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(54) Title: GROUP A STREP IMMUNOGENIC COMPOSITIONS WITH POLYSACCHARIDE-PROTEIN CONJUGATES

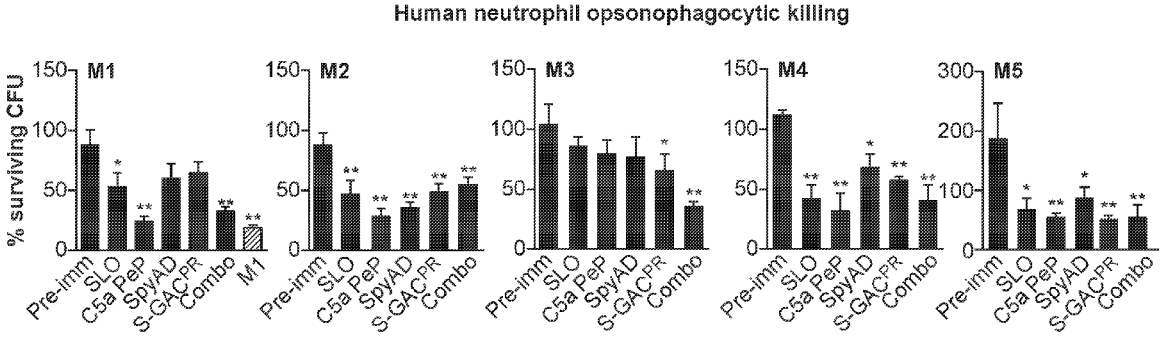


FIG. 24A

(57) Abstract: The present disclosure provides immunogenic compositions comprising Group A Streptococcus (GAS) polypeptide antigens and at least one polypeptide-polysaccharide conjugate. The present disclosure further provides methods of using such compositions to induce immune responses against GAS infections in subjects.

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GROUP A STREP IMMUNOGENIC COMPOSITIONS WITH POLYSACCHARIDE-PROTEIN CONJUGATES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/977,886, filed February 18, 2020; U.S. Provisional Application No. 63/013,924, filed April, 22, 2020; U.S. Provisional Application No. 63/090,069, filed October 9, 2020; and U.S. Provisional Application No. 63/123,293, filed December 9, 2020, the disclosures of which are hereby incorporated by reference in their entireties.

STATEMENT OF GOVERNMENT INTEREST

[0002] This invention was made with the support of the United States government under grant number 93.360, subaward 4500003905, awarded by the Health and Human Services Office of the Assistant Secretary for Preparedness and Response (HHS/ASPR) under the CARB-X Pass Through Entity. The government has certain rights in the invention.

DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

[0003] The sequence listing associated with this application is provided in text format in lieu of a paper copy, and is hereby incorporated by reference into the specification. The name of the text file containing the sequence listing is STRO_011_04WO_ST25.txt. The text file is 321 kb, was created on February 12, 2020, and is being submitted electronically via EFS-Web.

FIELD

[0004] The present application relates to the fields of microbiology and vaccine development.

BACKGROUND

[0005] Group A Streptococcus (GAS) is a preeminent human pathogen causing 700 million cases of pharyngitis ('strep throat') annually worldwide and increasing cases of severe invasive infections, sepsis, necrotizing fasciitis, otitis media, and toxic shock syndrome. Pharyngitis is highly prevalent in school-age children and a major source of antibiotic prescriptions worldwide; driving selective pressure for resistance throughout the human microflora. GAS is also responsible for post-infectious immune-mediated rheumatic heart disease (RHD), a leading cause of mortality in the developing world. Some 30 million people are currently

affected by RHD, with over 300,000 deaths annually (60% < age 70) and 11.5 million disability-adjusted life years lost.

BRIEF SUMMARY

[0006] In some embodiments, the present disclosure provides an immunogenic composition comprising a first Group A Streptococcus (GAS) polypeptide antigen; a second GAS polypeptide antigen; and at least one polypeptide-polysaccharide conjugate, wherein the conjugate polypeptide is a third GAS polypeptide antigen or a non-GAS carrier polypeptide and comprises at least one non-natural amino acid (nnAA), and wherein the conjugate polysaccharide is a GAS polysaccharide or a variant thereof that lacks an immunodominant N-acetyl Glucosamine (GlcNAc) side chain.

[0007] In some embodiments, at least one of the GAS polypeptide antigens is a full length GAS antigen. In some embodiments, at least one of the GAS polypeptide antigens is a peptide fragment of a full length GAS antigen. In some embodiments, the first and second GAS polypeptide antigens are independently selected from C5a peptidase, streptolysin O (SLO), Sib35, and Sfb1. In some embodiments, the first and second GAS polypeptide antigens are C5a peptidase and SLO.

[0008] In some embodiments, the C5a peptidase polypeptide antigen comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 1 or SEQ ID NO: 3. In some embodiments, the C5a peptidase polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 3. In some embodiments, the C5a peptidase polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 29 or SEQ ID NO: 30.

[0009] In some embodiments, the SLO polypeptide antigen comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 5 or SEQ ID NO: 7. In some embodiments, the SLO polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 7. In some embodiments, the SLO polypeptide antigen comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53. In some embodiments, the SLO polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 31, SEQ ID NO:

32, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53. In some embodiments, the SLO polypeptide antigen comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, or SEQ ID NO: 64. In some embodiments, the SLO polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, or SEQ ID NO: 64.

[0010] In some embodiments, the non-GAS carrier polypeptide is ferritin. In some embodiments, the third GAS polypeptide antigen or non-GAS carrier polypeptide is selected from Streptococcus pyogenes Adhesion and Division (SpyAD) polypeptide, arginine deiminase (ADI), SEQ ID NO: 25, and Protein D. In some embodiments, the third GAS polypeptide antigen is SLO.

[0011] In some embodiments, the at least one nnAA is substituted for a lysine, a leucine, or an isoleucine in the conjugate polypeptide. In some embodiments, the at least one nnAA is substituted for a lysine, a leucine, an arginine, or an isoleucine in the conjugate polypeptide. In some embodiments, the nnAA comprises a click chemistry reactive group. In some embodiments, the nnAA is selected from 2-amino-3-(4-azidophenyl)propanoic acid (pAF), 2-amino-4-azidobutanoic acid, 2-azido-3-phenylpropionic acid, 2-amino-3-azidopropanoic acid, 2-amino-3-(4-(azidomethyl)phenyl)propanoic acid (pAMF), 2-amino-3-(5-(azidomethyl)pyridin-2-yl)propanoic acid, 2-amino-3-(4-(azidomethyl)pyridin-2-yl)propanoic acid, 2-amino-3-(6-(azidomethyl)pyridin-3-yl)propanoic acid, and 2-amino-5-azidopentanoic acid. In some embodiments, the nnAA is pAMF.

[0012] In some embodiments, the conjugate polypeptide is a third GAS protein, which is a SpyAD polypeptide and comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 9. In some embodiments, the SpyAD polypeptide comprises a pAMF substitution at positions K64, K287, K386, and K657 of SEQ ID NO: 9. In some embodiments, the SpyAD polypeptide comprises the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 11. In some embodiments, the conjugate polypeptide is a third GAS protein, which is a SpyAD polypeptide and comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 33. In some

embodiments, the SpyAD polypeptide comprises a pAMF substitution at positions K64, K287, K386, and K657 of SEQ ID NO: 33. In some embodiments, the SpyAD polypeptide comprises the amino acid sequence of SEQ ID NO: 33 or SEQ ID NO: 34.

[0013] In some embodiments, the conjugate polypeptide is a third GAS polypeptide, which is an ADI polypeptide and comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 13 or SEQ ID NO: 15. In some embodiments, the ADI polypeptide comprises a pAMF substitution at positions K15, K193, and K316 of SEQ ID NO: 13 or SEQ ID NO: 15. In some embodiments, the ADI polypeptide comprises the amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 19. In some embodiments, the conjugate polypeptide is a third GAS polypeptide, which is an ADI polypeptide and comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 35 or SEQ ID NO: 36. In some embodiments, the ADI polypeptide comprises a pAMF substitution at positions K15, K193, and K316 of SEQ ID NO: 35 or SEQ ID NO: 36. In some embodiments, the ADI polypeptide comprises the amino acid sequence of SEQ ID NO: 35 or SEQ ID NO: 36.

[0014] In some embodiments, the conjugate polypeptide is a ferritin polypeptide and comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 21. In some embodiments, the ferritin polypeptide comprises a pAMF substitution at position I5 of SEQ ID NO: 21. In some embodiments, the ferritin polypeptide comprises the amino acid sequence of SEQ ID NO: 23.

[0015] In some embodiments, the conjugate polysaccharide comprises a polyrhamnose core. In some embodiments, the conjugate polysaccharide has an average molecular weight of about 5 kDa to about 7 kDa. In some embodiments, the conjugate polysaccharide is a tetramer, a hexamer, an octamer, or a decamer polysaccharide. In some embodiments, the conjugate polysaccharide has an average molecular weight of about 10 kDa to 40 kDa.

[0016] In some embodiments, the present disclosure provides an immunogenic composition comprising a Group A Streptococcus (GAS) C5a peptidase polypeptide antigen; a GAS streptolysin O (SLO) polypeptide antigen; and a polypeptide-polysaccharide conjugate comprising a Streptococcus pyogenes Adhesion and Division (SpyAD) conjugate polypeptide and a GAS conjugate polysaccharide or a variant thereof that lacks an immunodominant N-

acetyl Glucosamine (GlcNAc) side chain. In some embodiments, the SpyAD protein comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 9 and comprises a pAMF substitution at positions K64, K287, K386, and K657. In some embodiments, the SpyAD protein comprises the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 11. In some embodiments, the SpyAD protein comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 33 and comprises a pAMF substitution at positions K64, K287, K386, and K657. In some embodiments, the SpyAD protein comprises the amino acid sequence of SEQ ID NO: 33 or SEQ ID NO: 34. In some embodiments, the SpyAD protein comprises the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 34

[0017] In some embodiments, the present disclosure provides an immunogenic composition comprising a Group A Streptococcus (GAS) C5a peptidase polypeptide antigen; a GAS streptolysin O (SLO) polypeptide antigen; and a polypeptide-polysaccharide conjugate comprising an arginine deiminase (ADI) conjugate polypeptide and a GAS conjugate polysaccharide or a variant thereof that lacks an immunodominant N-acetyl Glucosamine (GlcNAc) side chain. In some embodiments, the ADI polypeptide comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 13 or SEQ ID NO: 15 and comprises a pAMF substitution at positions K15, K193, and K316. In some embodiments, the ADI polypeptide comprises the amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 19. In some embodiments, the ADI polypeptide comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 35 or SEQ ID NO: 36 and comprises a pAMF substitution at positions K15, K193, and K316. In some embodiments, the ADI polypeptide comprises the amino acid sequence of SEQ ID NO: 37 or SEQ ID NO: 38

[0018] In some embodiments, the present disclosure provides an immunogenic composition comprising a Group A Streptococcus (GAS) C5a peptidase polypeptide antigen; a GAS streptolysin O (SLO) polypeptide antigen; and a polypeptide-polysaccharide conjugate comprising a ferritin polypeptide and a GAS conjugate polysaccharide or a variant thereof that lacks an immunodominant N-acetyl Glucosamine (GlcNAc) side chain. In some embodiments, the ferritin protein comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 21 and comprises a pAMF substitution at position I5. In some embodiments, the ferritin protein comprises the amino acid sequence of SEQ ID NO: 23.

[0019] In some embodiments, the composition comprises about 10% to about 50% of the first GAS antigen, about 10% to about 50% of the second GAS antigen, and about 10% to about 50% of the polypeptide-polysaccharide conjugate. In some embodiments, the composition further comprises one or more adjuvants selected from alum, saponin, monophosphoryl lipid A (MPL), or combinations thereof. In some embodiments, the composition comprises Alhydogel.

[0020] In some embodiments, the present disclosure provides a method of inducing a protective immune response against a Group A Streptococcus (GAS) bacterium in a subject comprising administering an immunogenic composition described herein to the subject.

[0021] In some embodiments, the subject is 18 years or older. In some embodiments, the subject is less than 18 years old. In some embodiments, the subject is between 5 years and 17 years old, between 6 months and 9 years old, or between 5 years and 9 years old.

[0022] In some embodiments, the GAS bacterium is of a serotype selected from M1, M2, M3, M4, M6, M11, M12, M22, M28, M75, and M89. In some embodiments, the GAS bacterium is a serotype selected from M1, M3, M5, M9, M12, M18, M22, M25, M28, M71, M72, and M74. In some embodiments, the GAS bacterium is a serotype selected from M1, M4, M6, M11, M12, M22, M44, M75, M77, M77, and M81. In some embodiments, the GAS bacterium is a serotype selected from M1, M2, M3, M4, M6, M9, M12, M18, M22, M75, M77, M89, and M92. In some embodiments, the GAS bacterium is a serotype selected from M1, M2, M3, M4, M5, M6, M9, M11, M12, M13, M28, M62, and M89. In some embodiments, the GAS bacterium is a serotype selected from M1, M2, M3, M4, M6, M12, M22, M28, M49, M53, M68, M77, M80, M83, M87, M89, and M92.

[0023] In some embodiments, the immunogenic composition induces an antibody response in the subject against the Group A Streptococcus (GAS) bacterium and does not induce an antibody response in the subject against human tissue. In some embodiments, the immunogenic composition induces a protective immune response against a *Shigella* bacterium in the subject, wherein the Shigella bacterium comprises a polysaccharide with a polyrhmanose backbone.

[0024] In some embodiments, the present disclosure provides a method of inducing a protective immune response against a *Shigella* bacterium in a subject comprising administering

an immunogenic composition described herein to the subject. In some embodiments, the present disclosure provides a use of an immunogenic composition described herein for inducing a protective immune response against a *Shigella* bacterium in a subject. In some embodiments, the present disclosure provides a use of an immunogenic composition described herein in the manufacture of a medicament for inducing a protective immune response against a *Shigella* bacterium in a subject. In some embodiments, the subject is 18 years or older. In some embodiments, the subject is less than 18 years old. In some embodiments, the subject is between 5 years and 17 years old, between 6 months and 9 years old, or between 5 years and 9 years old.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] FIG. 1A and FIG. 1B show the expression levels of native polypeptide antigens and non-natural amino acids containing polypeptide antigens used for making conjugates with GAS polysaccharide .

[0026] FIG. 2 shows polypeptide antigen- and polypeptide-polysaccharide conjugate-immunized sera titers compared to a pre-bleed control.

[0027] FIG. 3A, FIG. 3B, FIG. 3C, FIG. 3D, and FIG. 3E show antibody titers and the IgG binding change of immune serum compared to a pre-immune serum control.

[0028] FIG. 3F shows rabbit IgG binding to SpyAD knockout GAS, confirming binding to native GAC.

[0029] FIG. 4 shows the increased killing capacity of immune serum vs. a pre-immune serum control.

[0030] FIG. 5A, FIG. 5B, FIG. 5C, FIG. 5D, FIG. 5E, and FIG. 5F show the results of active immunization experiments, including antibody titers, lesion size, and bacterial burden, and percent survival.

[0031] FIG. 6A and FIG. 6B show percent survival data following passive immunization of mice subjected to an IP challenge.

[0032] FIG. 7 shows lesion size and bacterial burden data following passive immunization of mice subjected to a subdermal challenge.

