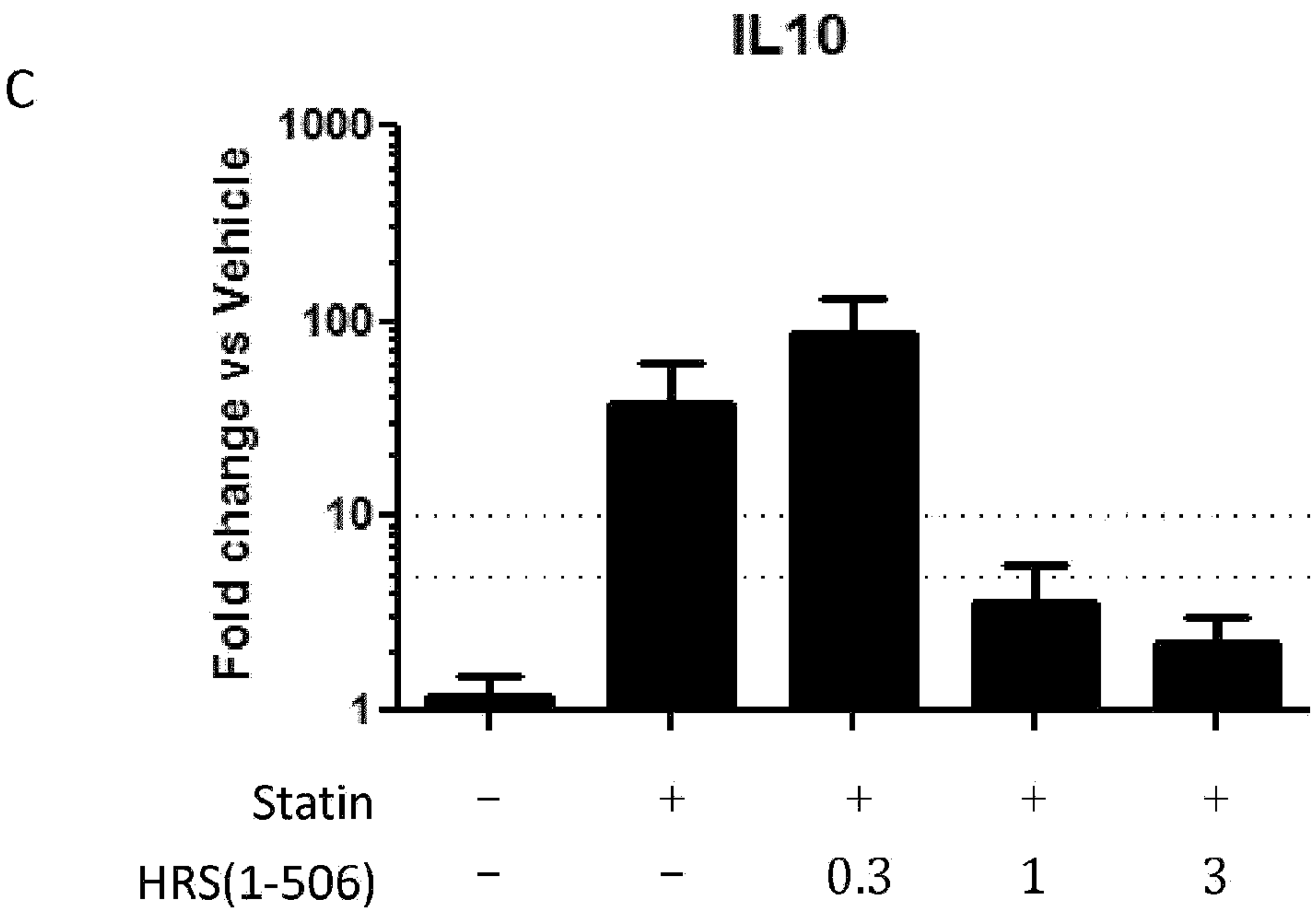


Fig. 16



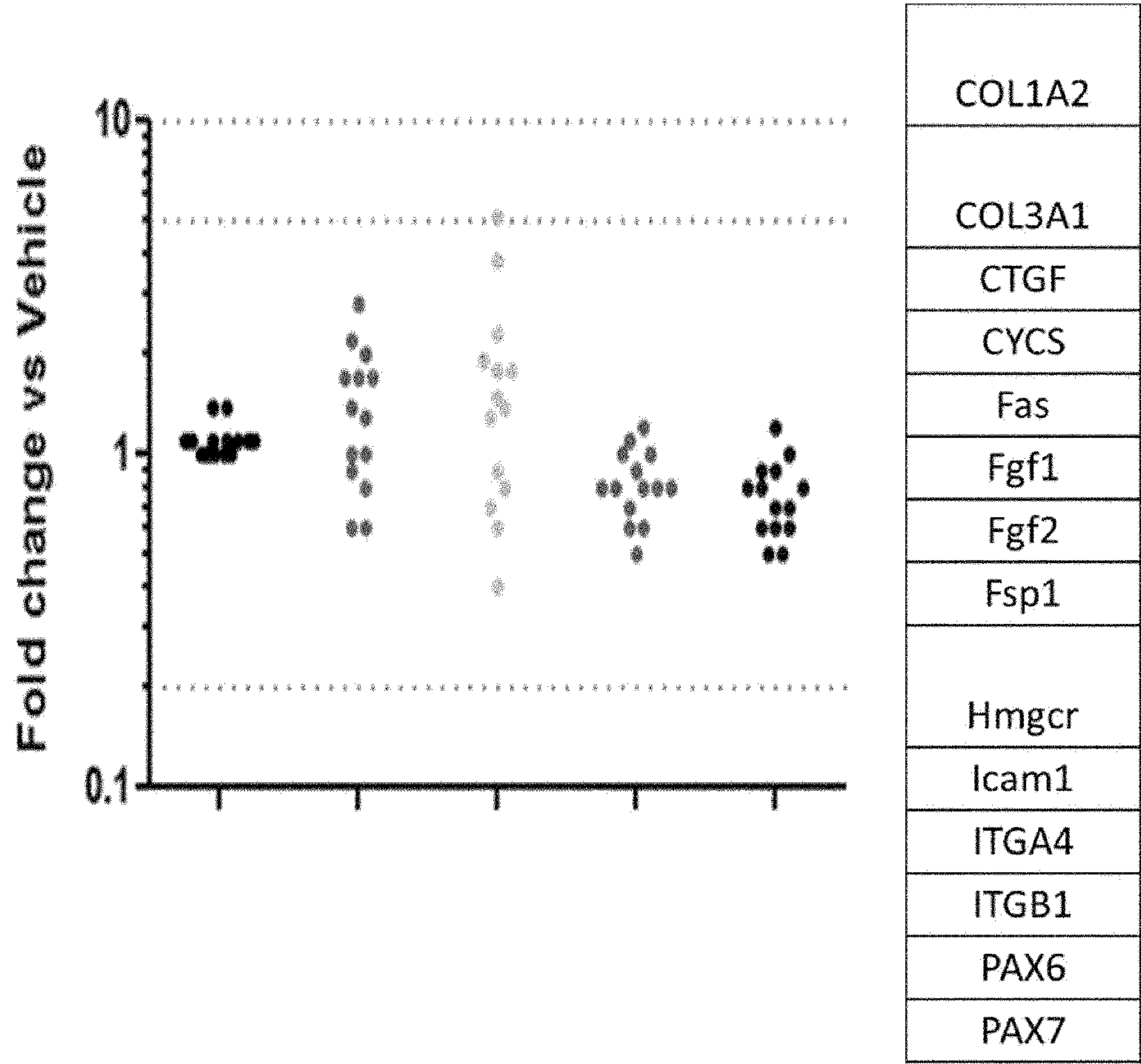


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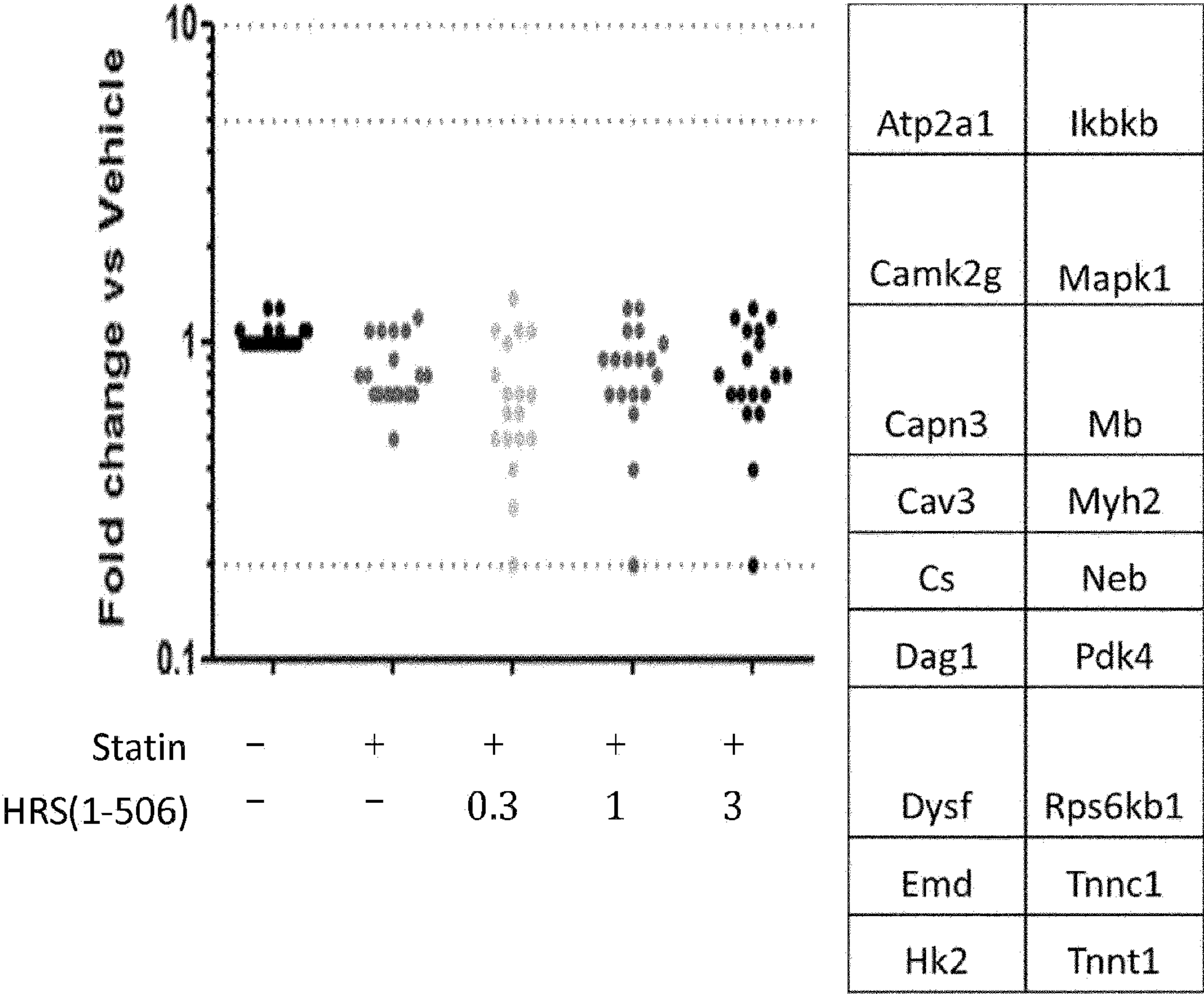