[0033] FIG. 8 shows an ELISA titer analysis of immune serum against *S. flexneri 2a* OPS.

[0034] FIG. 9 shows the general reaction scheme for derivatization of GAC with DBCO-PEG_4-NH_2 and subsequent conjugation to pAMF-modified SpyAD.

[0035] FIG. 10 shows SEC MALS analysis of purified native GAC, purified native SpyAD[4pAMF], and purified polypeptide-polysaccharide conjugates thereof.

[0036] FIG. 11 shows a Western blot analysis assessing antigen-specific antisera for cross-reactivity to human heart lysate.

[0037] FIG. 12 is a graphical representation of fragment sequences for select antigens.

[0038] FIG. 13 shows comparative expression data of select antigens.

[0039] FIG. 14 shows expression data of select single and double SLO mutants compared to GFP.

[0040] FIG. 15 is a graphical representation of truncated SLO sequences compared to the non-truncated SLO sequence.

[0041] FIG. 16 shows expression levels (total and soluble) for truncated SLO variants.

[0042] FIG. 17 shows purity of captured peptide fragments by HisTrap column chromatography.

[0043] FIG. 18 shows purity of select expressed peptides after TEV cleavage and HisTrap purification.

[0044] FIG. 19 shows the results of purification of expressed peptides by size exclusion chromatography.

[0045] FIG. 20 shows the *in vivo* assessment of immunogenicity of select SLO fragments in rabbits.

[0046] FIG. 21 shows the use of serum from mock and peptide combination-immunized mice to opsonize GAS in an OPK assay.

[0047] FIG. 22A, FIG. 22B, and FIG. 22C show the results of an intradermal challenge (with M1 89155 GAS) of wild-type CD-1 mice immunized with mock or a peptide combination, including lesion size and GAS burden data.

[0048] FIG. 23 shows the results of a red blood cell hemolysis assay to assess the presence of functional antibody against SLO variants.

[0049] FIG. 24A, FIG. 24B, and FIG. 24C show the opsonophagocytic killing of GAS of different human neutrophil serotypes in the presence of immune serum from immunized rabbits, the blocking of lysis by antisera, and the oxidative burst capacity of human neutrophils exposed to GAS supernatants containing SLO.

[0050] FIG. 25A and FIG25B shows differential IgG binding of immune sera to additional GAS serotype surface antigens.

[0051] FIG. 26 shows expression of 10 pAMF-containing SLO polypeptide variants.

[0052] FIGS. 27A and 27B shows SDS-PAGE analysis for SLO variants during two steps of purification.

[0053] FIG. 28 shows conditions for the conjugation of SLO variants to DBCO-GAC.

[0054] FIG. 29 shows SDS-PAGE analysis of 3- and 4-pAMF variant SLO variant conjugates.

[0055] FIG. 30 shows SEC-MALS data for 3- and 4-pAMF variant SLO variant conjugates.

[0056] FIG. 31A and FIG. 31B show the antibody titers of mice post-immunization using conjugates made with select SLO(ΔC101) variants as carrier proteins, and the results of a hemolysis assay using anti-sera generated from mice vaccinated with conjugates made with those same variants. FIG. 31C shows the antibody titers of mice post-immunization using conjugates made with select SLO(ΔC101) variants, and using long polysaccharide as the coating antigen in the ELISA.

[0057] FIG. 32 shows SEC-MALS data for a conjugate of SpyAD[4pAMF] and long polysaccharide.

[0058] FIG. 33A and FIG. 33B show the expression levels of 22 SLO variants with 3-8 pAMF, and the corresponding gels for purified 5-8 pAMF containing variants.

[0059] FIG. 34 shows SDS-PAGE analysis of 5- and 6-pAMF variant SLO conjugates with long GAC.

[0060] FIG. 35 shows SEC-MALS data for 5- and 6-pAMF variant SLO conjugates with long GAC.

[0061] FIG. 36A, FIG. 36B, and FIG. 36C show NMR analysis of purified polysaccharide originating from a GAS bacterial strain expressing PS lacking GlcNAc.

[0062] FIG. 37 shows a western blot demonstrating M-protein is removed from polysaccharide when purified as described in Example 19.

DETAILED DESCRIPTION

Overview

[0063] Despite high global demand, there is no safe and efficacious commercial vaccine against GAS. Features of the pathogen pose particular challenges to vaccination, including its invariant capsule of hyaluronic acid, an immunologically inert carbohydrate ubiquitous in connective tissues. Furthermore, the immunodominant surface-anchored GAS M proteins are highly polymorphic (>200 *emm* types), and regions of their dimeric coiled-coil structure may provoke an autoimmune response against cardiac tissue in rheumatic heart disease (RHD). The World Health Organization, International Vaccine Institute, Wellcome Trust, Gates Foundation and others have recently convened in advisories on GAS vaccine development to address these significant scientific challenges and meet this paramount global health need.

Group A Streptococcus

[0064] Group A *streptococcus* (GAS) bacteria is a Gram positive, beta-hemolytic coccus in chains. It is responsible for a range of diseases in humans. These diseases include strep throat (acute pharyngitis), otitis media, and skin and soft tissue infections such impetigo and cellulitis.

These can also include rare cases of invasive (serious) illnesses such as necrotizing fasciitis (flesh eating disease) and toxic shock syndrome (TSS). Several virulence factors contribute to the pathogenesis of GAS, such as M protein, hemolysins, and extracellular enzymes.

[0065] GAS vaccinology has been focused largely on the cell wall-anchored M protein. Historical studies administered crude whole M protein preparations to human volunteers, which was followed by challenge with live GAS bacteria in the pharynx. While these preparations decreased GAS colonization, an increased incidence of acute rheumatic fever (ARF) was reported for some of the immunized subjects. Two N-terminal M protein vaccine preparations have reached human clinical trials to date: a hexavalent preparation (Dale JB. 1999. Vaccine, 17:193–200; Kotloff et al, 2004. JAMA 292:709–715; Hall et al., 2004. Infect. Immun. 72:2507–2512) and a 26-valent preparation (Hu et al., 2002. Infect. Immun. 70:2171–2177; McNeil et al., 2005. Clin. Infect. Dis. 41:1114–1122). These preparations were well tolerated in adult volunteers. More recently, a similar multivalent vaccine was developed, which includes N-terminal peptides representing 30 GAS serotypes (Dale et al., 2011. Vaccine 29:8175–8178.). Sera raised against this experimental vaccine exhibited bactericidal activity against all 30 vaccine serotypes as well as 24 (of 40 tested) nonvaccine serotypes. While such preparations have been shown to have the capacity to protect against select nonvaccine serotypes, they do not protect against all globally circulating serotypes (Abdissa et al., 2006. Clin. Infect. Dis. 42:1362–1367; Ikebe et al., Working Group for Beta-Haemolytic Streptococci in Japan. 2007. 2001-2005. Epidemiol. Infect. 135:1227–1229; Nir-Paz et al., 2010. Epidemiol. Infect. 138:53–60; Steer et al., 2009. Lancet Infect. Dis. 9:611–616; O'Loughlin et al., 2007. Clin. Infect. Dis. 45:853–862). The 30 serotypes included are based on those prevalent in North America and Europe (Dale JB et al., 2011. Vaccine **29:**8175–8178.), which often differ from the diverse serotypes isolated in regions of endemicity. The use of GAS proteins and Protein-GAC conjugates as vaccines has been recently reported (Di Benedetto, R. et al., 2020. J. Mol. Sci. no 22: 8558), although these use non-specific conjugation methods that can disrupt both the B/T-cell epitopes on the carrier protein and the PS backbone. Furthermore, these conjugates were also made with thenative GAC , which harbors potentially cross-reactive GlcNAc immunodominant epitope.

[0066] Thus, there is a need in the art for improved vaccines against GAS.

Combination Vaccines

[0067] In some embodiments, the present disclosure provides an immunogenic composition comprising two or more GAS polypeptide antigens and a polypeptide-polysaccharide conjugate. For clarity, in compositions described herein, each polypeptide is different. For example, in compositions comprising a first, a second, and a third polypeptide antigen or polypeptide-polysaccharide conjugate, the first, second, and third polypeptides have different sequences.

GAS Antigens

[0068] In some embodiments, the immunogenic compositions of the present disclosure comprise two or more GAS polypeptide antigens. In some embodiments, the GAS polypeptide antigens are selected from C5a peptidase, streptolysin O (SLO), streptococcal immunoglobulin-binding protein 35 (Sib35), and Fibronectin binding protein F1 (Sfb1). In some embodiments, the GAS polypeptide antigens are selected from C5a peptidase, streptolysin O (SLO), streptococcal immunoglobulin-binding protein 35 (Sib35), Fibronectin binding protein F1 (Sfb1), and Adhesion and Division polypeptide (SpyAD). UniProt Reference Identifiers for each of these GAS antigens are provided in Table 1 below.

Table 1: UniProt References for exemplary GAS antigens

Antigen	UniProt Ref
C5a	P15926
SLO	P0C0I3
Sib35	Q1XG74
Sfb1	Q48VN7

[0069] In some embodiments, the GAS polypeptide antigens comprise the full length polypeptide antigen sequence. In some embodiments, the GAS polypeptide antigens comprise a fragment of the full length polypeptide antigen sequence. For example, in some embodiments, the immunogenic composition comprises a C5a GAS polypeptide antigen, wherein the C5a polypeptide is a fragment of the full length C5a polypeptide. In some embodiments, the C5a fragment comprises amino acids 90-1035 of the full length protein (*e.g.*, comprises SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 29, or SEQ ID NO: 30). In some embodiments, the immunogenic composition comprises an SLO GAS polypeptide antigen, wherein the SLO polypeptide is a fragment of the full length SLO polypeptide. In some embodiments, the SLO

fragment comprises amino acids 79-571 of the full length protein (*e.g.*, comprises SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 31, or SEQ ID NO: 32).

[0070] In some embodiments, the GAS polypeptide antigens comprise one or more amino acid mutations in the wild-type amino acid sequence. For example, in some embodiments, the immunogenic composition comprises a C5a GAS polypeptide antigen, wherein the C5a polypeptide comprises one or more amino acid mutations. In some embodiments, the amino acid mutations are D131A and S513A (*e.g.*, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 30). In some embodiments, the immunogenic composition comprises an SLO GAS polypeptide antigen, wherein the SLO polypeptide comprises one or more amino acid mutations. In some embodiments, the amino acid mutation is W535A (*e.g.*, SEQ ID NO: 7, SEQ ID NO: 8, or SEQ ID NO: 32).

[0071] Amino acid sequences of exemplary GAS antigen are provided below in Table 2. Mutated amino acids are indicated in bolded text. In some embodiments, the underlined and italicized amino acids are cleaved from the mature GAS antigens.

Table 2: Exemplary GAS Antigens

Antigen	Amino Acid Sequence	SEQ ID:
SLO [79-571 fragment] WT w/ His tag and TEV sequence	*MHHHHHHGSGENLYFQG*APKEMPLESAEKEEKKSEDKKKSEEDHTE EINDKIYSLNYNELEVLAKNGETIENFVPKEGVKKADKFIVIERKK KNINTTPVDISIIDSVTDRTYPAALQLANKGFTENKPDAVVTKRNP QKIHIDLPGMGDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNT LPARTQYTESMVYSKSQIEAALNVNSKILDGTLGIDFKSISKGEKK VMIAAYKQIFYTVSANLPNNPADVFDKSVTFKELQRKGVSNEAPPL FVSNVAYGRTVFVKLETSSKSNDVEAAFSAALKGTDVKTNGKYSDI LENSSFTAVVLGGDAAEHNKVVTKDFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSGKINLSHQGAYVA QYEILWDEINYDDKGKEVITKRRWDNNWYSKTSPFSTVIPLGANSR NIRIMARECTGLAWEWWRKVIDERDVKLSKEINVNISGSTLSPYGS ITYK	5
SLO [79-571 fragment] W535A w/ leader	*MHHHHHHGSGENLYFQG*APKEMPLESAEKEEKKSEDKKKSEEDHTE EINDKIYSLNYNELEVLAKNGETIENFVPKEGVKKADKFIVIERKK KNINTTPVDISIIDSVTDRTYPAALQLANKGFTENKPDAVVTKRNP QKIHIDLPGMGDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNT LPARTQYTESMVYSKSQIEAALNVNSKILDGTLGIDFKSISKGEKK VMIAAYKQIFYTVSANLPNNPADVFDKSVTFKELQRKGVSNEAPPL FVSNVAYGRTVFVKLETSSKSNDVEAAFSAALKGTDVKTNGKYSDI LENSSFTAVVLGGDAAEHNKVVTKDFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSGKINLSHQGAYVA QYEILWDEINYDDKGKEVITKRRWDNNWYSKTSPFSTVIPLGANSR	7

Antigen	Amino Acid Sequence	SEQ ID:
	NIRIMARECTGLA**A**EWWRKVIDERDVKLSKEINVNISGSTLSPYGS ITYK	
SLO(ΔC101) fragment W535A w/o leader, with G overhang from cleavage	GAPKEMPLESAEKEEKKSEDKKKSEEDHTEEINDKIYSLNYN ELEVLAKNGETIENFVPKEGVKKADKFIVIERKKKNINTTPV DISIIDSVTDRTYPAALQLANKGFTENKPDAVVTKRNPQKIH IDLPGMGDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNT LPARTQYTESMVYSKSQIEAALNVNSKILDGTLGIDFKSISK GEKKVMIAAYKQIFYTVSANLPNNPADVFDKSVTFKELQRKG VSNEAPPLFVSNVAYGRTVFVKLETSSKSNDVEAAFSAALKG TDVKTNGKYSDILENSSFTAVVLGGDAAEHNKVVTKDFDVIR NVIKDNATFSRKNPAYPISYTSVFLKNNKIAGVNNRTEYVET TSTEYTSGKINLSHQ	53
C5a [90-1035 fragment] WT w/ leader	*MHHHHHHGSGENLYFQG*KTADTPVTSKATIRDLNDPSQVKTLQEKA GKGAGTVVAVIDAGFDKNHEAWRLTDKTKARYQSKEDLEKAKKEHG ITYGEWVNDKVAYYHDYSKDGKTAVDQEHGTHVSGILSGNAPSETK EPYRLEGAMPEAQLLLMRVEIVNGLADYARNYAQAIRDAVNLGAKV INMSFGNAALAYANLPDETKKAFDYAKSKGVSIVTSAGNDSSFGGK TRLPLADHPDYGVVGTPAAADSTLTVASYSPDKQLTETATVKTADK QDKEMPVLSTNRFEPNKAYDYAYANRGMKEDDFKDVKGKIALIERG DIDFKDKIANAKKAGAVGVLIYDNQDKGFPIELPNVDQMPAAFISR KDGLLLKDNPKKTITFNATPKVLPTASGTKLSRFSSWGLTADGNIK PDIAAPGQDILSSVANNKYAKLSGTSMSAPLVAGIMGLLQKQYETQ YPDMTPSERLDLAKKVLMSSATALYDEDEKAYFSPRQQGAGAVDAK KASAATMYVTDKDNTSSKVHLNNVSDTFEVTVTVHNKSDKPQELYY QATVQTDKVDGKHFALAPKALYETSWQKITIPANSSKQVTVPIDAS RFSKDLLAQMKNGYFLEGFVRFKQDPTKEELMSIPYIGFRGDFGNL SALEKPIYDSKDGSSYYHEANSDAKDQLDGDGLQFYALKNNFTALT TESNPWTIIKAVKEGVENIEDIESSEITETIFAGTFAKQDDDSHYY IHRHANGKPYAAISPNGDGNRDYVQFQGTFLRNAKNLVAEVLDKEG NVVWTSEVTEQVVKNYNNDLASTLGSTRFEKTRWDGKDKDGKVVAN GTYTYRVRYTPISSGAKEQHTDFDVIVDNTTPEVATSATFSTEDRR LTLASKPKTSQPVYRERIAYTYMDEDLPTTEYISPNEDGTFTLPEE AETMEGATVPLKMSDFTYVVEDMAGNITYTPVTKLLEGHSNK	1
C5a [90-1035 fragment] D131A/S513A w/ leader	*MHHHHHHGSGENLYFQ*GKTADTPVTSKATIRDLNDPSQVKTLQEKA GKGAGTVVAVI**A**AGFDKNHEAWRLTDKTKARYQSKEDLEKAKKEHG ITYGEWVNDKVAYYHDYSKDGKTAVDQEHGTHVSGILSGNAPSETK EPYRLEGAMPEAQLLLMRVEIVNGLADYARNYAQAIRDAVNLGAKV INMSFGNAALAYANLPDETKKAFDYAKSKGVSIVTSAGNDSSFGGK TRLPLADHPDYGVVGTPAAADSTLTVASYSPDKQLTETATVKTADK QDKEMPVLSTNRFEPNKAYDYAYANRGMKEDDFKDVKGKIALIERG DIDFKDKIANAKKAGAVGVLIYDNQDKGFPIELPNVDQMPAAFISR KDGLLLKDNPKKTITFNATPKVLPTASGTKLSRFSSWGLTADGNIK PDIAAPGQDILSSVANNKYAKLSGT**A**MSAPLVAGIMGLLQKQYETQ YPDMTPSERLDLAKKVLMSSATALYDEDEKAYFSPRQQGAGAVDAK KASAATMYVTDKDNTSSKVHLNNVSDTFEVTVTVHNKSDKPQELYY QATVQTDKVDGKHFALAPKALYETSWQKITIPANSSKQVTVPIDAS RFSKDLLAQMKNGYFLEGFVRFKQDPTKEELMSIPYIGFRGDFGNL SALEKPIYDSKDGSSYYHEANSDAKDQLDGDGLQFYALKNNFTALT TESNPWTIIKAVKEGVENIEDIESSEITETIFAGTFAKQDDDSHYY IHRHANGKPYAAISPNGDGNRDYVQFQGTFLRNAKNLVAEVLDKEG	3

Antigen	Amino Acid Sequence	SEQ ID:
	NVVWTSEVTEQVVKNYNNDLASTLGSTRFEKTRWDGKDKDGKVVAN GTYTYRVRYTPISSGAKEQHTDFDVIVDNTTPEVATSATFSTEDRR LTLASKPKTSQPVYRERIAYTYMDEDLPTTEYISPNEDGTFTLPEE AETMEGATVPLKMSDFTYVVEDMAGNITYTPVTKLLEGHSNK	

[0072] Residue positions in Table 2, above, and elsewhere in this disclosure, refer to the residue numbering according to the residue position in the full length sequences (e.g., as identified in Tables 1 and 3 by reference to the UniProt sequence database) for identifying the fragments and/or lysine residues modified by nnAA (e.g., pAMF replacements of certain lysine residues).

[0073] In some embodiments, the immunogenic composition comprises a C5a GAS polypeptide antigen comprising or consisting of an amino acid sequence that is at least 95%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NOs: 1-4. In some embodiments, the immunogenic composition comprises a C5a GAS polypeptide antigen comprising or consisting of an amino acid sequence that is at least 95%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NOs: 29-30. In some embodiments, the immunogenic composition comprises a C5a GAS polypeptide antigen comprising or consisting of the amino acid sequence of any one of SEQ ID NOs: 1-4. In some embodiments, the immunogenic composition comprises a C5a GAS polypeptide antigen comprising or consisting of the amino acid sequence of any one of SEQ ID NOs: 29-30. In some embodiments, the immunogenic composition comprises an SLO GAS polypeptide antigen comprising or consisting of an amino acid sequence that is at least 95%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NOs: 5-8. In some embodiments, the immunogenic composition comprises an SLO GAS polypeptide antigen comprising or consisting of an amino acid sequence that is at least 95%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53. In some embodiments, the immunogenic composition comprises an SLO GAS polypeptide antigen comprising or consisting of the amino acid sequence of any one of SEQ ID NOs: 5-8. In some embodiments, the immunogenic composition comprises an SLO GAS polypeptide antigen comprising or consisting of the amino acid sequence of SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID

NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53. In some embodiments, the immunogenic composition comprises a SpyAD GAS polypeptide antigen comprising or consisting of an amino acid sequence that is at least 95%, 95%, 97%, 98%, or 99% identical to SEQ ID NO: 10 or 33. In some embodiments, the immunogenic composition comprises a SpyAD GAS polypeptide antigen comprising or consisting of the amino acid sequence of SEQ ID NO: 10 or 33.

Polypeptide-Polysaccharide Conjugates

[0074] In some embodiments, immunogenic compositions described herein comprise at least one polypeptide-polysaccharide conjugate. The polypeptide-polysaccharide conjugates comprise a conjugate polypeptide (also referred to as a carrier protein) and a polysaccharide conjugated to the conjugate polypeptide at one or more amino acid positions. In some embodiments, the conjugate polypeptide is selected from SpyAD, ADI, SEQ ID NO: 25, ferritin, and Protein D. UniProt Reference Identifiers for exemplary conjugate polypeptides are provided in Table 3 below.

Table 3: UniProt and CAS References for exemplary conjugate polypeptides

Antigen	UniProt (or CAS) Ref
SpyAD	Q9A1H3
ADI	P0C0B3
Protein D	R4R7Q5
ferritin	P02792
CRM197	(CAS: 1272033-67-6)

[0075] In some embodiments, the conjugate polypeptides comprise the full length polypeptide antigen sequence. In some embodiments, the conjugate polypeptides comprise a fragment of the full length polypeptide sequence. For example, in some embodiments, the immunogenic composition comprises a polypeptide-polysaccharide conjugate wherein the conjugate polypeptide is SpyAD and is a fragment of the full length SpyAD polypeptide (*e.g.*, SEQ ID NO: 9 or SEQ ID NO: 10). In some embodiments, the SpyAD fragment comprises amino acids 33-849 of the full length protein (*e.g.*, comprises SEQ ID NO: 9 or SEQ ID NO: 10). In some embodiments, the immunogenic composition comprises a polypeptide-polysaccharide conjugate wherein the conjugate polypeptide is ADI and is a fragment of the full length ADI polypeptide (*e.g.*, comprises SEQ ID NO: 13 or SEQ ID NO: 14). In some

embodiments, the immunogenic composition comprises a polypeptide-polysaccharide conjugate wherein the conjugate polypeptide is ferritin and is a fragment of the full length ferritin polypeptide (*e.g.*, SEQ ID NO: 21 or SEQ ID NO: 22).

[0076] In some embodiments, the conjugate polypeptide comprises one or more amino acid mutations in the wild-type amino acid sequence. For example, in some embodiments, the conjugate polypeptide is SpyAD, wherein the SpyAD polypeptide comprises one or more amino acid mutations. In some embodiments, the conjugate polypeptide is ADI, wherein the ADI polypeptide comprises one or more amino acid mutations. In some embodiments, the amino acid mutation is D277A (*e.g.*, SEQ ID NO: 15 or SEQ ID NO: 16).

[0077] In some embodiments, the conjugate polypeptide comprises one or more non-natural amino acids (nnAA). In some embodiments, the one or more nnAA comprise a click chemistry reactive group. Herein, a "click chemistry reactive group" refers to a moiety, such as an azide or an alkyne, capable of undergoing a click chemistry reaction with a second click chemistry reactive group. In some embodiments, one click chemistry reactive group reacts with a second click chemistry reactive group to form a substituted triazole. Examples of this type of click reaction can be found, for instance, in International PCT Publication No. WO 2018/126229. General examples of metal-free click reactions used in biomedical applications can be found, for instance, in Kim, *et al., Chemical Science*, 2019, 10, 7835-7851. Examples of nnAAs comprising click chemistry reactive groups include 2-amino-3-(4-azidophenyl)propanoic acid (pAF), 2-amino-4-azidobutanoic acid, 2-azido-3-phenylpropionic acid, 2-amino-3-azidopropanoic acid, 2-amino-3-(4-(azidomethyl)phenyl)propanoic acid (pAMF), 2-amino-3-(5-(azidomethyl)pyridin-2-yl)propanoic acid, 2-amino-3-(4-(azidomethyl)pyridin-2-yl)propanoic acid, 2-amino-3-(6-(azidomethyl)pyridin-3-yl)propanoic acid, and 2-amino-5-azidopentanoic acid. In some embodiments, the conjugate polypeptide comprises one or more nnAAs, wherein each of the nnAAs are pAMF.

[0078] Amino acid sequences of exemplary conjugate polypeptides are provided in Table 4 below. Sites suitable for substitution with an nnAA are indicated in bolded and underlined text. In some embodiments, the underlined and italicized amino acids are cleaved from the mature protein during production.

Table 4: Exemplary Carrier Proteins

Protein	Amino Acid Sequence	SEQ ID:
ADI [full length] WT w/ leader	*MHHHHHHGSGENLYFQG*TAQTPIHVYSEIG**K**LKKVLLHRPG KEIENLMPDYLERLLFDDIPFLEDAQKEHDAFAQALRDEGIEVLYL ETLAAESLVTPEIREAFIDEYLSEANIRGRATKKAIRELLMAIEDN QELIEKTMAGVQKSELPEIPASEKGLTDLVESNYPFAIDPMPNLYF TRDPFATIGTGVSLNHMFSETRNRETLYG**K**YIFTHHPIYGGGKVPM VYDRNETTRIEGGDELVLSKDVLAVGISQRTDAASIEKLLVNIFKQ NLGFKKVLAFEFANNRKFMHLDTVFTMVDYDKFTIHPEIEGDLRVY SVTYDNEELHIVEE**K**GDLAELLAANLGVEKVDLIRCGGDNLVAAGR EQWNDGSNTLTIAPGVVVVYNRNTITNAILESKGLKLIKIHGSELV RGRGGPRCMSMPFEREDI	13
ADI [full length] D277A w/ leader	*MHHHHHHGSGENLYFQG*TAQTPIHVYSEIG**K**LKKVLLHRPG KEIENLMPDYLERLLFDDIPFLEDAQKEHDAFAQALRDEGIEVLYL ETLAAESLVTPEIREAFIDEYLSEANIRGRATKKAIRELLMAIEDN QELIEKTMAGVQKSELPEIPASEKGLTDLVESNYPFAIDPMPNLYF TRDPFATIGTGVSLNHMFSETRNRETLYG**K**YIFTHHPIYGGGKVPM VYDRNETTRIEGGDELVLSKDVLAVGISQRTDAASIEKLLVNIFKQ NLGFKKVLAFEFANNRKFMHL**A**TVFTMVDYDKFTIHPEIEGDLRVY SVTYDNEELHIVEE**K**GDLAELLAANLGVEKVDLIRCGGDNLVAAGR EQWNDGSNTLTIAPGVVVVYNRNTITNAILESKGLKLIKIHGSELV RGRGGPRCMSMPFEREDI	15
SpyAD [33-849 fragment] WT w/ leader	*MHHHHHHGSGENLYFQG*QVKADDRASGETKASNTHDDSLPK PETIQEA**K**ATIDAVEKTLSQQKAELTELATALTKTTAEINHLKEQQ DNEQKALTSAQEIYTNTLASSEETLLAQGAEHQRELTATETELHNA QADQHSKETALSEQKASISAETTRAQDLVEQVKTSEQNIAKLNAMI SNPDAITKAAQTANDNTKALSSELEKAKADLENQKAKVKKQLTEEL AAQKAALAEKEAELSRLKSSAPSTQDSIVGNNTMKAPQGYPLEELK **K**LEASGYIGSASYNNYYKEHADQIIAKASPGNQLNQYQDIPADRNR FVDPDNLTPEVQNELAQFAAHMINSVRRQLGLPPVTVTAGSQEFAR LLSTSYK**K**THGNTRPSFVYGQPGVSGHYGVGPHDKTIIEDSAGASG LIRNDDNMYENIGAFNDVHTVNGIKRGIYDSIKYMLFTDHLHGNTY GHAINFLRVDKHNPNAPVYLGFSTSNVGSLNEHFVMFPESNIANHQ RFNKTPIKAVGSTKDYAQRVGTVSDTIAAIKGKVSSLENRLSAIHQ EADIMAAQAKVSQLQGKLASTLKQSDSLNLQVRQLNDTKGSLRTEL LAAKAKQAQLEATRDQSLAKLASLKAALHQTEALAEQAAARVTALV AK**K**AHLQYLRDFKLNPNRLQVIRERIDNTKQDLAKTTSSLLNAQEA LAALQAKQSSLEATIATTEHQLTLLKTLANEKEYRHLDEDIATVPD LQVAPPLTGVKPLSYSKIDTTPLVQEMVKETKQLLEASARLAAENT SLVAEALVGQTSEMVASNAIVSKITSSITQPSSKTSYGSGSSTTSN LISDVDESTQR	9
hFL [full length] WT w/ leader	*MHHHHHHGSG*SSQ**I**RQNYSTDVEAAVNSLVNLYLQASYTYL SLGFYFDRDDVALEGVSHFFRELAEEKREGYERLLKMQNQRGGRAL FQDIKKPAEDEWGKTPDAMKAAMALEKKLNQALLDLHALGSARTDP HLCDFLETHFLDEEVKLIKKMGDHLTNLHRLGGPEAGLGEYLFERL TLKHD	21
eCRM-pAMF6 mutant	MGADDVVDSSKSFVMENFSSYHGTKPGYVDSIQXGIQKPKS GTQGNYDDDWKEFYSTDNKYDAAGYSVDNENPLSGKAGGVVKVTYP GLTKVLALKVDNAETIKKELGLSLTEPLMEQVGTEEFIKRFGDGAS RVVLSLPFAEGSSSVEYINNWEQAKALSVELEINFETRGKRGQDAM YEYMAQACAGNRVRNSVGSSLSCINLDWDVIRDXTKTKIESLKEHG	25

Protein	Amino Acid Sequence	SEQ ID:
	PIKNKMSESPNKTVSEEKAXQYLEEFHQTALEHPELSELXTVTGTN PVFAGANYAAWAVNVAQVIDSETADNLEKTTAALSILPGIGSVMGI ADGAVHHNTEEIVAQSIALSSLMVAQAIPLVGELVDIGFAAYNFVE SIINLFQVVHNSYNRPAYSPGHXTQPFLHDGYAVSWNTVEDSIIRT GFQGESGHDIKITAENTPLPIAGVLLPTIPGKLDVNKSKTHISVNG RKIRMRCRAIDGDVTFCRPKSPVYVGNGVHANLHVAFHRSSSEKIH SNEISSDSIGVLGYQKTVDHTKVNSXLSLFFEIKS	

[0079] In some embodiments, the conjugate polypeptide is SpyAD and comprises four nnAAs substituted at positions K64, K287, K396, and K657 of SEQ ID NO: 9. In some embodiments, the conjugate polypeptide is SpyAD and comprises four nnAAs substituted at positions K64, K287, K396, and K657 of SEQ ID NO: 33. In some embodiments, the four nnAAs are each pAMF. In some embodiments, the conjugate polypeptide is SpyAD and comprises or consists of an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 11 or SEQ ID NO: 12. In some embodiments, the four nnAAs are each pAMF. In some embodiments, the conjugate polypeptide is SpyAD and comprises or consists an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 34. In some embodiments, the conjugate polypeptide is SpyAD and comprises or consists of the amino acid sequence of SEQ ID NO: 11 or SEQ ID NO: 12. In some embodiments, the conjugate polypeptide is SpyAD and comprises or consists of the amino acid sequence of SEQ ID NO: 34.