Fig. 18



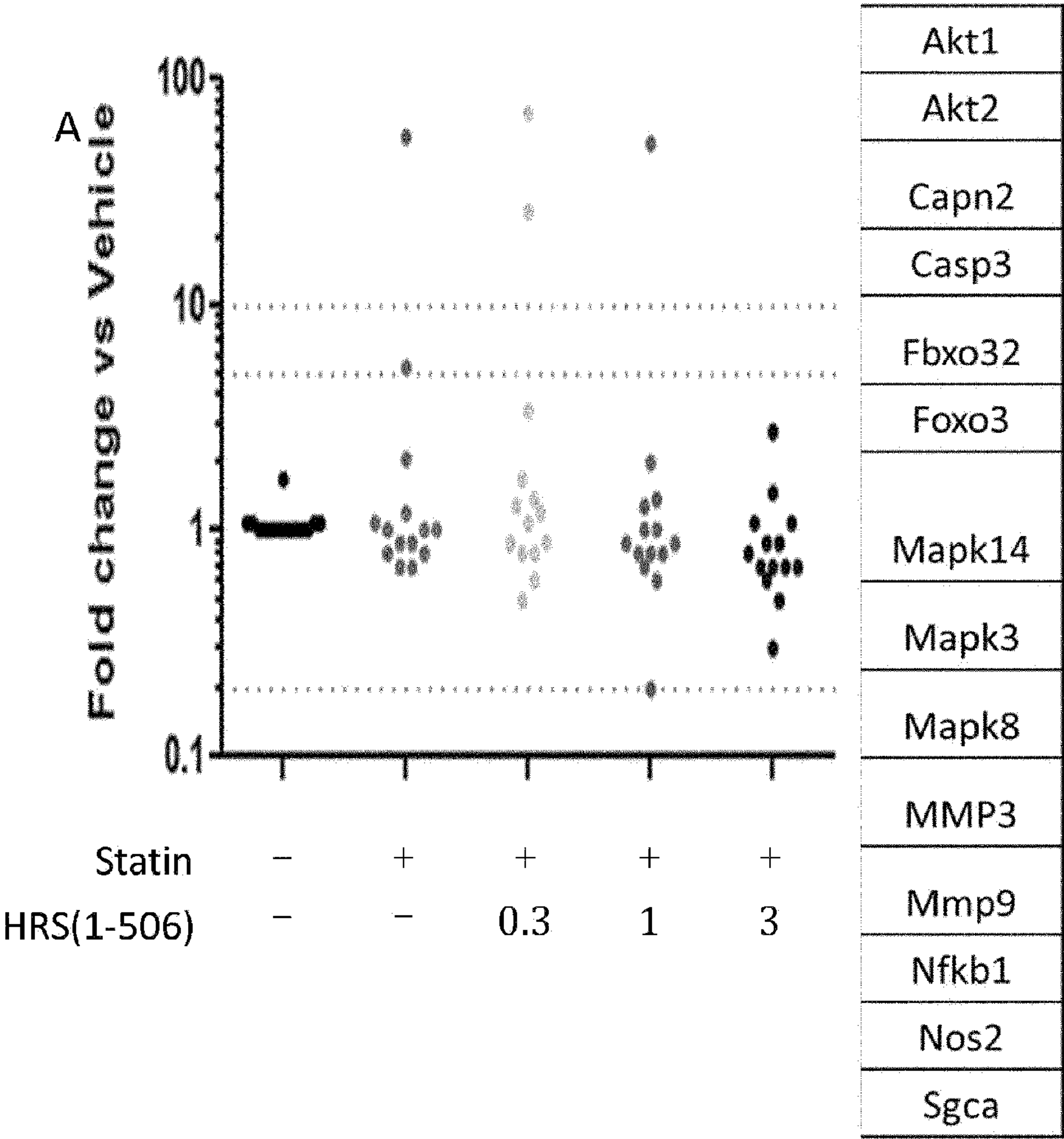


Fig. 19



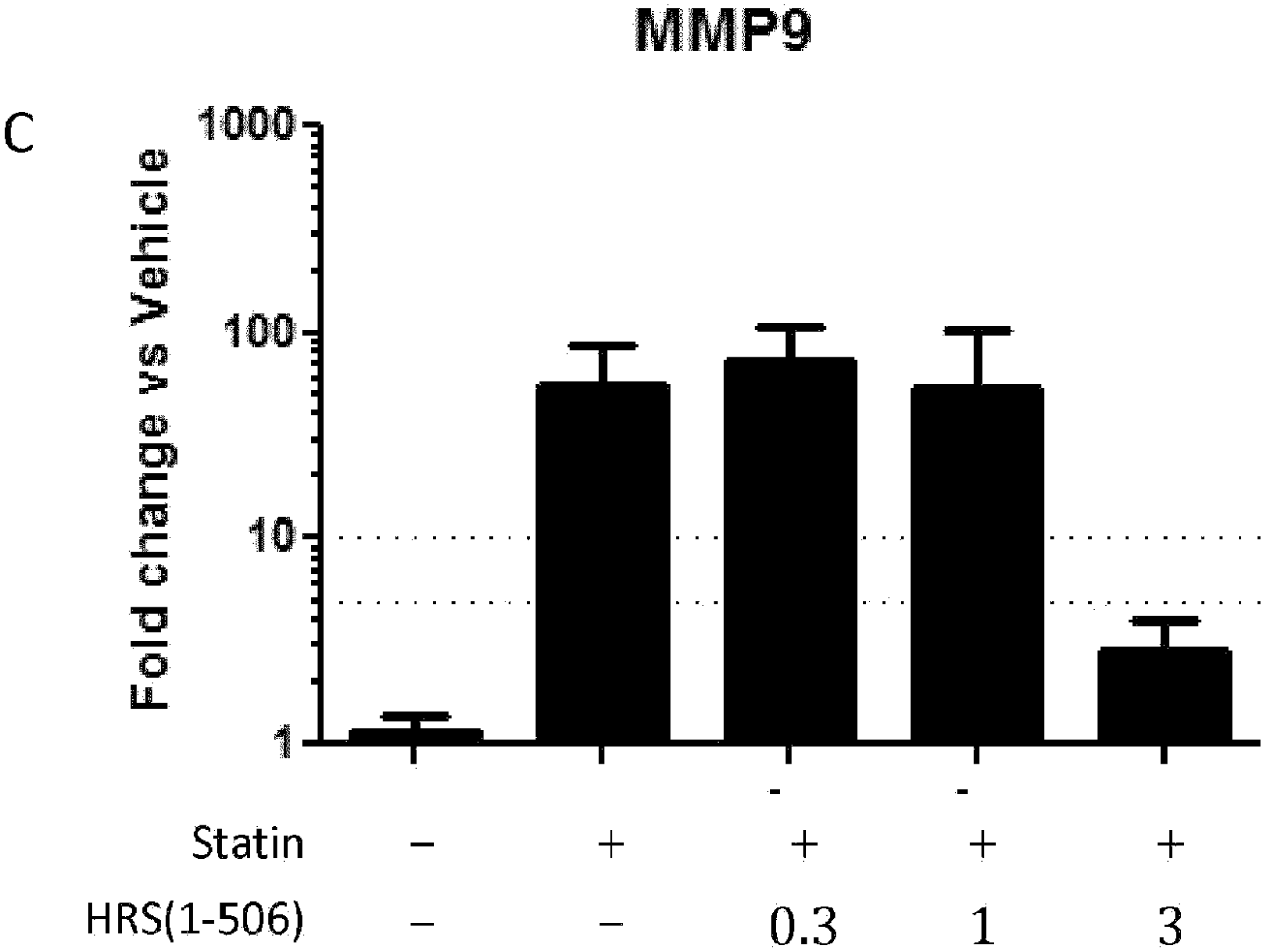
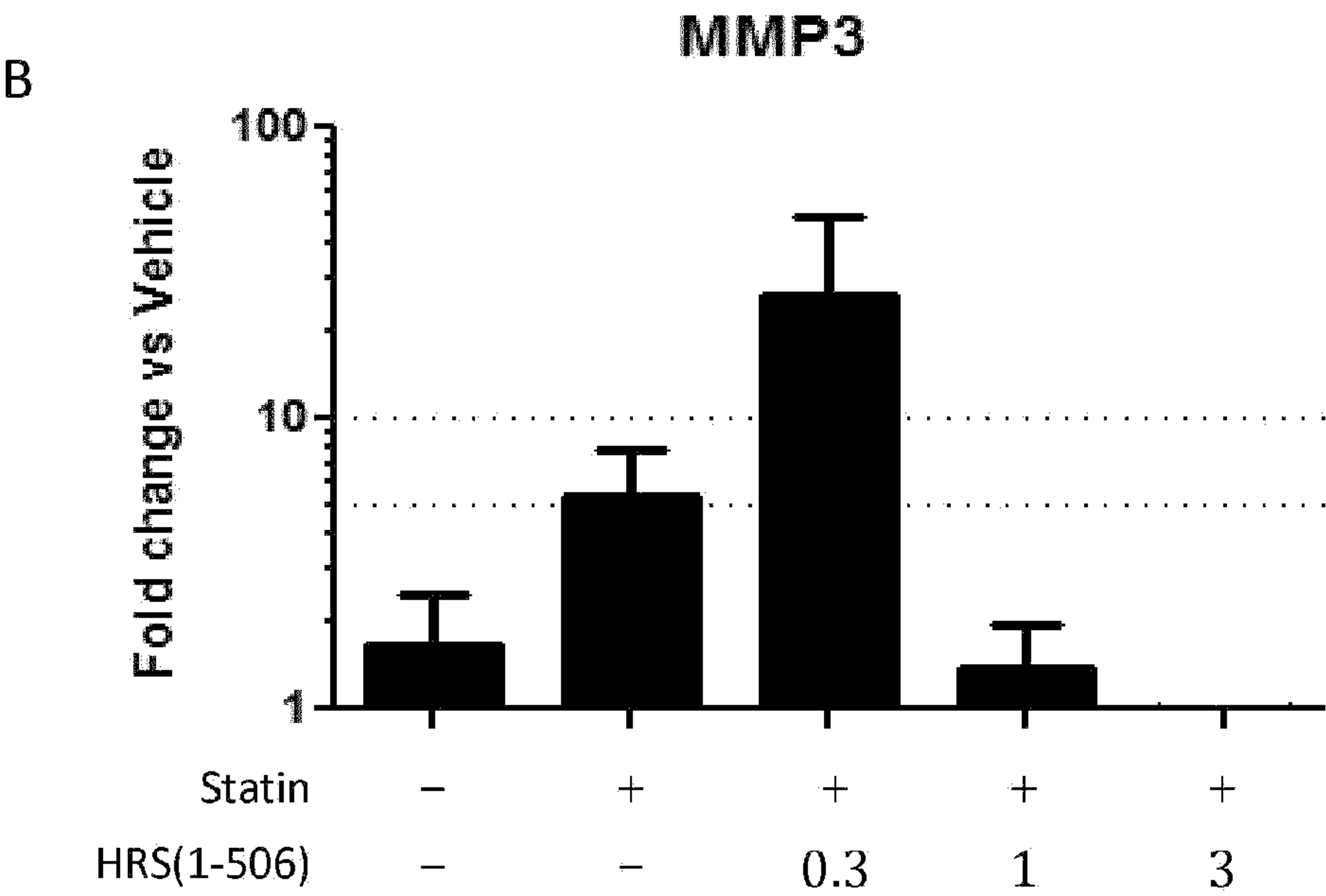


Fig. 19



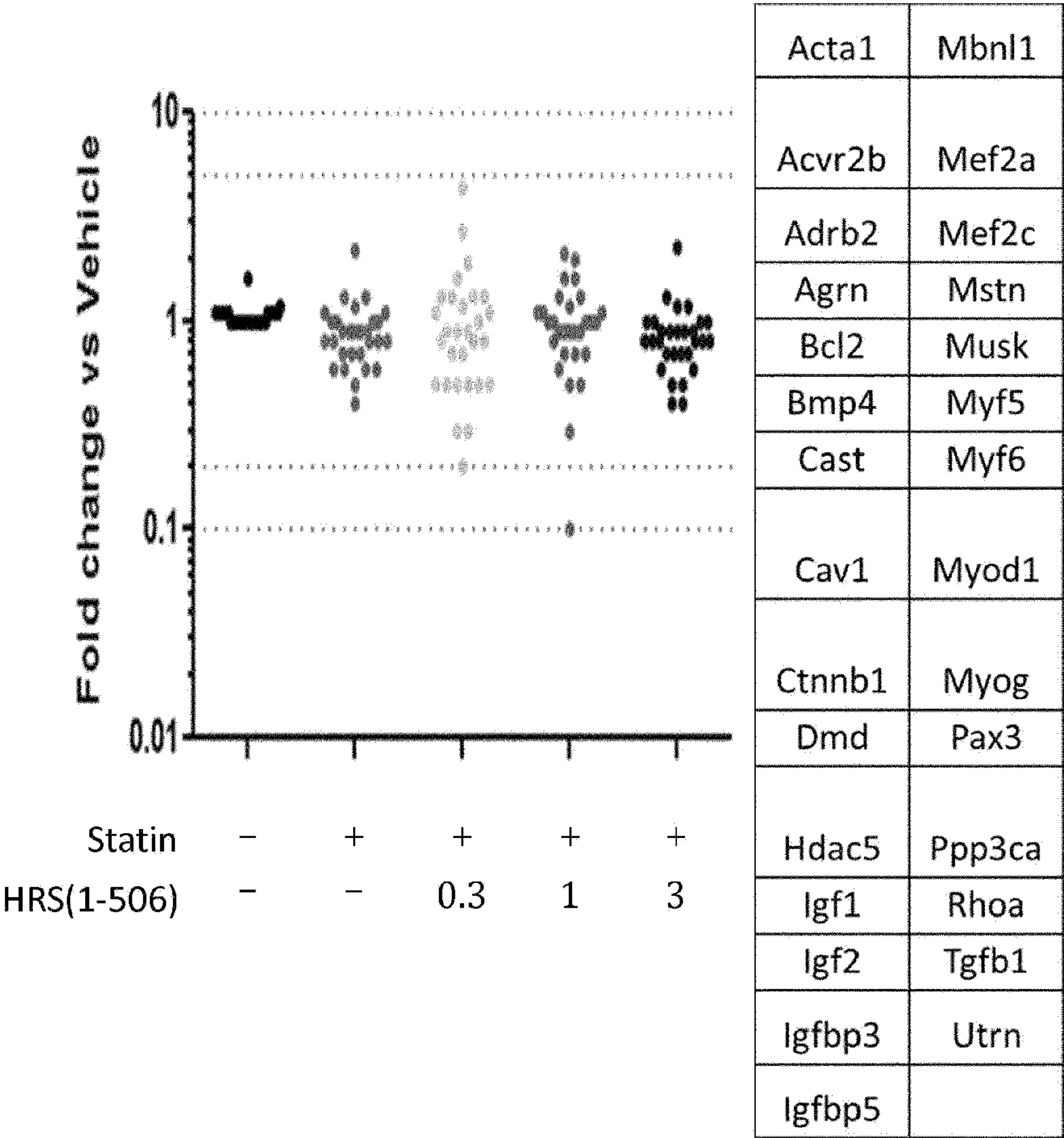


Fig. 20



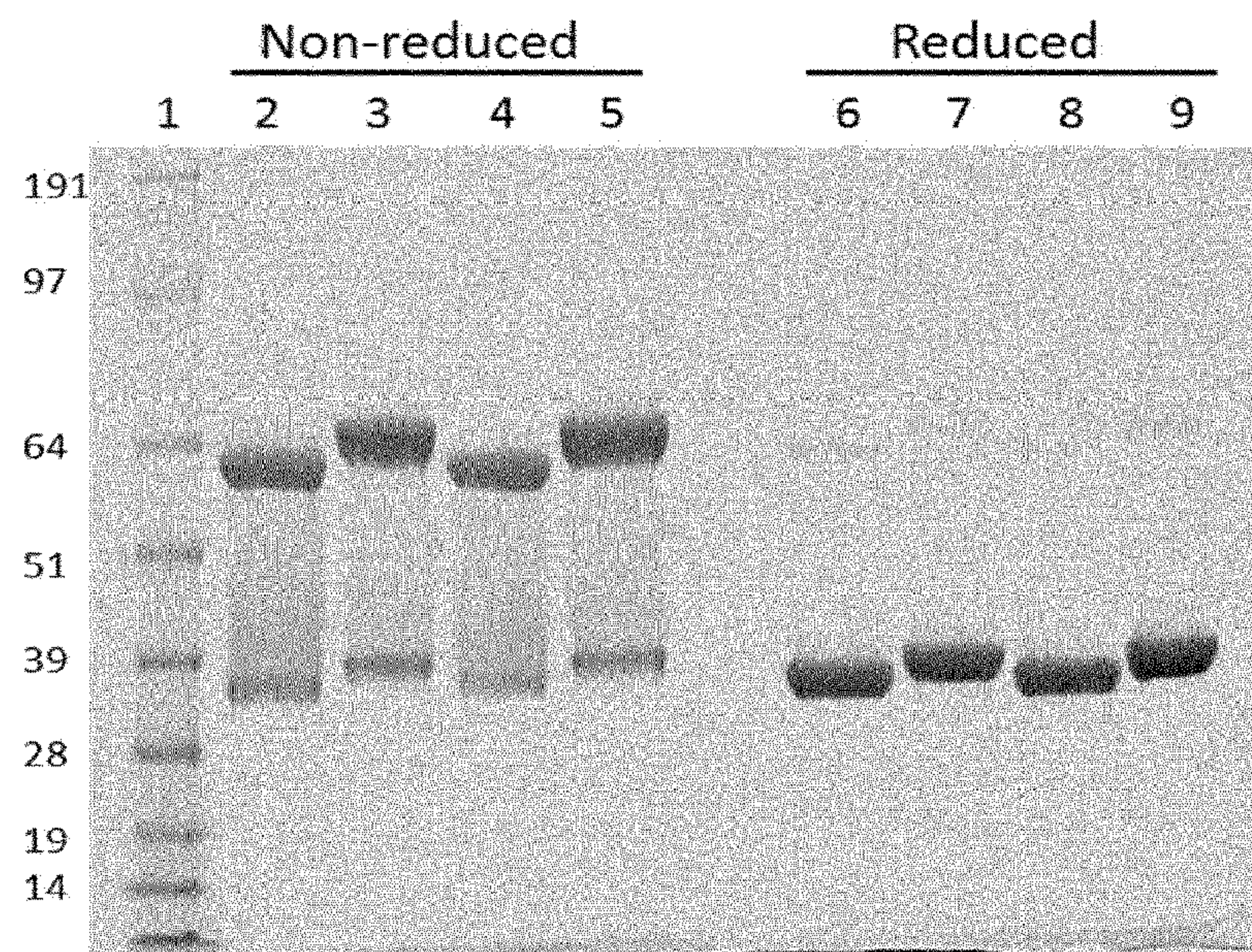


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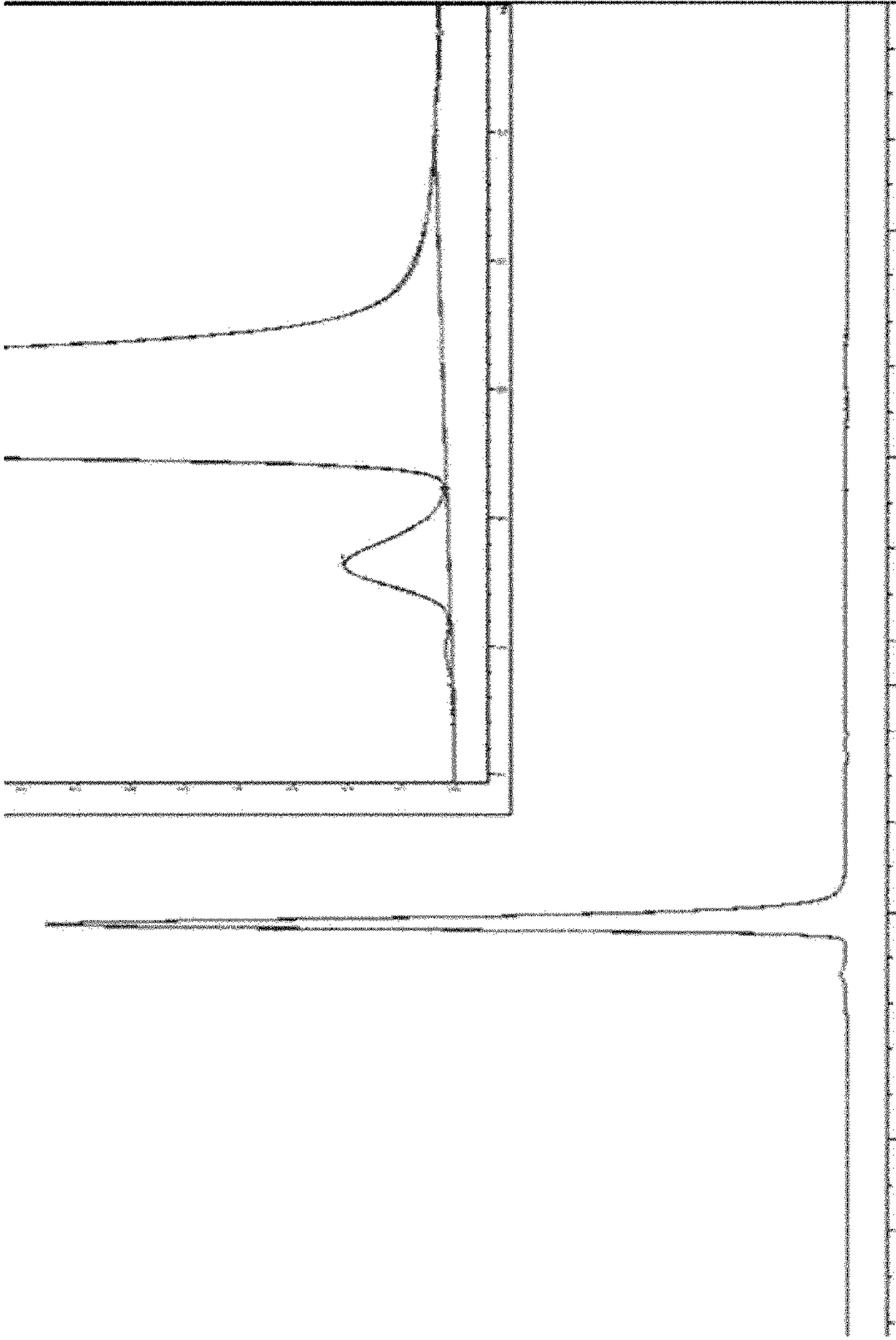