[0080] In some embodiments, the conjugate polypeptide is ADI and comprises three nnAAs substituted at positions K15, K193, and K316 of SEQ ID NO: 13. In some embodiments, the conjugate polypeptide is ADI and comprises three nnAAs substituted at positions K15, K193, and K316 of SEQ ID NO: 35. In some embodiments, the three nnAAs are each pAMF. In some embodiments, the conjugate polypeptide is ADI and comprises or consists of an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to any one of SEQ ID NOs: 17, 18, 19, or 20. In some embodiments, the three nnAAs are each pAMF. In some embodiments, the conjugate polypeptide is SpyAD and comprises or consists of an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to any one of SEQ ID NOs: 26, 27, 37, or 38. In some embodiments, the conjugate polypeptide is ADI and comprises or consists of the amino acid sequence of any one of SEQ ID NOs: 17, 18, 19, or 20. In some

embodiments, the conjugate polypeptide is ADI and comprises or consists of the amino acid sequence of any one of SEQ ID NOs: 26, 27, 37, 38.

[0081] In some embodiments, the conjugate polypeptide is a SLO polypeptide. In some embodiments, the conjugate polypeptide is SLO(ΔC101) and comprises three or four nnAAs substituted at positions selected from selected from K98, K112, R151, K189, K272, K323, K357, K375, K407, and K464 of SEQ ID NO: 53. In some embodiments, the conjugate polypeptide is SLO(ΔC101) and comprises or consists of nnAAs substituted at positions K98, R151 , K272, and K357; positions K112, K189, K323, and K375; positions R151, K272, K357, and K407; positions R151, K272, K375, and K464; positions K112, K272, K357, and K464; positions K98, K189, and K357; positions K112, K189, and K323; positions K98, R151, and K272; positions K112, K272, and K375; or positions K112, K323, and K407. In some embodiments, the three or four nnAAs are each pAMF. In some embodiments, the conjugate polypeptide is a SLO polypeptide and comprises or consists of an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to any one of SEQ ID NOs: 55, 56, 57, 58, 59, 60, 61, 62, 63, or 64. In some embodiments, the conjugate polypeptide is a SLO polypeptide and comprises or consists of the amino acid sequence of any one of SEQ ID NOs: 55, 56, 57, 58, 59, 60, 61, 62, 63, or 64.

[0082] In some embodiments, the conjugate polypeptide is a SLO polypeptide. In some embodiments, the conjugate polypeptide is SLO(ΔC101) and comprises five, six, seven, or eight nnAAs substituted at positions selected from selected from K98, K112, R151, K189, K272, K323, K357, K375, K407, and K464 of SEQ ID NO: 53. In some embodiments, the conjugate polypeptide is SLO(ΔC101) and comprises or consists of nnAAs substituted at positions K98, R151, K272, K357, and K407; positions K112, K189, K323, K375, and K464; positions K112, R151, K272, K357, and K407; positions K98, R151, K272, K357, K407, and K464; positions K112, R151, K189, K323, K375, and K464; positions K98, K112, K189, K323, K375, and K464; positions K112, R151, K189, K272, K357, K407, and K464; positions K98, R151, K189, K323, K375, K407, and K464; positions K112, K189, K272, K357, K375, K407, and K464; positions K98, K112, R151, K189, K272, K323, K357, and K375; positions K98, R151, K189, K272, K323, K357, K407, and K464; or positions K112, K189, K272, K323, K357, K375, K407, and K464. In some embodiments, the five, six, seven, or eight

nnAAs are each pAMF. In some embodiments, the conjugate polypeptide is a SLO polypeptide and comprises or consists of an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to any one of SEQ ID NOs: 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, or 76. In some embodiments, the conjugate polypeptide is a SLO polypeptide and comprises or consists of the amino acid sequence of any one of SEQ ID NOs: 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, or 76.

[0083] In some embodiments, the conjugate polypeptide is ferritin and comprises one nnAA substituted at position I5 of SEQ ID NO: 21. In some embodiments, the nnAA is pAMF. In some embodiments, the conjugate polypeptide is ferritin and comprises or consists of an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 23 or SEQ ID NO: 24. In some embodiments, the conjugate polypeptide is ADI and comprises or consists of SEQ ID NO: 23 or SEQ ID NO: 24.

Polysaccharides

[0084] In some embodiments, the conjugate polysaccharide is a GAS polysaccharide, such as the group A carbohydrate (GAC). The GAC is composed of a polyrhamnose backbone with an immunodominant GlcNAc side chain and is present on the surface of strains of all GAS serotypes irrespective of M type and has been examined as a GAS vaccine candidate. The conjugate polysaccharide may be defined as the polysaccharide component of a conjugate. Affinity-purified anti-GAC antibodies successfully opsonized three tested GAS serotypes (Salvadori et al., 1995. J. Infect. Dis. 171:593–600.), and mice immunized with GAC were protected against both intraperitoneal and intranasal GAS challenge (Sabharwal et al., 2006. J. Infect. Dis. 193:129–135). However, immunological cross-reactivity between anti-GAC antibodies and host heart valve proteins (Goldstein et al., 1967. Nature 213:44–47) and cytoskeletal proteins, such as actin, keratin, myosin, and vimentin, raises important potential safety concerns regarding the use of GAC as a GAS vaccine constituent.

[0085] In some embodiments, a *Streptococcus pyogenes* bacterial cell may be engineered to produce a polysaccharide or a variant thereof that lacks an immunodominant N-acetyl Glucosamine (GLcNAc) side chain. Therefore, in some embodiments, the conjugate polysaccharide is a variant of the GAC that lacks the immunodominant GlcNAc side chain (See *e.g.*, International PCT Publication No. WO 2013/020090 US Patent No. 10,780,155, and

Gao, N. J. et al., December 29, 2020. Infectious Microbes and Diseases, doi: 10.1097/IM9.0000000000000044). In some embodiments, the conjugate polysaccharide is a GAC comprising a polyrhamanose core. In some embodiments, the conjugate polysaccharide has an average molecular weight of about 3kDa to about 10kDa, about 5kDa to about 10kDa, about 9kDa to about 10kDa, about 9kDa to about 10kDa, about 3kDa to about 9kDa, about 3kDa to about 7kDa, about 3kDa to about 5kDa, about 5kDa to about 9kDa, or about 5kDa to about 7kDa. In some embodiments, the conjugate polysaccharide has an average molecular weight of about 3kDa, 4kDa, 5kDa, 6kDa, 7kDa, 8kDa, 9kDa, or about 10kDa. In some embodiments, the conjugate polysaccharide is a tetramer, a pentamer, a hexamer, a septamer, an octamer, a nonomer, or a decamer polysaccharide.

[0086] In some embodiments, the conjugate polysaccharide has an average molecular weight of between about 10 kDa to about 45 kDa. In some embodiments, the conjugate polysaccharide has an average molecular weight of about 10 kDa to about 15 kDa, about 15 kDa to about 20 kDa, about 20 kDa to about 25 kDa, about 25 kDa to about 30 kDa, about 30 kDa to about 35 kDa, or about about 35 kDa to about 40 kDa. In some embodiments, the conjugate polysaccharide has an average molecular weight of about 15 kDa to about 40 kDa, about 15 kDa to about 35 kDa, about 15 kDa to about 30 kDa, about 15 kDa to about 25 kDa, or about 15 kDa to about 45 kDa. In some embodiments, the conjugate polysaccharide has an average molecular weight of about 20 kDa to about 40 kDa, about 20 kDa to about 35 kDa, about 20 kDa to about 30 kDa, or about 20 kDa to about 45 kDa. In some embodiments, the conjugate polysaccharide has an average molecular weight of about 10 kDa, 15 kDa, 20 kDa, 25 kDa, 30 kDa, 35 kDa, 40 kD or about 45 kDa. In some embodiments, the conjugate polysaccharide has an average molecular weight of at least about 10 kDa, 15 kDa, 20 kDa, 25 kDa, 30 kDa, or about 35 kDa.

[0087] In some embodiments, the conjugate polysaccharide is purified from GAS bacterial cultures or bacterial stocks. Methods of such purification are known in the art, *see e.g.*, WO 2010/049806, International PCT Publication No. WO 2013/020090, and van Sorge, *et al.*, *Cell Host Microbe.*, 2014, 15(6), 729-740. *See also* Example 1. In some embodiments, the conjugate polysaccharide is a synthesized polysaccharide. Methods of polysaccharide synthesis are known in the art, *see e.g.*, Zhao, *et al.*, *Org. Chem. Front.*, 2019, 6, 3589-3596. *See also*

Example 3. In some embodiments, the conjugate polysaccharides are modified with a click chemistry reactive group to facilitate conjugation to the conjugate protein. Examples of click chemistry reactive groups can be found, for instance, in International PCT Publication No. WO 2018/126229, incorporated by reference herein by reference in its entirety. For example, in some embodiments, the conjugate polysaccharides are modified with DBCO or DBCO-PEG (*e.g.,* DBCO-PEG-NH_2). In some embodiments, the conjugate polysaccharides are modified with DBCO-$(PEG)_4$-NH_2.

[0088] In some embodiments, the polypeptide-polysaccharide conjugate has an average molecular mass of about 10 kDa to about 5000 kDa. In some embodiments, the polypeptide-polysaccharide conjugate has an average molecular mass of about 10 kDa to about 50 kDa. In some embodiments, the polypeptide-polysaccharide conjugate has an average molecular mass of about 10 kDa to about 100 kDa. In some embodiments, the polypeptide-polysaccharide conjugate has an average molecular mass of about 10 kDa to about 200 kDa. In some embodiments, the polypeptide-polysaccharide conjugate has an average molecular mass of about 10 kDa to about 500 kDa. In some embodiments, the polypeptide-polysaccharide conjugate has an average molecular mass of about 10 kDa to about 1000 kDa. In some embodiments, the polypeptide-polysaccharide conjugate has an average molecular mass of about 100 kDa to about 200 kDa. In some embodiments, the polypeptide-polysaccharide conjugate has an average molecular mass of about 100 kDa to about 300 kDa. In some embodiments, the polypeptide-polysaccharide conjugate has an average molecular mass of about 100 kDa to about 400 kDa. In some embodiments, the polypeptide-polysaccharide conjugate has an average molecular mass of about 100 kDa to about 500 kDa. In some embodiments, the polypeptide-polysaccharide conjugate has an average molecular mass of about 100 kDa to about 5000 kDa. In some embodiments, the polypeptide-polysaccharide conjugate has an average molecular mass of about 500 kDa to about 5000 kDa. In some embodiments, the polypeptide-polysaccharide conjugate has an average molecular mass of about 1000 kDa to about 5000 kDa. In some embodiments, the polypeptide-polysaccharide conjugate has an average molecular mass of about 2000 kDa to about 5000 kDa. In some embodiments, the polypeptide-polysaccharide conjugate has an average molecular mass of about 3000 kDa to about 5000 kDa. In some embodiments, the polypeptide-polysaccharide conjugate has an average molecular mass of about 4000 kDa to about 5000 kDa. In some

embodiments, the polypeptide-polysaccharide conjugate has an average molecular mass of about 1000 kDa to about 3000 kDa. In some embodiments, the polypeptide-polysaccharide conjugate has an average molecular mass of about 1000 kDa to about 2000 kDa. In some embodiments, the polypeptide-polysaccharide conjugate has an average molecular mass of about 2000 kDa to about 3000 kDa. In some embodiments, the polypeptide-polysaccharide conjugate has an average molecular mass of about 5000 kDa or greater.

Exemplary Combination Vaccines

[0089] In some embodiments, the present disclosure provides an immunogenic composition comprising a GAS C5a peptidase polypeptide antigen; a GAS streptolysin O (SLO) polypeptide antigen; and a polypeptide-polysaccharide conjugate comprising a SpyAD conjugate polypeptide and a GAS conjugate polysaccharide or a variant thereof that lacks an immunodominant GlcNAc side chain. In some embodiments, the SpyAD protein comprises or consists of an amino acid sequence that is at least 95% identical to SEQ ID NO: 9 or SEQ ID NO: 10 and comprises a pAMF substitution at positions K64, K287, K386, and K657. In some embodiments, the SpyAD protein comprises the amino acid sequence of SEQ ID NO: 11 or SEQ ID NO: 12. In some embodiments, the SpyAD protein comprises or consists of an amino acid sequence that is at least 95% identical to SEQ ID NO: 33 and comprises a pAMF substitution at positions K64, K287, K386, and K657. In some embodiments, the SpyAD protein comprises the amino acid sequence of SEQ ID NO: 34. In some embodiments, the SpyAD protein comprises or consists of the amino acid sequence of SEQ ID NO: 11 or SEQ ID NO: 12. In some embodiments, the SpyAD protein comprises or consists of the amino acid sequence of SEQ ID NO: 34.

[0090] In some embodiments, the present disclosure provides an immunogenic composition comprising a GAS C5a peptidase polypeptide antigen; a GAS streptolysin O (SLO) polypeptide antigen; and a polypeptide-polysaccharide conjugate comprising a SpyAD conjugate polypeptide and a GAS conjugate polysaccharide or a variant thereof that lacks an immunodominant GlcNAc side chain. In some embodiments, the SLO polypeptide antigen comprises or consists of an amino acid sequence that is at least 95% identical to SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID

NO: 53. In some embodiments, the SLO polypeptide antigen comprises or consists of an amino acid sequence that is at least 95% identical to SEQ ID NO: 31. In some embodiments, the SLO polypeptide antigen comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 32. In some embodiments, the SLO polypeptide antigen comprises or consists of an amino acid sequence that is at least 95% identical to SEQ ID NO: 44. In some embodiments, the SLO polypeptide antigen comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 45. In some embodiments, the SLO polypeptide antigen comprises or consists of an amino acid sequence that is at least 95% identical to SEQ ID NO: 46. In some embodiments, the SLO polypeptide antigen comprises or consists of an amino acid sequence that is at least 95% identical to SEQ ID NO: 47. In some embodiments, the SLO polypeptide antigen comprises or consists of an amino acid sequence that is at least 95% identical to SEQ ID NO: 48. In some embodiments, the SLO polypeptide antigen comprises or consists of an amino acid sequence that is at least 95% identical to SEQ ID NO: 49. In some embodiments, the SLO polypeptide antigen comprises or consists of an amino acid sequence that is at least 95% identical to SEQ ID NO: 50. In some embodiments, the SLO polypeptide antigen comprises or consists of an amino acid sequence that is at least 95% identical to SEQ ID NO: 51. In some embodiments, the SLO polypeptide antigen comprises or consists of an amino acid sequence that is at least 95% identical to SEQ ID NO: 52. In some embodiments, the SLO polypeptide antigen comprises or consists of an amino acid sequence that is at least 95% identical to SEQ ID NO: 53. In some embodiments, the SLO polypeptide antigen comprises or consists of the amino acid sequence of SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53.

[0091] In some embodiments, the present disclosure provides an immunogenic composition comprising a GAS C5a peptidase polypeptide antigen; a GAS streptolysin O (SLO) polypeptide antigen; and a polypeptide-polysaccharide conjugate comprising a SpyAD conjugate polypeptide and a GAS conjugate polysaccharide or a variant thereof that lacks an immunodominant GlcNAc side chain. In some embodiments, the C5a peptidase polypeptide antigen comprises or consists of an amino acid sequence that is at least 95% identical to SEQ ID NO: 29 or SEQ ID NO: 30; the SLO polypeptide antigen comprises or consists of an amino acid sequence that is at least 95% identical to SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO:

44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53; and the SpyAD conjugate protein comprises or consists of an amino acid sequence that is at least 95% identical to SEQ ID NO: 33 and comprises a pAMF substitution at positions K64, K287, K386, and K657. In some embodiments, the SpyAD protein comprises or consists of the amino acid sequence of SEQ ID NO: 34. In some embodiments, the C5a peptidase polypeptide antigen comprises or consists of the amino acid sequence of SEQ ID NO: 29 or SEQ ID NO: 30; the SLO polypeptide antigen comprises or consists of an amino acid sequence of SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53; and the SpyAD conjugate protein comprises or consists of an amino acid sequence of SEQ ID NO: 33 and comprises a pAMF substitution at positions K64, K287, K386, and K657. In some embodiments, the SpyAD protein comprises or consists of the amino acid sequence of SEQ ID NO: 34.

[0092] In some embodiments, the present disclosure provides an immunogenic composition comprising a C5a peptidase polypeptide antigen of SEQ ID NO: 30; a SLO polypeptide antigen of SEQ ID NO: 53; and a polypeptide-polysaccharide conjugate comprising a SpyAD conjugate polypeptide of SEQ ID NO: 34 and a GAS conjugate polysaccharide. In some embodiments, the present disclosure provides an immunogenic composition comprising a C5a peptidase polypeptide antigen of SEQ ID NO: 30; a SLO polypeptide antigen of SEQ ID NO: 52; and a polypeptide-polysaccharide conjugate comprising a SpyAD conjugate polypeptide of SEQ ID NO: 34 and a GAS conjugate polysaccharide. In some embodiments, the present disclosure provides an immunogenic composition comprising a C5a peptidase polypeptide antigen of SEQ ID NO: 30; a SLO polypeptide antigen of SEQ ID NO: 32; and a polypeptide-polysaccharide conjugate comprising a SpyAD conjugate polypeptide of SEQ ID NO: 34 and a GAS conjugate polysaccharide. In some embodiments, the present disclosure provides an immunogenic composition comprising a C5a peptidase polypeptide antigen of SEQ ID NO: 29; a SLO polypeptide antigen of SEQ ID NO: 53; and a polypeptide-polysaccharide conjugate comprising a SpyAD conjugate polypeptide of SEQ ID NO: 34 and a GAS conjugate polysaccharide. In some embodiments, the present disclosure provides an immunogenic composition comprising a C5a peptidase polypeptide antigen comprising or consisting of the

amino acid sequence of SEQ ID NO: 30; a SLO polypeptide antigen comprising or consisting of the amino acid sequence of SEQ ID NO: 53; and a polypeptide-polysaccharide conjugate comprising a SpyAD conjugate polypeptide comprising or consisting of the amino acid sequence of SEQ ID NO: 34 and a GAS conjugate polysaccharide. In some embodiments, the present disclosure provides an immunogenic composition comprising a C5a peptidase polypeptide antigen comprising or consisting of the amino acid sequence of SEQ ID NO: 30; a SLO polypeptide antigen comprising or consisting of the amino acid sequence of SEQ ID NO: 52; and a polypeptide-polysaccharide conjugate comprising a SpyAD conjugate polypeptide comprising or consisting of the amino acid sequence of SEQ ID NO: 34 and a GAS conjugate polysaccharide. In some embodiments, the present disclosure provides an immunogenic composition comprising a C5a peptidase polypeptide antigen comprising or consisting of the amino acid sequence of SEQ ID NO: 30; a SLO polypeptide antigen comprising or consisting of the amino acid sequence of SEQ ID NO: 32; and a polypeptide-polysaccharide conjugate comprising a SpyAD conjugate polypeptide comprising or consisting of the amino acid sequence of SEQ ID NO: 34 and a GAS conjugate polysaccharide. In some embodiments, the present disclosure provides an immunogenic composition comprising a C5a peptidase polypeptide antigen comprising or consisting of the amino acid sequence of SEQ ID NO: 29; a SLO polypeptide antigen comprising or consisting of the amino acid sequence of SEQ ID NO: 53; and a polypeptide-polysaccharide conjugate comprising a SpyAD conjugate polypeptide comprising or consisting of the amino acid sequence of SEQ ID NO: 34 and a GAS conjugate polysaccharide.

[0093] In some embodiments, the present disclosure provides an immunogenic composition comprising a C5a peptidase polypeptide antigen; a GAS SLO polypeptide antigen; and a polypeptide-polysaccharide conjugate comprising an ADI conjugate polypeptide and a GAS conjugate polysaccharide or a variant thereof that lacks an immunodominant GlcNAc side chain. In some embodiments, the ADI polypeptide comprises or consists of an amino acid sequence that is at least 95% identical to any one of SEQ ID NOs: 13-16 and comprises a pAMF substitution at positions K15, K193, and K316. In some embodiments, the ADI polypeptide comprises the amino acid sequence of any one of SEQ ID NOs: 17-20. In some embodiments, the ADI polypeptide comprises or consists of an amino acid sequence that is at least 95% identical to any one of SEQ ID NOs: 35-36 and comprises a pAMF substitution at

positions K15, K193, and K316. In some embodiments, the ADI polypeptide comprises or consists of the amino acid sequence of any one of SEQ ID NOs: 37-38. In some embodiments, the ADI polypeptide comprises or consists of the amino acid sequence of any one of SEQ ID NOs: 17-20. In some embodiments, the ADI polypeptide comprises or consists of an amino acid sequence that is at least 95% identical to any one of SEQ ID NOs: 35-36 and comprises a pAMF substitution at positions K15, K193, and K316. In some embodiments, the ADI polypeptide comprises or consists of the amino acid sequence of any one of SEQ ID NOs: 37-38.

[0094] In some embodiments, the present disclosure provides an immunogenic composition comprising a C5a peptidase polypeptide antigen; a GAS SLO polypeptide antigen; and a polypeptide-polysaccharide conjugate comprising a ferritin polypeptide and a GAS conjugate polysaccharide or a variant thereof that lacks an immunodominant GlcNAc side chain. In some embodiments, the ferritin polypeptide comprises or consists of an amino acid sequence that is at least 95% identical to SEQ ID NO: 21 or SEQ ID NO: 22 and comprises a pAMF substitution at position I5. In some embodiments, the ferritin polypeptide comprises the amino acid sequence of SEQ ID NO: 23 or SEQ ID NO: 24. In some embodiments, the ferritin polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 23 or SEQ ID NO: 24.

Therapeutic Methods

[0095] In some embodiments, the present disclosure provides a method of inducing a protective immune response against a GAS bacterium in a subject comprising administering an immunogenic composition described herein to the subject. In certain embodiments are provided the use of the immunogenic compositions described herein for inducing a protective immune response against a GAS bacterium in a subject. In some embodiments, provided herein are the use of the immunogenic compositions described herein in the manufacture of a medicament for inducing a protective immune response against a GAS bacterium in a subject.

[0096] Herein, the term “subject” refers to a mammal. In some embodiments, the subject is a mouse, a rat, a dog, a guinea pig, a sheep, a non-human primate, or a human. In some embodiments, the subject is a human. In some embodiments, the human subjects are 18 years of age or older. In some embodiments, the human subjects are less than 18 years of age.

[0097] In some embodiments, the human subjects are between 6 months of age and 17 years of age. In some embodiments, the human subjects are between 6 months of age and 9 years of age, between 6 months of age and 8 years of age, between 6 months of age and 7 years of age, between 6 months of age and 6 years of age, between 6 months of age and 5 years of age, between 6 months of age and 4 years of age, between 6 months of age and 3 years of age, between 6 months of age and 2 years of age, or between 6 months of age and 1 year of age. In some embodiments, the human subjects are between 5 years of age and 17 years of age, between 7 years of age and 17 years of age, between 9 years of age and 17 years of age, between 11 years of age and 17 years of age, between 13 years of age and 17 years of age, or between 15 years of age and 17 years of age. In some embodiments, the human subjects are 6 months, 1 year, 2 years, 3 years, 4 years, 5 years, 6 years, 7 years, 8 years, 9 years, 10 years, 11 years, 12 years, 13 years, 14 years, 15 years, 16 years, 17 years, or 18 years of age.

[0098] Herein, the term "protective immune response" encompasses eliciting an anti-GAS antibody response in the subject. Antibody titers generated after administration of the immunogenic compositions described herein can be determined by means known in the art, for example by ELISA assays of serum samples derived from immunized subjects (See Example 6). In some embodiments, the immunogenic compositions described herein elicit antibody responses in treated subjects, wherein the antibodies generated bind to multiple (*i.e.,* two or more) GAS serotypes. In some embodiments, the immunogenic compositions described herein elicit antibody responses in treated subjects, wherein the antibodies generated bind to 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, or more GAS serotypes. In some embodiments, the immunogenic compositions described herein do not elicit antibody responses against human proteins or tissue.

[0099] In some embodiments, the immunogenic compositions described herein elicit antibody responses in treated subjects, wherein the antibodies generated bind to at least one GAS serotype selected from M1, M2, M3, M4, M5, M6, M9, M11, M12, M13, M18, M22, M25, M28, M62, M71, M72, M74, M75, M77, M80, M81, M83, M87, M89, and M92. In some embodiments, the immunogenic compositions described herein elicit antibody responses in treated subjects, wherein the antibodies generated bind to two or more GAS serotypes selected

from M1, M2, M3, M4, M5, M6, M9, M11, M12, M13, M18, M22, M25, M28, M62, M71, M72, M74, M75, M77, M80, M81, M83, M87, M89, and M92.

[0100] In some embodiments, the immunogenic compositions described herein elicit antibody responses in treated subjects, wherein the antibodies generated bind to at least one GAS serotype selected from M1, M2, M3, M4, M6, M11, M12, M22, M28, M75, and M89. In some embodiments, the immunogenic compositions described herein elicit antibody responses in treated subjects, wherein the antibodies generated bind to two or more GAS serotypes selected from M1, M2, M3, M4, M6, M11, M12, M22, M28, M75, and M89.

[0101] In some embodiments, the immunogenic compositions described herein elicit antibody responses in treated subjects, wherein the antibodies generated bind to at least one GAS serotype selected from M1, M3, M5, M9, M12, M18, M22, M25, M28, M71, M72, and M74. In some embodiments, the immunogenic compositions described herein elicit antibody responses in treated subjects, wherein the antibodies generated bind to two or more GAS serotypes selected from M1, M3, M5, M9, M12, M18, M22, M25, M28, M71, M72, and M74.

[0102] In some embodiments, the immunogenic compositions described herein elicit antibody responses in treated subjects, wherein the antibodies generated bind to at least one GAS serotype selected from M1, M4, M6, M11, M12, M22, M44, M75, M77, M77, and M81. In some embodiments, the immunogenic compositions described herein elicit antibody responses in treated subjects, wherein the antibodies generated bind to two or more GAS serotypes selected from M1, M4, M6, M11, M12, M22, M44, M75, M77, M77, and M81.

[0103] In some embodiments, the immunogenic compositions described herein elicit antibody responses in treated subjects, wherein the antibodies generated bind to at least one GAS serotype selected from M1, M2, M3, M4, M6, M9, M12, M18, M22, M75, M77, M89, and M92. In some embodiments, the immunogenic compositions described herein elicit antibody responses in treated subjects, wherein the antibodies generated bind to two or more GAS serotypes selected from M1, M2, M3, M4, M6, M9, M12, M18, M22, M75, M77, M89, and M92.

[0104] In some embodiments, the immunogenic compositions described herein elicit antibody responses in treated subjects, wherein the antibodies generated bind to at least one

GAS serotype selected from M1, M2, M3, M4, M5, M6, M9, M11, M12, M13, M28, M62, and M89. In some embodiments, the immunogenic compositions described herein elicit antibody responses in treated subjects, wherein the antibodies generated bind to two or more GAS serotypes selected from M1, M2, M3, M4, M5, M6, M9, M11, M12, M13, M28, M62, and M89.

[0105] In some embodiments, the immunogenic compositions described herein elicit antibody responses in treated subjects, wherein the antibodies generated bind to at least one GAS serotype selected from M1, M2, M3, M4, M6, M12, M22, M28, M49, M53, M68, M77, M80, M83, M87, M89, and M92. In some embodiments, the immunogenic compositions described herein elicit antibody responses in treated subjects, wherein the antibodies generated bind to two or more GAS serotypes selected from M1, M2, M3, M4, M6, M12, M22, M28, M49, M53, M68, M77, M80, M83, M87, M89, and M92.

[0106] In some embodiments, the immunogenic compositions described herein elicit antibody responses in treated subjects that bind to one or more *Shigella* serotypes. In some embodiments, the immunogenic compositions described herein elicit antibody responses in treated subjects that bind to one or more GAS serotypes and also bind to one or more *Shigella* serotypes. In some embodiments, the *Shigella* serotypes comprise a polysaccharide comprising a polyrhamanose backbone. Exemplary *Shigella* serotypes comprising such polysaccharides include *S. flexneri* such as *S. flexneri* 2A and 3A and *S. flexneri 6*.

FURTHER NUMBERED EMBODIMENTS

[0107] Further embodiments of the instant disclosure are provided in the numbered embodiments below:

[0108] Embodiment 1: An immunogenic composition comprising a first Group A Streptococcus (GAS) polypeptide antigen; a second GAS polypeptide antigen; and at least one polypeptide-polysaccharide conjugate, wherein the conjugate polypeptide is a third GAS polypeptide antigen or a non-GAS carrier polypeptide and comprises at least one non-natural amino acid, and wherein the conjugate polysaccharide is a GAS polysaccharide or a variant thereof that lacks an immunodominant N-acetyl Glucosamine (GlcNAc) side chain.

[0109] Embodiment 2: The immunogenic composition of Embodiment 1, wherein at least one of the GAS polypeptide antigens is a full length GAS antigen.

[0110] Embodiment 3: The immunogenic composition of Embodiment 1, wherein at least one of the GAS polypeptide antigens is a peptide fragment of a full length GAS antigen.

[0111] Embodiment 4: The immunogenic composition of any one of Embodiments 1-3, wherein the first and second GAS polypeptide antigens are independently selected from C5a peptidase, streptolysin O (SLO), Sib35, and Sfb1.

[0112] Embodiment 5: The immunogenic composition of any one of Embodiments 1-3, wherein the first and second GAS polypeptide antigens are C5a peptidase and SLO.