Fig. 22



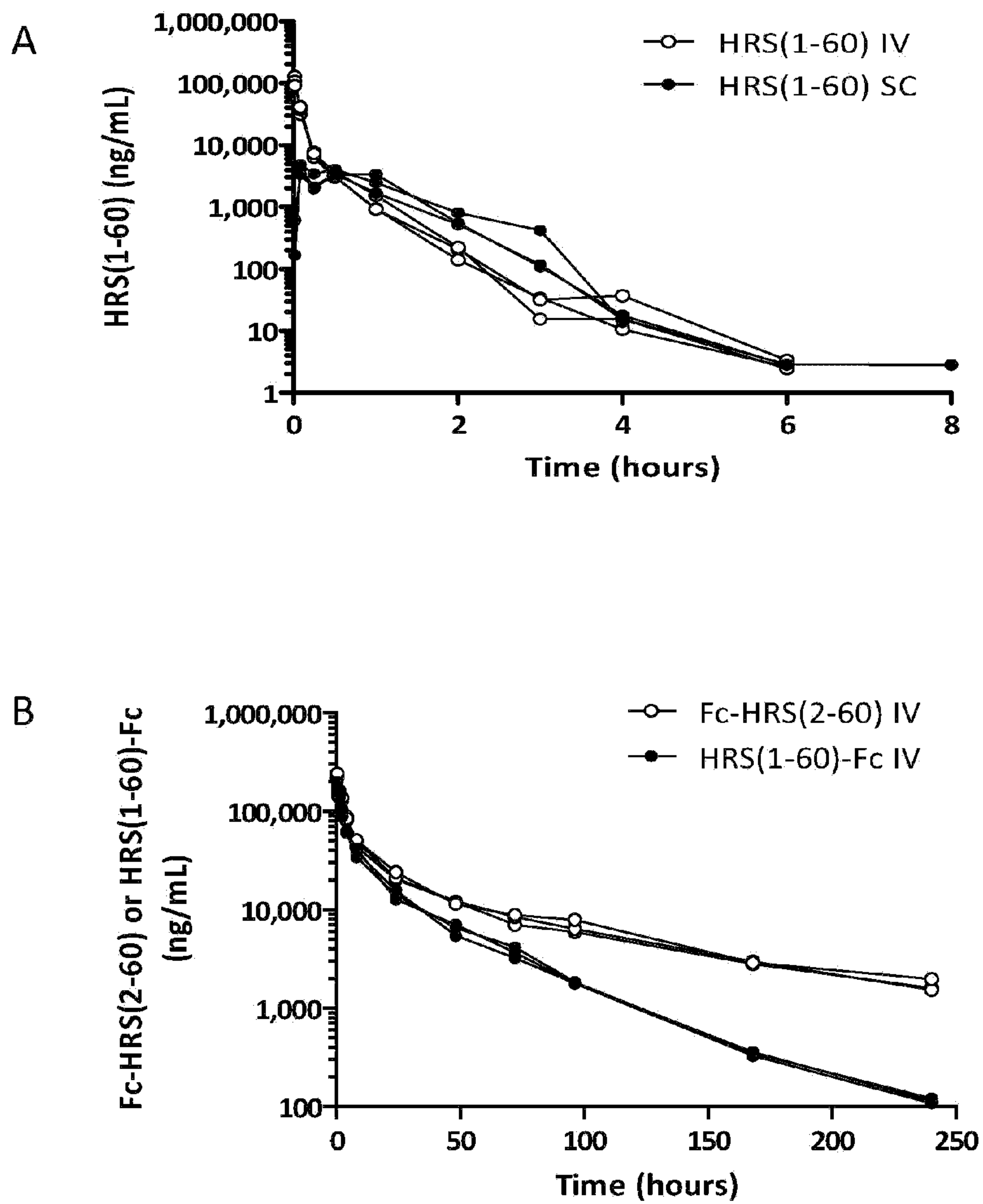


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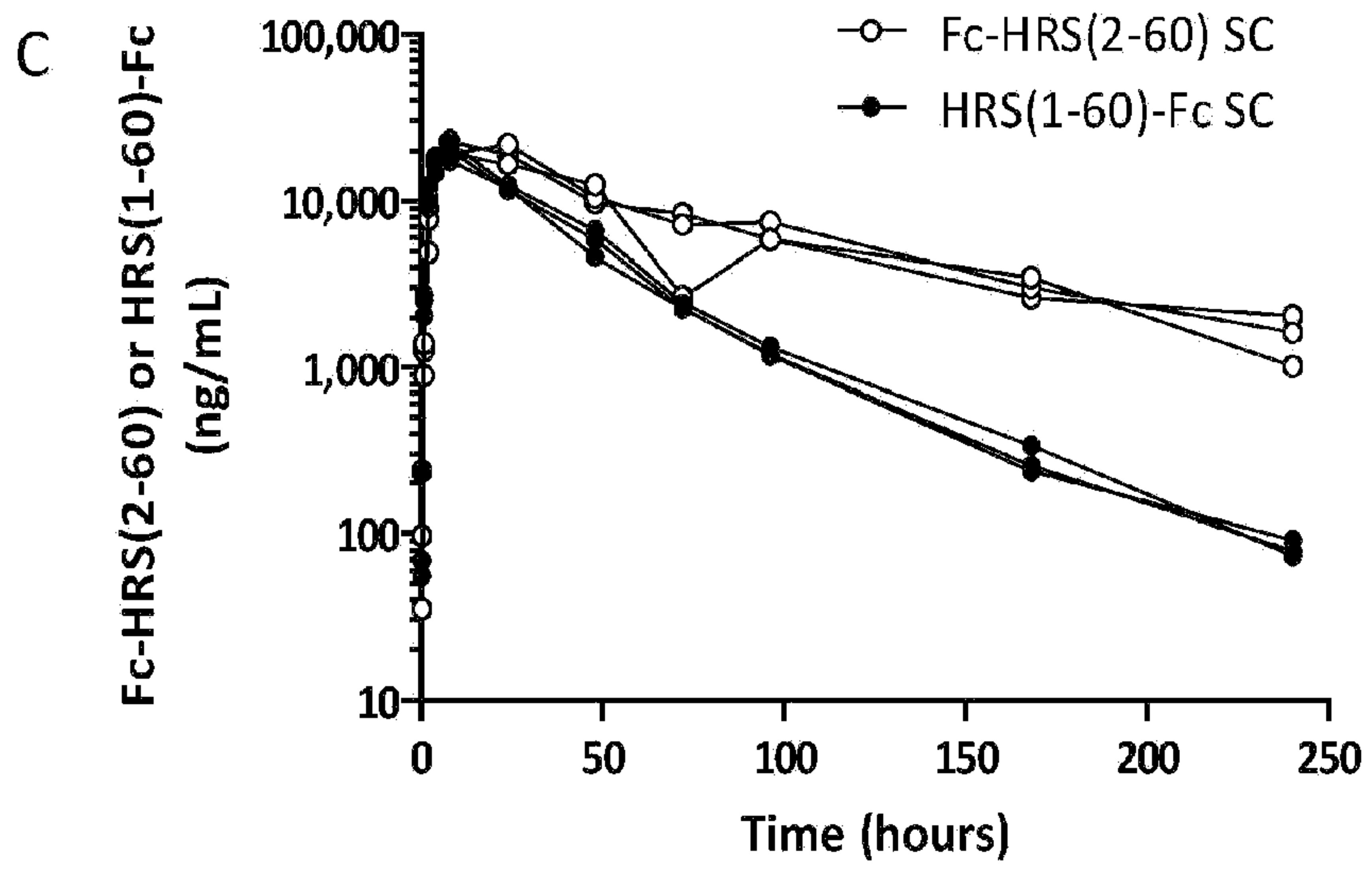