[0113] Embodiment 6: The immunogenic composition of Embodiment 5, wherein the C5a peptidase polypeptide antigen comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 1 or SEQ ID NO: 3.

[0114] Embodiment 7: The immunogenic composition of Embodiment 5, wherein the C5a peptidase polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 3.

[0115] Embodiment 8: The immunogenic composition of Embodiment 5, wherein the SLO polypeptide antigen comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 5 or SEQ ID NO: 7.

[0116] Embodiment 9: The immunogenic composition of Embodiment 5, wherein the SLO polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 7.

[0117] Embodiment 10: The immunogenic composition of any one of Embodiments 1-9, wherein the non-GAS carrier polypeptide is ferritin.

[0118] Embodiment 11: The immunogenic composition of any one of Embodiments 1-10, wherein the third GAS polypeptide antigen is selected from Streptococcus pyogenes Adhesion and Division (SpyAD) polypeptide, arginine deiminase (ADI), eCRM, and Protein D.

[0119] Embodiment 12: The immunogenic composition of any one of Embodiments 1-11, wherein the at least one nnAA is substituted for a lysine, a leucine, or an isoleucine in the conjugate polypeptide.

[0120] Embodiment 13: The immunogenic composition of any one of Embodiments 1-11, wherein the nnAA comprises a click chemistry reactive group.

[0121] Embodiment 14: The immunogenic composition of Embodiment 13, wherein the nnAA is selected from 2-amino-3-(4-azidophenyl)propanoic acid(4-azidophenyl)propanoic acid (pAF), 2-amino-4-azidobutanoic acid, 2-azido-3-phenylpropionic acid, 2-amino-3-azidopropanoic acid, 2-amino-3-(4-(azidomethyl)phenyl)propanoic acid (pAMF), 2-amino-3-(5-(azidomethyl)pyridin-2-yl)propanoic acid, 2-amino-3-(4-(azidomethyl)pyridin-2-yl)propanoic acid, 2-amino-3-(6-(azidomethyl)pyridin-3-yl)propanoic acid, and 2-amino-5-azidopentanoic acid.

[0122] Embodiment 15: The immunogenic composition of Embodiment 13, wherein the nnAA is pAMF.

[0123] Embodiment 16: The immunogenic composition of any one of Embodiments 12-15, wherein the conjugate polypeptide is a third GAS protein, which is a SpyAD polypeptide and comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 9.

[0124] Embodiment 17: The immunogenic composition of Embodiment 16, wherein the SpyAD polypeptide comprises a pAMF substitution at positions K64, K287, K386, and K657 of SEQ ID NO: 9.

[0125] Embodiment 18: The immunogenic composition of Embodiment 17, wherein the SpyAD polypeptide comprises the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 11.

[0126] Embodiment 19: The immunogenic composition of any one of Embodiments 12-15, wherein the conjugate polypeptide is a third GAS polypeptide, which is an ADI polypeptide and comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 13 or SEQ ID NO: 15.

[0127] Embodiment 20: The immunogenic composition of Embodiment 19, wherein the ADI polypeptide comprises a pAMF substitution at positions K15, K193, and K316 of SEQ ID NO: 13 or SEQ ID NO: 15.

[0128] Embodiment 21: The immunogenic composition of Embodiment 20, wherein the ADI polypeptide comprises the amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 19.

[0129] Embodiment 22: The immunogenic composition of any one of Embodiments 12-15, wherein the conjugate polypeptide is a ferritin polypeptide and comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 21.

[0130] Embodiment 23: The immunogenic composition of Embodiment 22, wherein the ferritin polypeptide comprises a pAMF substitution at position I5 of SEQ ID NO: 21.

[0131] Embodiment 24: The immunogenic composition of Embodiment 23, wherein the ferritin polypeptide comprises the amino acid sequence of SEQ ID NO: 23.

[0132] Embodiment 25: The immunogenic composition of any one of Embodiments 1-23, wherein the conjugate polysaccharide comprises a polyrhamnose core.

[0133] Embodiment 26: The immunogenic composition of any one of Embodiments 1-23, wherein the conjugate polysaccharide has an average molecular weight of about 5KDa to about 7 KDa.

[0134] Embodiment 27: The immunogenic composition of any one of Embodiments 1-26, wherein the conjugate polysaccharide is a tetramer, a hexamer, an octamer, or a decamer polysaccharide.

[0135] Embodiment 28: An immunogenic composition comprising a Group A Streptococcus (GAS) C5a peptidase polypeptide antigen; a GAS streptolysin O (SLO) polypeptide antigen; and a polypeptide-polysaccharide conjugate comprising a Streptococcus pyogenes Adhesion and Division (SpyAD) conjugate polypeptide and a GAS conjugate polysaccharide or a variant thereof that lacks an immunodominant N-acetyl Glucosamine (GlcNAc) side chain.

[0136] Embodiment 29: The immunogenic composition of Embodiment 28, wherein the SpyAD protein comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 9 and comprises a pAMF substitution at positions K64, K287, K386, and K657.

[0137] Embodiment 30: The immunogenic composition of Embodiment 28, wherein the SpyAD protein comprises the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 11.

[0138] Embodiment 31: An immunogenic composition comprising a Group A Streptococcus (GAS) C5a peptidase polypeptide antigen; a GAS streptolysin O (SLO) polypeptide antigen; and a polypeptide-polysaccharide conjugate comprising an arginine deiminase (ADI) conjugate polypeptide and a GAS conjugate polysaccharide or a variant thereof that lacks an immunodominant N-acetyl Glucosamine (GlcNAc) side chain.

[0139] Embodiment 32: The immunogenic composition of Embodiment 31, wherein the ADI polypeptide comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 13 or SEQ ID NO: 15 and comprises a pAMF substitution at positions K15, K193, and K316.

[0140] Embodiment 33: The immunogenic composition of Embodiment 31, wherein the ADI polypeptide comprises the amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 19.

[0141] Embodiment 34: An immunogenic composition comprising a Group A Streptococcus (GAS) C5a peptidase polypeptide antigen; a GAS streptolysin O (SLO) polypeptide antigen; and a polypeptide-polysaccharide conjugate comprising a ferritin polypeptide and a GAS conjugate polysaccharide or a variant thereof that lacks an immunodominant N-acetyl Glucosamine (GlcNAc) side chain.

[0142] Embodiment 35: The immunogenic composition of Embodiment 34, wherein the ferritin protein comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 21 and comprises a pAMF substitution at position I5.

[0143] Embodiment 35A: The immunogenic composition Embodiment 34, wherein the ferritin protein comprises the amino acid sequence of SEQ ID NO: 23.

[0144] Embodiment 36: The immunogenic composition of any one of Embodiments 1-35, wherein the composition comprises about 10% to about 50% of the first GAS antigen, about

10% to about 50% of the second GAS antigen, and about 10% to about 50% of the polypeptide-polysaccharide conjugate.

[0145] Embodiment 37: The immunogenic composition of any one of Embodiments 1-36, further comprising one or more adjuvants selected from alum, saponin, monophosphoryl lipid A (MPL), or combinations thereof.

[0146] Embodiment 38: A method of inducing a protective immune response against a Group A Streptococcus (GAS) bacterium in a subject comprising administering the immunogenic composition of any one of Embodiments 1-37 to the subject.

[0147] Embodiment 38A: Use of the immunogenic composition of any one of Embodiments 1-37 for inducing a protective immune response against a Group A Streptococcus (GAS) bacterium in a subject.

[0148] Embodiment 38B: Use of the immunogenic composition of any one of Embodiments 1-37 in the manufacture of a medicament for inducing a protective immune response against a Group A Streptococcus (GAS) bacterium in a subject.

[0149] Embodiment 39: The method of any one of Embodiments 38-38B, wherein the subject is 18 years or older.

[0150] Embodiment 40: The method of any one of Embodiments 38-38B, wherein the subject is less than 18 years old.

[0151] Embodiment 41: The method of any one of Embodiments 38-38B, wherein the subject is between 5 years and 17 years old, between 6 months and 9 years old, or between 5 years and 9 years old.

[0152] Embodiment 42: The method of any one of Embodiments 38-41, wherein the GAS bacterium is of a serotype selected from M1, M2, M3, M4, M6, M11, M12, M22, M28, M75, and M89.

[0153] Embodiment 43: The method of any one of Embodiments 38-41, wherein the GAS bacterium is a serotype selected from M1, M3, M5, M9, M12, M18, M22, M25, M28, M71, M72, and M74.

[0154] Embodiment 44: The method of any one of Embodiments 38-41, wherein the GAS bacterium is a serotype selected from M1, M4, M6, M11, M12, M22, M44, M75, M77, M77, and M81.

[0155] Embodiment 45: The method of any one of Embodiments 38-41, wherein the GAS bacterium is a serotype selected from M1, M2, M3, M4, M6, M9, M12, M18, M22, M75, M77, M89, and M92.

[0156] Embodiment 46: The method of any one of Embodiments 38-41, wherein the GAS bacterium is a serotype selected from M1, M2, M3, M4, M5, M6, M9, M11, M12, M13, M28, M62, and M89.

[0157] Embodiment 47: The method of any one of Embodiments 38-41, wherein the GAS bacterium is a serotype selected from M1, M2, M3, M4, M6, M12, M22, M28, M49, M53, M68, M77, M80, M83, M87, M89, and M92.

[0158] Embodiment 48: The method of any one of Embodiments 38-47, wherein the immunogenic composition induces an antibody response in the subject against the Group A Streptococcus (GAS) bacterium and does not induce an antibody response in the subject against human tissue.

[0159] Embodiment 49: The method of any one of Embodiments 38-48, wherein the immunogenic composition induces a protective immune response against a *Shigella* bacterium in the subject, wherein the Shigella bacterium comprises a polysaccharide with a polyrhmanose backbone.

[0160] Embodiment 50: A method of inducing a protective immune response against a Shigella bacterium in a subject comprising administering the immunogenic composition of any one of Embodiments 1-37 to the subject.

[0161] Embodiment 51: Use of the immunogenic composition of any one of Embodiments 1-37 for inducing a protective immune response against a *Shigella* bacterium in a subject.

[0162] Embodiment 52: Use of the immunogenic composition of any one of Embodiments 1-37 in the manufacture of a medicament for inducing a protective immune response against a *Shigella* bacterium in a subject.

[0163] Embodiment 53: The method or use of any one of Embodiments 50-52, wherein the subject is 18 years or older.

[0164] Embodiment 54: The method or use of Embodiment 53, wherein the subject is less than 18 years old.

[0165] Embodiment 55: The method or use of Embodiment 54, wherein the subject is between 5 years and 17 years old, between 6 months and 9 years old, or between 5 years and 9 years old.

[0166] Embodiment II-1. An immunogenic composition comprising

a. a first Group A Streptococcus (GAS) polypeptide antigen;

b. a second GAS polypeptide antigen; and

c. at least one polypeptide-polysaccharide conjugate, wherein the conjugate polypeptide is a third GAS polypeptide antigen or a non-GAS carrier polypeptide and comprises at least one non-natural amino acid, and wherein the conjugate polysaccharide is a GAS polysaccharide or a variant thereof that lacks an immunodominant N-acetyl Glucosamine (GlcNAc) side chain.

[0167] Embodiment II-2. The immunogenic composition of embodiment II-1, wherein at least one of the GAS polypeptide antigens is a full length GAS antigen.

[0168] Embodiment II-3. The immunogenic composition of embodiment II-1, wherein at least one of the GAS polypeptide antigens is a peptide fragment of a full length GAS antigen.

[0169] Embodiment II-4. The immunogenic composition of any one of embodiments II-1 to II-3, wherein the first and second GAS polypeptide antigens are independently selected from C5a peptidase, streptolysin O (SLO), Sib35, and Sfb1.

[0170] Embodiment II-5. The immunogenic composition of any one of embodiments II-1 to II-3, wherein the first and second GAS polypeptide antigens are C5a peptidase and SLO.

[0171] Embodiment II-6. The immunogenic composition of embodiment II-5, wherein the C5a peptidase polypeptide antigen comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 1 or SEQ ID NO: 3.

[0172] Embodiment II-7. The immunogenic composition of embodiment II-5, wherein the C5a peptidase polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 3.

[0173] Embodiment II-8. The immunogenic composition of any one of embodiments II-1 to II-6 wherein the first and second GAS polypeptide antigens are C5a peptidase and SLO, and the C5a peptidase polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 29 or SEQ ID NO: 30.

[0174] Embodiment II-9. The immunogenic composition of embodiment II-5, wherein the SLO polypeptide antigen comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 5 or SEQ ID NO: 7.

[0175] Embodiment II-10. The immunogenic composition of any one of embodiments II-1 to II-6 or II-9, wherein the first and second GAS polypeptide antigens are C5a peptidase and SLO, and the SLO polypeptide antigen comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53.

[0176] Embodiment II-11. The immunogenic composition of embodiment II-5, wherein the SLO polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 7.

[0177] Embodiment II-12. The immunogenic composition of any one of embodiments II-1 to II-6, wherein the first and second GAS polypeptide antigens are C5a peptidase and SLO, and the SLO polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 31, SEQ

ID NO: 32, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53.

[0178] Embodiment II-13. The immunogenic composition of any one of embodiments II-1 to II-12, wherein the non-GAS carrier polypeptide is ferritin.

[0179] Embodiment II-14. The immunogenic composition of any one of embodiments II-1 to II-13, wherein the third GAS polypeptide antigen is selected from Streptococcus pyogenes Adhesion and Division (SpyAD) polypeptide, arginine deiminase (ADI), eCRM, and Protein D.

[0180] Embodiment II-15. The immunogenic composition of any one of embodiments II-1 to II-14, wherein the at least one nnAA is substituted for a lysine, a leucine, or an isoleucine in the conjugate polypeptide.

[0181] Embodiment II-16. The immunogenic composition of any one of embodiments II-1 to II-14, wherein the nnAA comprises a click chemistry reactive group.

[0182] Embodiment II-17. The immunogenic composition of embodiment II-16, wherein the nnAA is selected from 2-amino-3-(4-azidophenyl)propanoic acid (pAF), 2-amino-4-azidobutanoic acid, 2-azido-3-phenylpropionic acid, 2-amino-3-azidopropanoic acid, 2-amino-3-(4-(azidomethyl)phenyl)propanoic acid (pAMF), 2-amino-3-(5-(azidomethyl)pyridin-2-yl)propanoic acid, 2-amino-3-(4-(azidomethyl)pyridin-2-yl)propanoic acid, 2-amino-3-(6-(azidomethyl)pyridin-3-yl)propanoic acid, and 2-amino-5-azidopentanoic acid.

[0183] Embodiment II-18. The immunogenic composition of embodiment II-16, wherein the nnAA is pAMF.

[0184] Embodiment II-19. The immunogenic composition of any one of embodiments II-15 to II-18, wherein the conjugate polypeptide is a third GAS protein, which is a SpyAD polypeptide and comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 9.

[0185] Embodiment II-20. The immunogenic composition of embodiment II-19, wherein the SpyAD polypeptide comprises a pAMF substitution at positions K64, K287, K386, and K657 of SEQ ID NO: 9.

[0186] Embodiment II-21. The immunogenic composition of embodiment II-20, wherein the SpyAD polypeptide comprises the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 11.

[0187] Embodiment II-22. The immunogenic composition of any one of embodiments II-15 to II-18, wherein the conjugate polypeptide is a third GAS protein, which is a SpyAD polypeptide and comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 33.

[0188] Embodiment II-23. The immunogenic composition of embodiment II-22, wherein the SpyAD polypeptide comprises a pAMF substitution at positions K64, K287, K386, and K657 of SEQ ID NO: 33.

[0189] Embodiment II-24. The immunogenic composition of embodiment II-23, wherein the SpyAD polypeptide comprises the amino acid sequence of SEQ ID NO: 33 or SEQ ID NO: 34.

[0190] Embodiment II-25. The immunogenic composition of any one of embodiments II-15 to II-18, wherein the conjugate polypeptide is a third GAS polypeptide, which is an ADI polypeptide and comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 13 or SEQ ID NO: 15.

[0191] Embodiment II-26. The immunogenic composition of embodiment II-25, wherein the ADI polypeptide comprises a pAMF substitution at positions K15, K193, and K316 of SEQ ID NO: 13 or SEQ ID NO: 15.

[0192] Embodiment II-27. The immunogenic composition of embodiment II-26, wherein the ADI polypeptide comprises the amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 19.

[0193] Embodiment II-28. The immunogenic composition of any one of embodiments II-15 to II-18, wherein the conjugate polypeptide is a third GAS polypeptide, which is an ADI polypeptide and comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 35 or SEQ ID NO: 36.

[0194] Embodiment II-29. The immunogenic composition of embodiment II-28, wherein the ADI polypeptide comprises a pAMF substitution at positions K15, K193, and K316 of SEQ ID NO: 35 or SEQ ID NO: 36.

[0195] Embodiment II-30. The immunogenic composition of embodiment II-29, wherein the ADI polypeptide comprises the amino acid sequence of SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 37 or SEQ ID NO: 38.

[0196] Embodiment II-31. The immunogenic composition of any one of embodiments II-15 to II-18, wherein the conjugate polypeptide is a ferritin polypeptide and comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 21.

[0197] Embodiment II-32. The immunogenic composition of embodiment II-31, wherein the ferritin polypeptide comprises a pAMF substitution at position I5 of SEQ ID NO: 21.

[0198] Embodiment II-33. The immunogenic composition of embodiment II-32, wherein the ferritin polypeptide comprises the amino acid sequence of SEQ ID NO: 23.

[0199] Embodiment II-34. The immunogenic composition of any one of embodiments II-1 to II-33, wherein the conjugate polysaccharide comprises a polyrhamnose core.

[0200] Embodiment II-35. The immunogenic composition of any one of embodiments II-1 to II-33, wherein the conjugate polysaccharide has an average molecular weight of about 5KDa to about 7 KDa.

[0201] Embodiment II-36. The immunogenic composition of any one of embodiments II-1 to II-35, wherein the conjugate polysaccharide is a tetramer, a hexamer, an octamer, or a decamer polysaccharide.

[0202] Embodiment II-37. An immunogenic composition comprising

a. a Group A Streptococcus (GAS) C5a peptidase polypeptide antigen;

b. a GAS streptolysin O (SLO) polypeptide antigen; and

c. a polypeptide-polysaccharide conjugate comprising a Streptococcus pyogenes Adhesion and Division (SpyAD) conjugate polypeptide and a GAS conjugate polysaccharide or a variant thereof that lacks an immunodominant N-acetyl Glucosamine (GlcNAc) side chain.

[0203] Embodiment II-38. The immunogenic composition of embodiment II-37, wherein the SpyAD protein comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 9 and comprises a pAMF substitution at positions K64, K287, K386, and K657.

[0204] Embodiment II-39. The immunogenic composition of embodiment II-37, wherein the SpyAD protein comprises the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 11.

[0205] Embodiment II-40. The immunogenic composition of embodiment II-37, wherein the SpyAD protein comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 33 and comprises a pAMF substitution at positions K64, K287, K386, and K657.

[0206] Embodiment II-41. The immunogenic composition of embodiment II-37, wherein the SpyAD protein comprises the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 34.

[0207] Embodiment II-42. The immunogenic composition of embodiment II-41, wherein the SLO polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53.

[0208] Embodiment II-43. The immunogenic composition of embodiment II-1 or II-37, comprising a C5a peptidase polypeptide antigen of SEQ ID NO: 30; a SLO polypeptide antigen of SEQ ID NO: 53; and a polypeptide-polysaccharide conjugate comprising a SpyAD conjugate polypeptide of SEQ ID NO: 34 and a GAS conjugate polysaccharide.

[0209] Embodiment II-44. The immunogenic composition of embodiment II-1 or II-37, comprising a C5a peptidase polypeptide antigen of SEQ ID NO: 30; a SLO polypeptide antigen

of SEQ ID NO: 52; and a polypeptide-polysaccharide conjugate comprising a SpyAD conjugate polypeptide of SEQ ID NO: 34 and a GAS conjugate polysaccharide.

[0210] Embodiment II-45. The immunogenic composition of embodiment II-1 or II-37, comprising a C5a peptidase polypeptide antigen of SEQ ID NO: 30; a SLO polypeptide antigen of SEQ ID NO: 32; and a polypeptide-polysaccharide conjugate comprising a SpyAD conjugate polypeptide of SEQ ID NO: 34 and a GAS conjugate polysaccharide.

[0211] Embodiment II-46. The immunogenic composition of embodiment II-1 or II-37, comprising a C5a peptidase polypeptide antigen of SEQ ID NO: 29; a SLO polypeptide antigen of SEQ ID NO: 53; and a polypeptide-polysaccharide conjugate comprising a SpyAD conjugate polypeptide of SEQ ID NO: 34 and a GAS conjugate polysaccharide.

[0212] Embodiment II-47. An immunogenic composition comprising

a. a Group A Streptococcus (GAS) C5a peptidase polypeptide antigen;
b. a GAS streptolysin O (SLO) polypeptide antigen; and
c. a polypeptide-polysaccharide conjugate comprising an arginine deiminase (ADI) conjugate polypeptide and a GAS conjugate polysaccharide or a variant thereof that lacks an immunodominant N-acetyl Glucosamine (GlcNAc) side chain.

[0213] Embodiment II-48. The immunogenic composition of embodiment II-47, wherein the ADI polypeptide comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 13 or SEQ ID NO: 15 and comprises a pAMF substitution at positions K15, K193, and K316.

[0214] Embodiment II-49. The immunogenic composition of embodiment II-47, wherein the ADI polypeptide comprises the amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 19.

[0215] Embodiment II-50. The immunogenic composition of embodiments II-47 to II-48, wherein the ADI polypeptide comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 35 or SEQ ID NO: 36 and comprises a pAMF substitution at positions K15, K193, and K316.

[0216] Embodiment II-51. The immunogenic composition of embodiments II-47 to II-48, wherein the ADI polypeptide comprises the amino acid sequence of SEQ ID NO: 37 or SEQ ID NO: 38.

[0217] Embodiment II-52. An immunogenic composition comprising

a. a Group A Streptococcus (GAS) C5a peptidase polypeptide antigen;

b. a GAS streptolysin O (SLO) polypeptide antigen; and

c. a polypeptide-polysaccharide conjugate comprising a ferritin polypeptide and a GAS conjugate polysaccharide or a variant thereof that lacks an immunodominant N-acetyl Glucosamine (GlcNAc) side chain.

[0218] Embodiment II-53. The immunogenic composition of embodiment II-52, wherein the ferritin protein comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 21 and comprises a pAMF substitution at position I5.

[0219] Embodiment II-54. The immunogenic composition of embodiment II-52, wherein the ferritin protein comprises the amino acid sequence of SEQ ID NO: 23.

[0220] Embodiment II-55. The immunogenic composition of any one of embodiments II-1 to II-54, wherein the composition comprises about 10% to about 50% of the first GAS antigen, about 10% to about 50%of the second GAS antigen, and about 10% to about 50% of the polypeptide-polysaccharide conjugate.

[0221] Embodiment II-56. The immunogenic composition of any one of embodiments II-1 to II-55, further comprising one or more adjuvants selected from alum, saponin, monophosphoryl lipid A (MPL), or combinations thereof.

[0222] Embodiment II-57. A method of inducing a protective immune response against a Group A Streptococcus (GAS) bacterium in a subject comprising administering the immunogenic composition of any one of embodiments II-1 to II-56 to the subject.

[0223] Embodiment II-58. The method of embodiment II-57, wherein the subject is 18 years or older.

[0224] Embodiment II-59. The method of embodiment II-57, wherein the subject is less than 18 years old.

[0225] Embodiment II-60. The method of embodiment II-57, wherein the subject is between 5 years and 17 years old, between 6 months and 9 years old, or between 5 years and 9 years old.

[0226] Embodiment II-61. The method of any one of embodiments II-57 to II-60, wherein the GAS bacterium is of a serotype selected from M1, M2, M3, M4, M6, M11, M12, M22, M28, M75, and M89.

[0227] Embodiment II-62. The method of any one of embodiments II-57 to II-60, wherein the GAS bacterium is a serotype selected from M1, M3, M5, M9, M12, M18, M22, M25, M28, M71, M72, and M74.

[0228] Embodiment II-63. The method of any one of embodiments II-57 to II-60, wherein the GAS bacterium is a serotype selected from M1, M4, M6, M11, M12, M22, M44, M75, M77, M77, and M81.

[0229] Embodiment II-64. The method of any one of embodiments II-57 to II-60, wherein the GAS bacterium is a serotype selected from M1, M2, M3, M4, M6, M9, M12, M18, M22, M75, M77, M89, and M92.

[0230] Embodiment II-65. The method of any one of embodiments II-57 to II-60, wherein the GAS bacterium is a serotype selected from M1, M2, M3, M4, M5, M6, M9, M11, M12, M13, M28, M62, and M89.

[0231] Embodiment II-66. The method of any one of embodiments II-57 to II-60, wherein the GAS bacterium is a serotype selected from M1, M2, M3, M4, M6, M12, M22, M28, M49, M53, M68, M77, M80, M83, M87, M89, and M92.

[0232] Embodiment II-67. The method of any one of embodiments II-57 to II-66, wherein the immunogenic composition induces an antibody response in the subject against the Group A Streptococcus (GAS) bacterium and does not induce an antibody response in the subject against human tissue.

[0233] Embodiment II-68. The method of any one of embodiments II-57 to II-67, wherein the immunogenic composition induces a protective immune response against a Shigella bacterium in the subject, wherein the Shigella bacterium comprises a polysaccharide with a polyrhmanose backbone.

[0234] Embodiment II-69. A method of inducing a protective immune response against a Shigella bacterium in a subject comprising administering the immunogenic composition of any one of embodiments II-1 to II-56 to the subject.

[0235] Embodiment II-70. Use of the immunogenic composition of any one of embodiments II-1 to II-56 for inducing a protective immune response against a Shigella bacterium in a subject.

[0236] Embodiment II-71. Use of the immunogenic composition of any one of embodiments II-1 to II-56 in the manufacture of a medicament for inducing a protective immune response against a Shigella bacterium in a subject.

[0237] Embodiment II-72. The method or use of any one of embodiments II-69 to II-71, wherein the subject is 18 years or older.

[0238] Embodiment II-73. The method or use of embodiment II-72, wherein the subject is less than 18 years old.

[0239] Embodiment II-74. The method or use of embodiment II-73, wherein the subject is between 5 years and 17 years old, between 6 months and 9 years old, or between 5 years and 9 years old.

[0240] Embodiment III-1. An immunogenic composition comprising

a. a first Group A Streptococcus (GAS) polypeptide antigen;

b. a second GAS polypeptide antigen; and

c. at least one polypeptide-polysaccharide conjugate, wherein the conjugate polypeptide is a third GAS polypeptide antigen or a non-GAS carrier polypeptide and comprises at least one non-natural amino acid, and wherein the conjugate polysaccharide is a

GAS polysaccharide or a variant thereof that lacks an immunodominant N-acetyl Glucosamine (GlcNAc) side chain.

[0241] Embodiment III-2. The immunogenic composition of embodiment III-1, wherein at least one of the GAS polypeptide antigens is a full length GAS antigen.

[0242] Embodiment III-3. The immunogenic composition of embodiment III-1, wherein at least one of the GAS polypeptide antigens is a peptide fragment of a full length GAS antigen.

[0243] Embodiment III-4. The immunogenic composition of any one of embodiments III-1 to III-3, wherein the first and second GAS polypeptide antigens are independently selected from C5a peptidase, streptolysin O (SLO), Sib35, Sfb1, and SpyAD.

[0244] Embodiment III-5. The immunogenic composition of any one of embodiments III-1 to III-3, wherein the first and second GAS polypeptide antigens are independently selected from C5a peptidase, streptolysin O (SLO), Sib35, and Sfb1.

[0245] Embodiment III-6. The immunogenic composition of any one of embodiments III-1 to III-3, wherein the first and second GAS polypeptide antigens are C5a peptidase and SLO.

[0246] Embodiment III-7. The immunogenic composition of embodiment III-6, wherein the C5a peptidase polypeptide antigen comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 1 or SEQ ID NO: 3.

[0247] Embodiment III-7A. The immunogenic composition of embodiment III-6, wherein the C5a peptidase polypeptide antigen is at least 95% identical to SEQ ID NO: 1 or SEQ ID NO: 3.

[0248] Embodiment III-8. The immunogenic composition of embodiment III-6, wherein the C5a peptidase polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 3.

[0249] Embodiment III-9. The immunogenic composition of any one of embodiments III-1 to III-6 wherein the first and second GAS polypeptide antigens are C5a peptidase and SLO,

and the C5a peptidase polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 29 or SEQ ID NO: 30.

[0250] Embodiment III-9A. The immunogenic composition of any one of embodiments III-1 to III-6 wherein the first and second GAS polypeptide antigens are C5a peptidase and SLO, and the C5a peptidase polypeptide antigen consisting of the amino acid sequence of SEQ ID NO: 29 or SEQ ID NO: 30.

[0251] Embodiment III-10. The immunogenic composition of embodiment III-6, wherein the SLO polypeptide antigen comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 5 or SEQ ID NO: 7.

[0252] Embodiment III-10A. The immunogenic composition of embodiment III-6, wherein the SLO polypeptide antigen is at least 95% identical to SEQ ID NO: 5 or SEQ ID NO: 7.

[0253] Embodiment III-11. The immunogenic composition of any one of embodiments III-1 to III-7 or III-10, wherein the first and second GAS polypeptide antigens are C5a peptidase and SLO, and the SLO polypeptide antigen comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53.

[0254] Embodiment III-11A. The immunogenic composition of any one of embodiment III-17, III-10, III-7A or III-10A, wherein the first and second GAS polypeptide antigens are C5a peptidase and SLO, and the SLO polypeptide antigen is at least 95% identical to SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53.

[0255] Embodiment III-12. The immunogenic composition of embodiment III-6, wherein the SLO polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 7.

[0256] Embodiment III-12A. The immunogenic composition of embodiment III-6, wherein the SLO polypeptide antigen consisting of the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 7.

[0257] Embodiment III-13. The immunogenic composition of any one of embodiments III-1 to III-7, wherein the first and second GAS polypeptide antigens are C5a peptidase and SLO, and the SLO polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53.

[0258] Embodiment III-13A. The immunogenic composition of any one of embodiments III-1 to III-7 or III-7A, wherein the first and second GAS polypeptide antigens are C5a peptidase and SLO, and the SLO polypeptide antigen consisting of the amino acid sequence of SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53.