Fig. 23



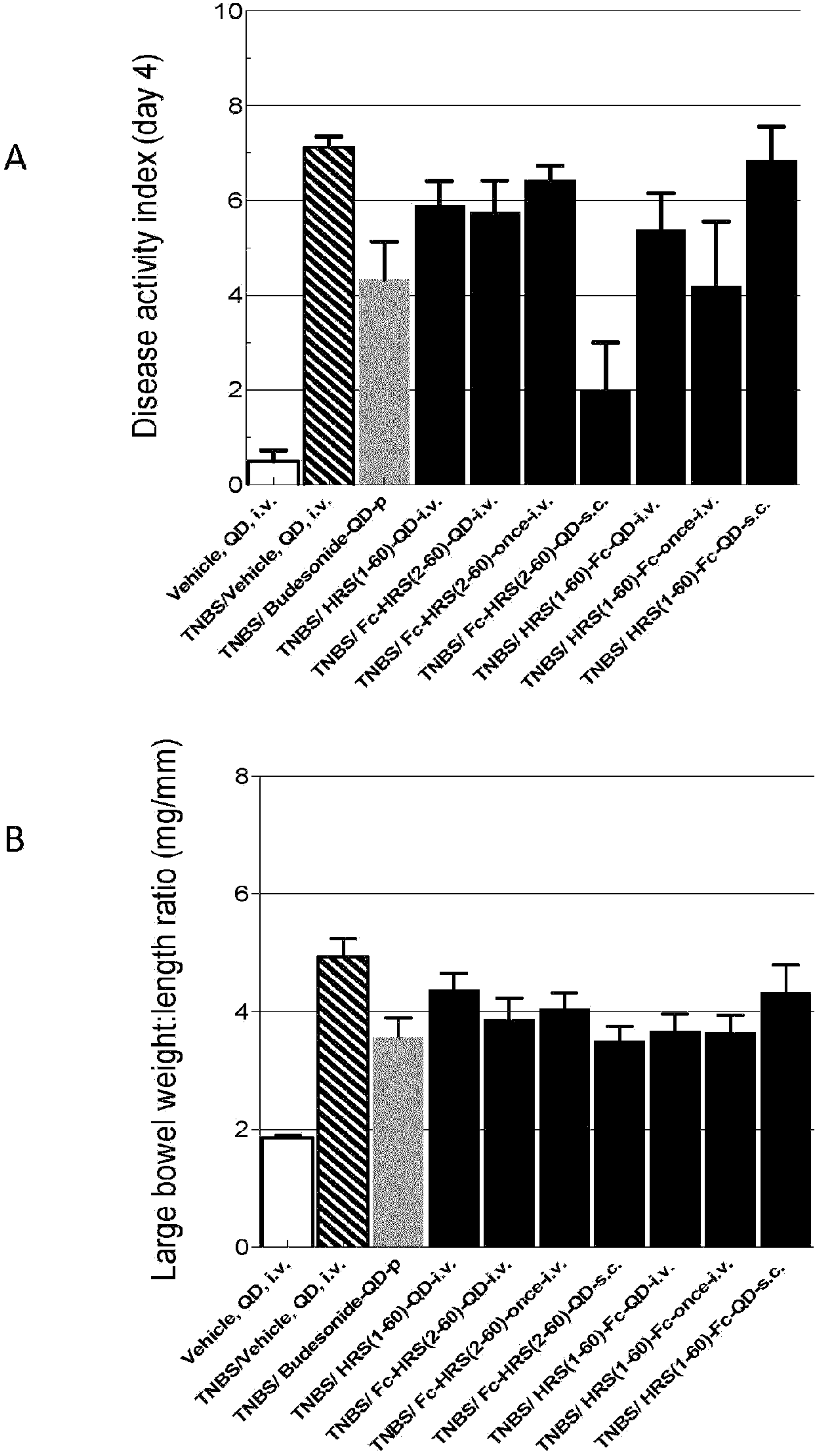


Fig. 24



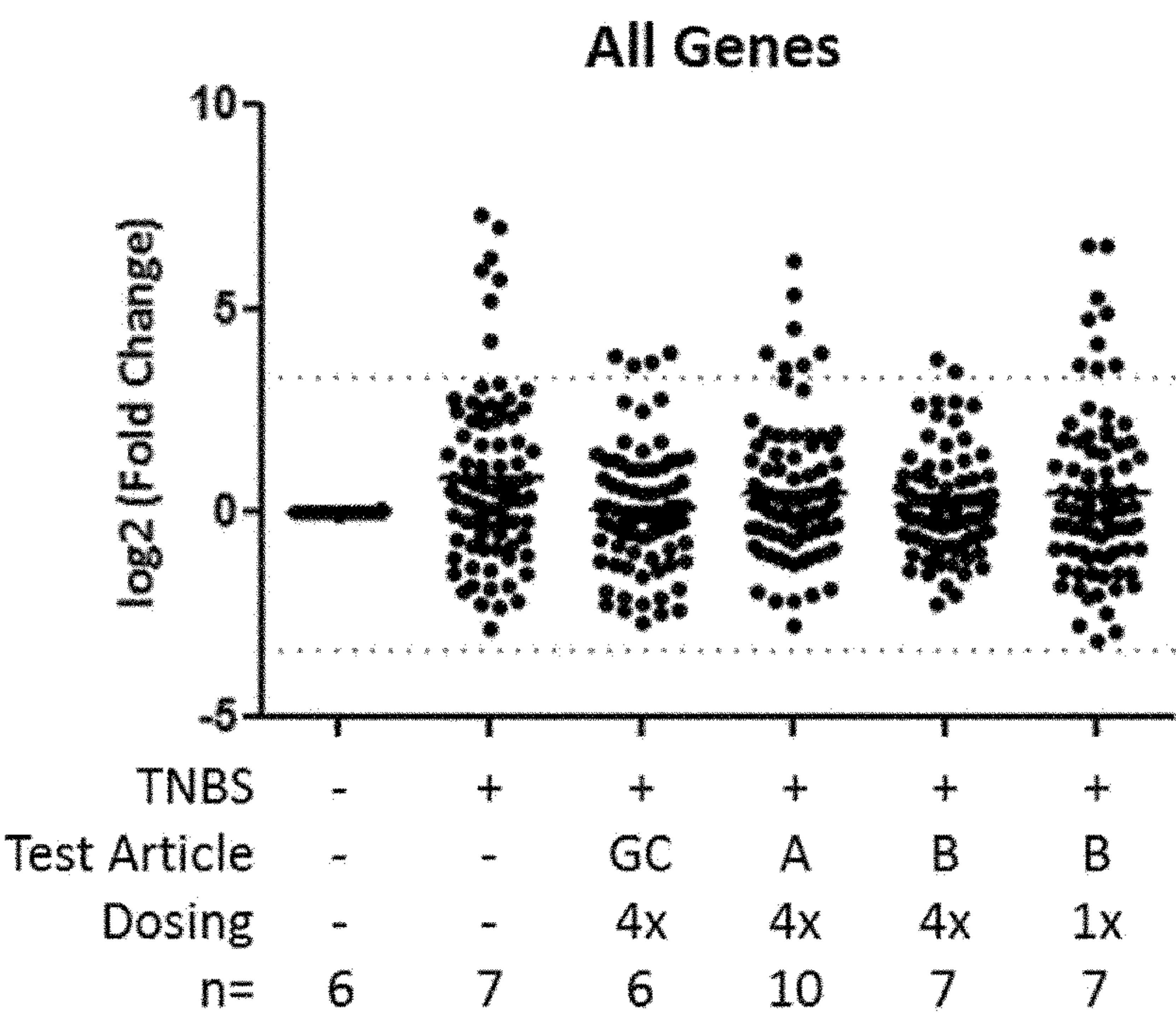


Fig. 25



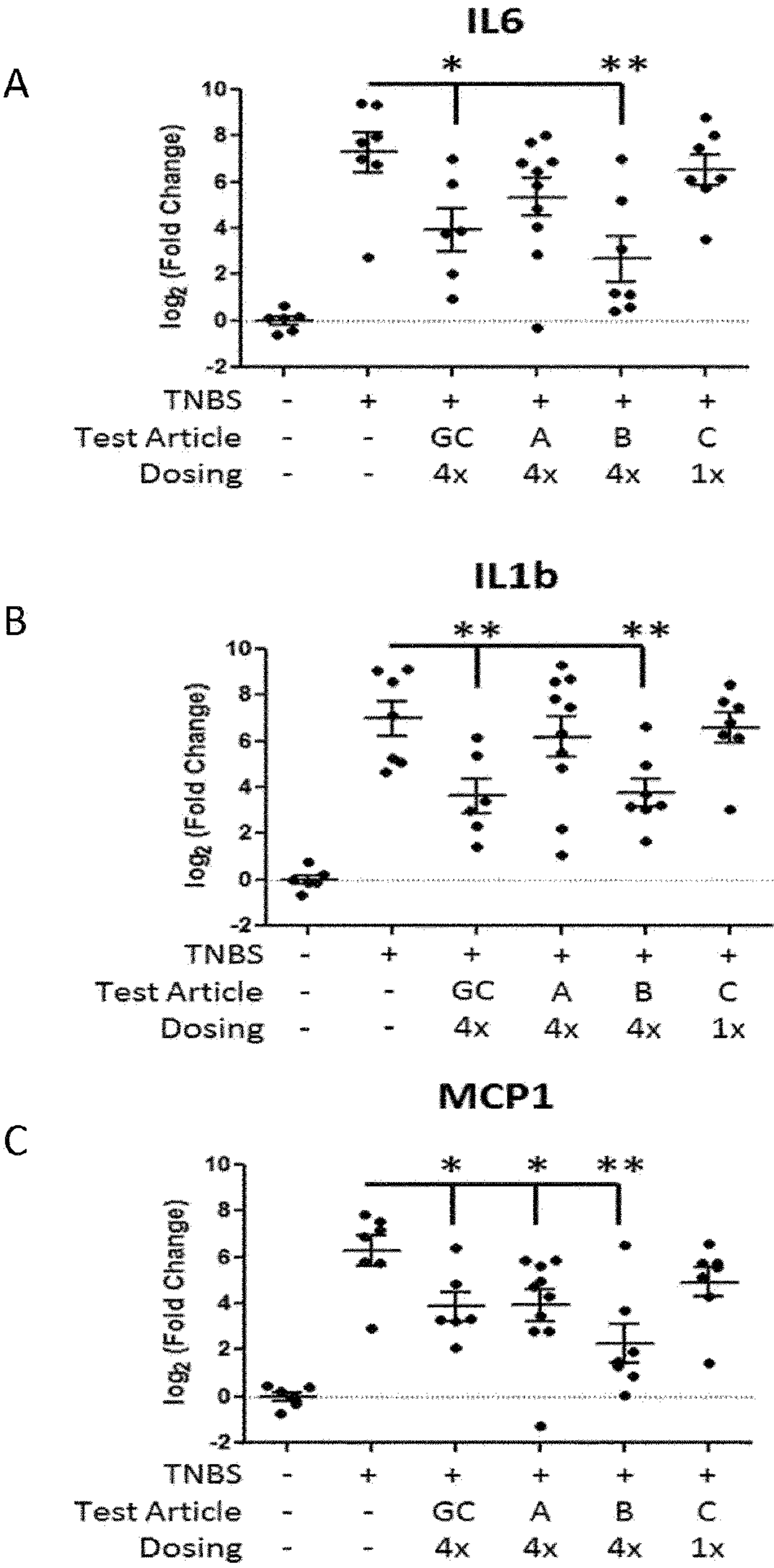
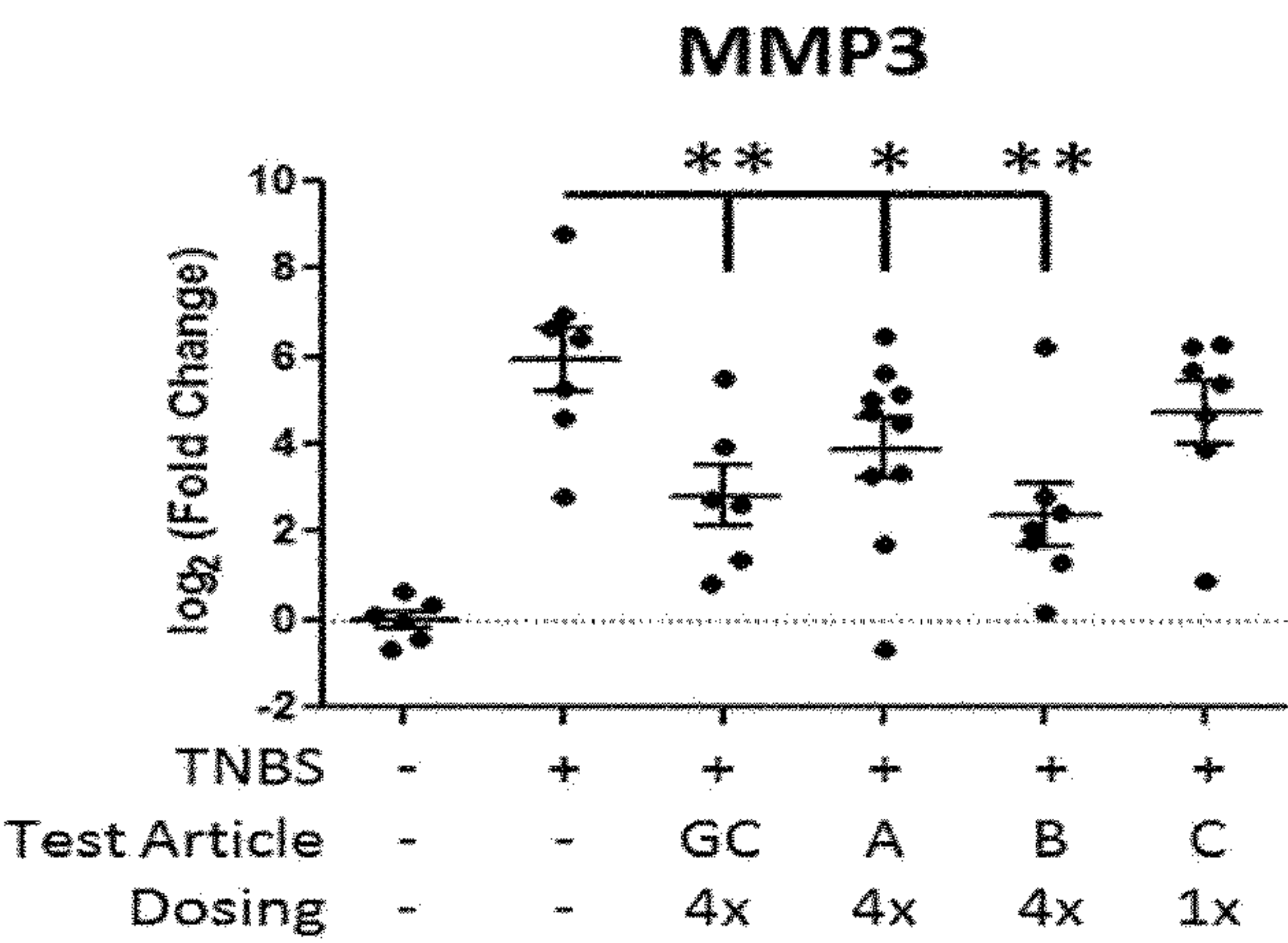


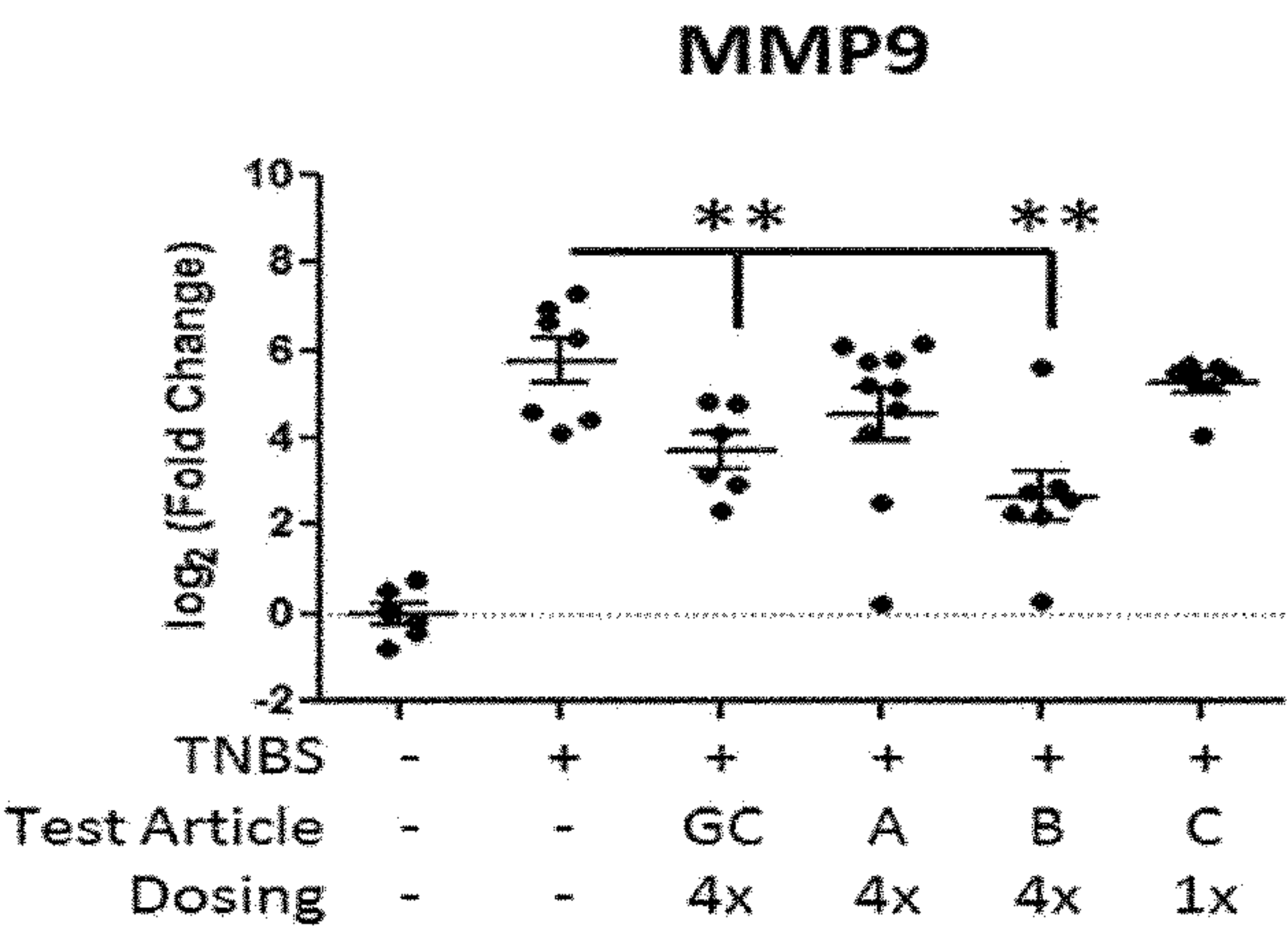
Fig. 26



D



E



F

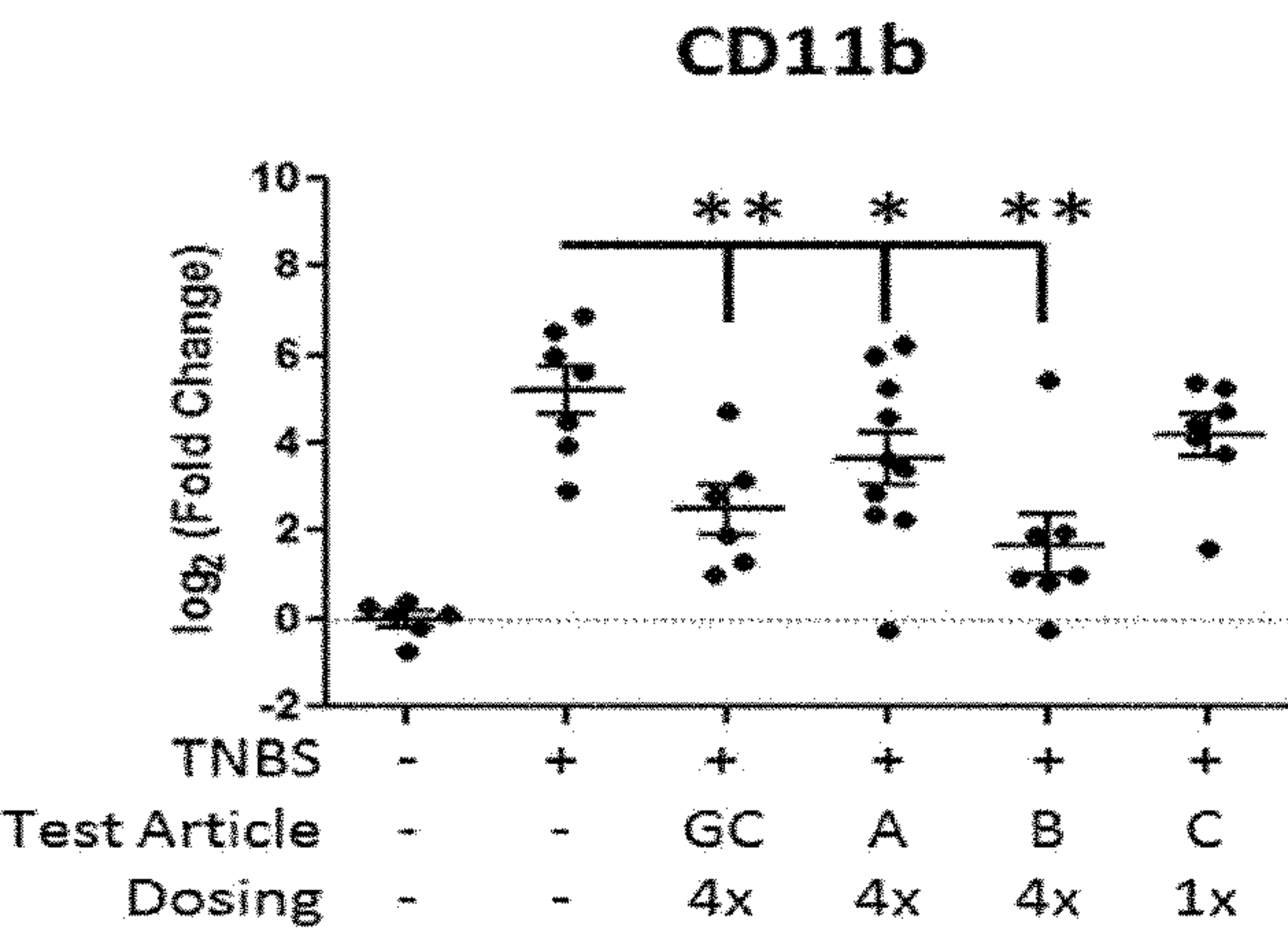


Fig. 26



H

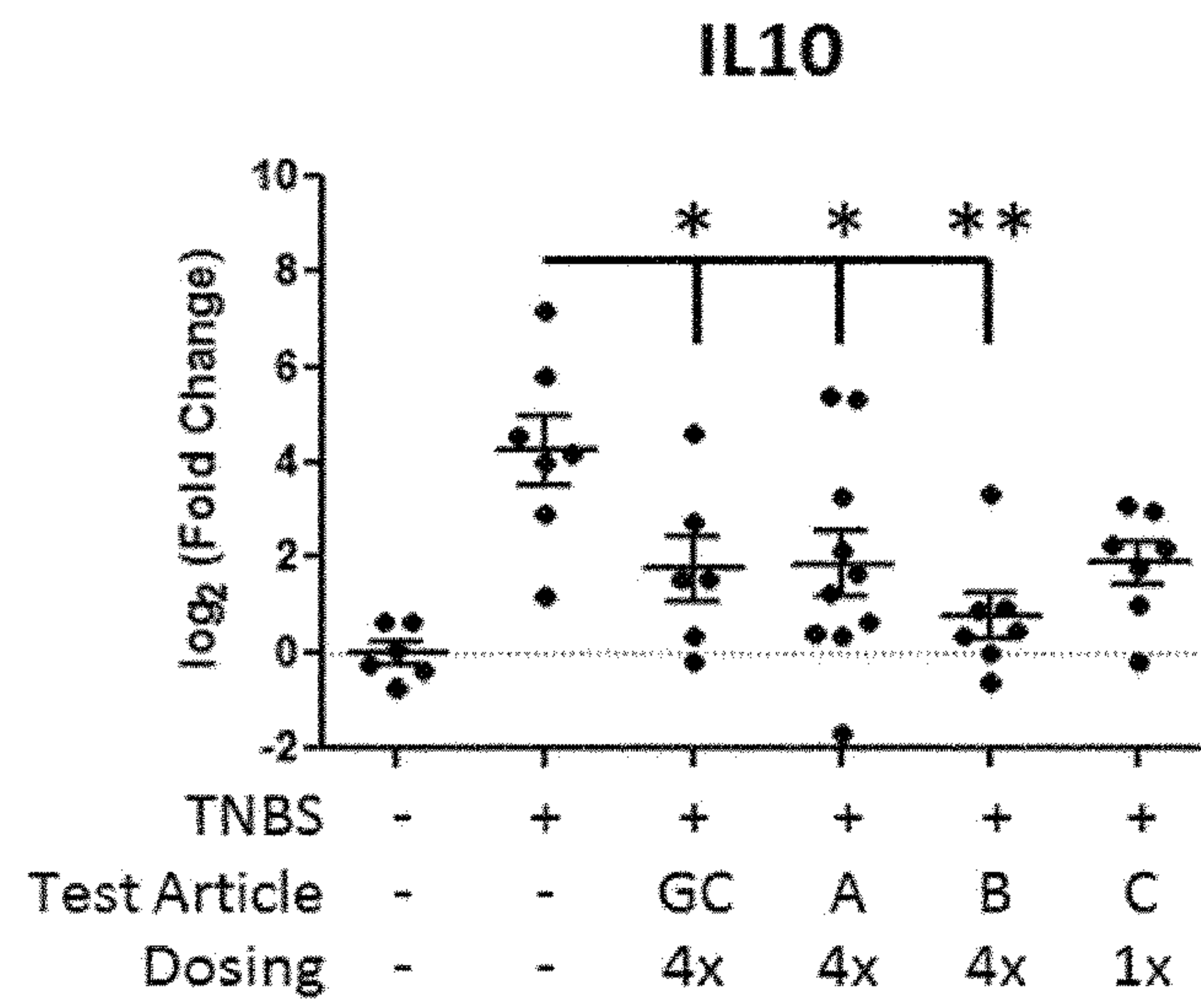


Fig. 26



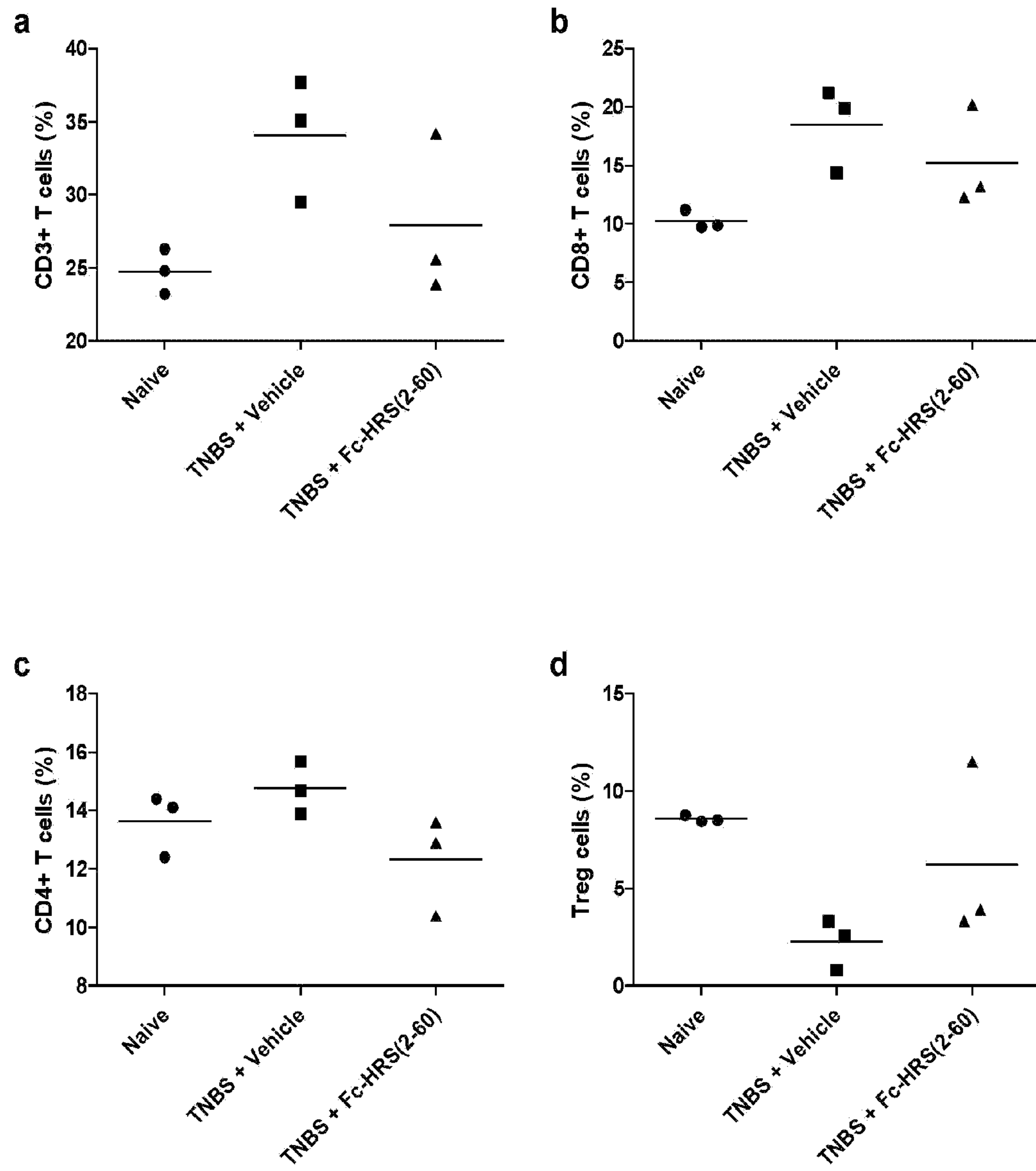


Fig. 27