[0259] Embodiment III-14. The immunogenic composition of any one of embodiments III-1 to III-3, wherein the first and second GAS polypeptide antigens are C5a peptidase and SpyAD.

[0260] Embodiment III-15. The immunogenic composition of embodiment III-14, wherein the C5a peptidase polypeptide antigen comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 29 or SEQ ID NO: 30.

[0261] Embodiment III-15A. The immunogenic composition of embodiment III-14, wherein the C5a peptidase polypeptide antigen is at least 95% identical to SEQ ID NO: 1 or SEQ ID NO: 3.

[0262] Embodiment III-16. The immunogenic composition of embodiment III-14, wherein the C5a peptidase polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 29 or SEQ ID NO: 30.

[0263] Embodiment III-16A. The immunogenic composition of embodiment III-14, wherein the C5a peptidase polypeptide antigen consisting of the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 3.

[0264] Embodiment III-17. The immunogenic composition of any one of embodiments III-1 to III-16, wherein the non-GAS carrier polypeptide is ferritin.

[0265] Embodiment III-18. The immunogenic composition of any one of embodiments III-1 to III-16, wherein the third GAS polypeptide antigen is selected from Streptococcus pyogenes Adhesion and Division (SpyAD) polypeptide, arginine deiminase (ADI), eCRM, and Protein D.

[0266] Embodiment III-19. The immunogenic composition of any one of embodiments III-1 to III-16, wherein the third GAS polypeptide antigen is selected from Streptococcus pyogenes Adhesion and Division (SpyAD) polypeptide, arginine deiminase (ADI), eCRM, Protein D, and SLO.

[0267] Embodiment III-20. The immunogenic composition of any one of embodiments III-1 to III-19, wherein the at least one nnAA is substituted for a lysine, a leucine, or an isoleucine in the conjugate polypeptide.

[0268] Embodiment III-21. The immunogenic composition of any one of embodiments III-1 to III-19, wherein the nnAA comprises a click chemistry reactive group.

[0269] Embodiment III-22. The immunogenic composition of embodiment III-16, wherein the nnAA is selected from 2-amino-3-(4-azidophenyl)propanoic acid (pAF), 2-amino-4-azidobutanoic acid, 2-azido-3-phenylpropionic acid, 2-amino-3-azidopropanoic acid, 2-amino-3-(4-(azidomethyl)phenyl)propanoic acid (pAMF), 2-amino-3-(5-(azidomethyl)pyridin-2-yl)propanoic acid, 2-amino-3-(4-(azidomethyl)pyridin-2-yl)propanoic acid, 2-amino-3-(6-(azidomethyl)pyridin-3-yl)propanoic acid, and 2-amino-5-azidopentanoic acid.

[0270] Embodiment III-23. The immunogenic composition of embodiment III-21, wherein the nnAA is pAMF.

[0271] Embodiment III-24. The immunogenic composition of any one of embodiments III-20 to III-23, wherein the conjugate polypeptide is a third GAS protein, which is a SpyAD polypeptide and comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 9.

[0272] Embodiment III-24A. The immunogenic composition of any one of embodiments III-20 to III-23, wherein the conjugate polypeptide is a third GAS protein, which is a SpyAD polypeptide is at least 95% identical to SEQ ID NO: 9.

[0273] Embodiment III-25. The immunogenic composition of embodiment III-24, wherein the SpyAD polypeptide comprises a pAMF substitution at positions K64, K287, K386, and K657 of SEQ ID NO: 9.

[0274] Embodiment III-26. The immunogenic composition of embodiment III-25, wherein the SpyAD polypeptide comprises the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 11.

[0275] Embodiment III-26A. The immunogenic composition of embodiment III-25, wherein the SpyAD polypeptide comprises consisting of the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 11.

[0276] Embodiment III-27. The immunogenic composition of any one of embodiments III-20 to III-23, wherein the conjugate polypeptide is a third GAS protein, which is a SpyAD polypeptide and comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 33.

[0277] Embodiment III-27A. The immunogenic composition of any one of embodiments III-20 to III-23, wherein the conjugate polypeptide is a third GAS protein, which is a SpyAD polypeptide and is at least 95% identical to SEQ ID NO: 33.

[0278] Embodiment III-28. The immunogenic composition of embodiment III-27, wherein the SpyAD polypeptide comprises a pAMF substitution at positions K64, K287, K386, and K657 of SEQ ID NO: 33.

[0279] Embodiment III-29. The immunogenic composition of embodiment III-28, wherein the SpyAD polypeptide comprises the amino acid sequence of SEQ ID NO: 33 or SEQ ID NO: 34.

[0280] Embodiment III-30. The immunogenic composition of any one of embodiments III-20 to III-23, wherein the conjugate polypeptide is a third GAS polypeptide, which is an ADI polypeptide and comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 13 or SEQ ID NO: 15.

[0281] Embodiment III-30A. The immunogenic composition of any one of embodiments III-20 to III-23, wherein the conjugate polypeptide is a third GAS polypeptide, which is an ADI polypeptide and is at least 95% identical to SEQ ID NO: 13 or SEQ ID NO: 15.

[0282] Embodiment III-31. The immunogenic composition of embodiment III-30, wherein the ADI polypeptide comprises a pAMF substitution at positions K15, K193, and K316 of SEQ ID NO: 13 or SEQ ID NO: 15.

[0283] Embodiment III-32. The immunogenic composition of embodiment III-31, wherein the ADI polypeptide comprises the amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 19.

[0284] Embodiment III-32A. The immunogenic composition of embodiment III-31, wherein the ADI polypeptide consisting of the amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 19.

[0285] Embodiment III-33. The immunogenic composition of any one of embodiments III-20 to III-23, wherein the conjugate polypeptide is a third GAS polypeptide, which is an ADI polypeptide and comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 35 or SEQ ID NO: 36.

[0286] Embodiment III-33A. The immunogenic composition of any one of embodiments III-20 to III-23, wherein the conjugate polypeptide is a third GAS polypeptide, which is an ADI polypeptide and is at least 95% identical to SEQ ID NO: 35 or SEQ ID NO: 36.

[0287] Embodiment III-34. The immunogenic composition of embodiment III-33, wherein the ADI polypeptide comprises a pAMF substitution at positions K15, K193, and K316 of SEQ ID NO: 35 or SEQ ID NO: 36.

[0288] Embodiment III-35. The immunogenic composition of embodiment III-34, wherein the ADI polypeptide comprises the amino acid sequence of SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 37 or SEQ ID NO: 38.

[0289] Embodiment III-35A. The immunogenic composition of embodiment III-34, wherein the ADI polypeptide consisting of the amino acid sequence of SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 37 or SEQ ID NO: 38.

[0290] Embodiment III-36. The immunogenic composition of any one of embodiments III-20 to III-23, wherein the conjugate polypeptide is a third GAS polypeptide, which is a SLO polypeptide and comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 31, SEQ ID NO: 32, or SEQ ID NO: 53.

[0291] Embodiment III-36A. The immunogenic composition of any one of embodiments III-20 to III-23, wherein the conjugate polypeptide is a third GAS polypeptide, which is a SLO polypeptide and is at least 95% identical to SEQ ID NO: 6, SEQ ID NO: 8, or SEQ ID NO: 53.

[0292] Embodiment III-37. The immunogenic composition of embodiment III-36, wherein the SLO polypeptide comprises 3 or 4 pAMF substitutions at positions selected from K98, K112, R151, K189, K272, K323, K357, K375, K407, or K464.

[0293] Embodiment III-38. The immunogenic composition of embodiment III-36 or III-37, wherein the SLO polypeptide comprises the amino acid sequence of SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, or SEQ ID NO: 64.

[0294] Embodiment III-38A. The immunogenic composition of embodiment III-36 or III-37, wherein the SLO polypeptide consisting of the amino acid sequence of SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, or SEQ ID NO: 64.

[0295] Embodiment III-39. The immunogenic composition of any one of embodiments III-20 to III-23, wherein the conjugate polypeptide is a ferritin polypeptide and comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 21.

[0296] Embodiment III-39A. The immunogenic composition of any one of embodiments III-20 to III-23, wherein the conjugate polypeptide is a ferritin polypeptide and is at least 95% identical to SEQ ID NO: 21.

[0297] Embodiment III-40. The immunogenic composition of embodiment III-39, wherein the ferritin polypeptide comprises a pAMF substitution at position I5 of SEQ ID NO: 21.

[0298] Embodiment III-41. The immunogenic composition of embodiment III-40, wherein the ferritin polypeptide comprises the amino acid sequence of SEQ ID NO: 23.

[0299] Embodiment III-41A. The immunogenic composition of embodiment III-40, wherein the ferritin polypeptide consisting of the amino acid sequence of SEQ ID NO: 23.

[0300] Embodiment III-42. The immunogenic composition of any one of embodiments III-1 to III-41, wherein the conjugate polysaccharide comprises a polyrhamnose core.

[0301] Embodiment III-43. The immunogenic composition of any one of embodiments III-1 to III-41, wherein the conjugate polysaccharide has an average molecular weight of about 5KDa to about 7 KDa.

[0302] Embodiment III-44. The immunogenic composition of any one of embodiments III-1 to III-43, wherein the conjugate polysaccharide is a tetramer, a hexamer, an octamer, or a decamer polysaccharide.

[0303] Embodiment III-45. An immunogenic composition comprising

a. a Group A Streptococcus (GAS) C5a peptidase polypeptide antigen;

b. a GAS streptolysin O (SLO) polypeptide antigen; and

c. a polypeptide-polysaccharide conjugate comprising a Streptococcus pyogenes Adhesion and Division (SpyAD) conjugate polypeptide and a GAS conjugate polysaccharide or a variant thereof that lacks an immunodominant N-acetyl Glucosamine (GlcNAc) side chain.

[0304] Embodiment III-46. The immunogenic composition of embodiment III-45, wherein the SpyAD protein comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 9 and comprises a pAMF substitution at positions K64, K287, K386, and K657.

[0305] Embodiment III-46A. The immunogenic composition of embodiment III-45, wherein the SpyAD protein is at least 95% identical to SEQ ID NO: 9 and comprises a pAMF substitution at positions K64, K287, K386, and K657.

[0306] Embodiment III-47. The immunogenic composition of embodiment III-45, wherein the SpyAD protein comprises the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 11.

[0307] Embodiment III-47A. The immunogenic composition of embodiment III-45, wherein the SpyAD protein consisting of the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 11.

[0308] Embodiment III-48. The immunogenic composition of embodiment III-45, wherein the SpyAD protein comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 33 and comprises a pAMF substitution at positions K64, K287, K386, and K657.

[0309] Embodiment III-48A. The immunogenic composition of embodiment III-45, wherein the SpyAD protein is at least 95% identical to SEQ ID NO: 33 and comprises a pAMF substitution at positions K64, K287, K386, and K657.

[0310] Embodiment III-49. The immunogenic composition of embodiment III-45, wherein the SpyAD protein comprises the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 34.

[0311] Embodiment III-49A. The immunogenic composition of embodiment III-45, wherein the SpyAD protein consisting of the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 34.

[0312] Embodiment III-50. The immunogenic composition of embodiment III-49, wherein the SLO polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53.

[0313] Embodiment III-50A. The immunogenic composition of embodiment III-49 or III-49A, wherein the SLO polypeptide antigen consisting of the amino acid sequence of SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53.

[0314] Embodiment III-51. The immunogenic composition of embodiment III-1 or III-45, comprising a C5a peptidase polypeptide antigen of SEQ ID NO: 30; a SLO polypeptide antigen of SEQ ID NO: 53; and a polypeptide-polysaccharide conjugate comprising a SpyAD conjugate polypeptide of SEQ ID NO: 34 and a GAS conjugate polysaccharide.

[0315] Embodiment III-52. The immunogenic composition of embodiment III-1 or III-45, comprising a C5a peptidase polypeptide antigen of SEQ ID NO: 30; a SLO polypeptide antigen of SEQ ID NO: 52; and a polypeptide-polysaccharide conjugate comprising a SpyAD conjugate polypeptide of SEQ ID NO: 34 and a GAS conjugate polysaccharide.

[0316] Embodiment III-53. The immunogenic composition of embodiment III-1 or III-45, comprising a C5a peptidase polypeptide antigen of SEQ ID NO: 30; a SLO polypeptide antigen of SEQ ID NO: 32; and a polypeptide-polysaccharide conjugate comprising a SpyAD conjugate polypeptide of SEQ ID NO: 34 and a GAS conjugate polysaccharide.

[0317] Embodiment III-54. The immunogenic composition of embodiment III-1 or III-45, comprising a C5a peptidase polypeptide antigen of SEQ ID NO: 29; a SLO polypeptide antigen of SEQ ID NO: 53; and a polypeptide-polysaccharide conjugate comprising a SpyAD conjugate polypeptide of SEQ ID NO: 34 and a GAS conjugate polysaccharide.

[0318] Embodiment III-55. An immunogenic composition comprising

a. a Group A Streptococcus (GAS) C5a peptidase polypeptide antigen;
b. a GAS streptolysin O (SLO) polypeptide antigen; and
c. a polypeptide-polysaccharide conjugate comprising an arginine deiminase (ADI) conjugate polypeptide and a GAS conjugate polysaccharide or a variant thereof that lacks an immunodominant N-acetyl Glucosamine (GlcNAc) side chain.

[0319] Embodiment III-56. The immunogenic composition of embodiment III-55, wherein the ADI polypeptide comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 13 or SEQ ID NO: 15 and comprises a pAMF substitution at positions K15, K193, and K316.

[0320] Embodiment III-56A. The immunogenic composition of embodiment III-55, wherein the ADI polypeptide is at least 95% identical to SEQ ID NO: 13 or SEQ ID NO: 15 and comprises a pAMF substitution at positions K15, K193, and K316.

[0321] Embodiment III-57. The immunogenic composition of embodiment III-55, wherein the ADI polypeptide comprises the amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 19.

[0322] Embodiment III-57A. The immunogenic composition of embodiment III-55, wherein the ADI polypeptide consisting of the amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 19.

[0323] Embodiment III-58. The immunogenic composition of embodiments III-55 to III-56, wherein the ADI polypeptide comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 35 or SEQ ID NO: 36 and comprises a pAMF substitution at positions K15, K193, and K316.

[0324] Embodiment III-58A. The immunogenic composition of any one of embodiments III-55 to III-56 or III-56A, wherein the ADI polypeptide is at least 95% identical to SEQ ID NO: 35 or SEQ ID NO: 36 and comprises a pAMF substitution at positions K15, K193, and K316.

[0325] Embodiment III-59. The immunogenic composition of embodiments III-55 to III-56, wherein the ADI polypeptide comprises the amino acid sequence of SEQ ID NO: 37 or SEQ ID NO: 38.

[0326] Embodiment III-59A. The immunogenic composition of any one of embodiments III-55 to III-56 or III-56A, wherein the ADI polypeptide consisting of the amino acid sequence of SEQ ID NO: 37 or SEQ ID NO: 38.

[0327] Embodiment III-60. An immunogenic composition comprising

a. a Group A Streptococcus (GAS) C5a peptidase polypeptide antigen;

b. a GAS streptolysin O (SLO) polypeptide antigen; and

c. a polypeptide-polysaccharide conjugate comprising a ferritin polypeptide and a GAS conjugate polysaccharide or a variant thereof that lacks an immunodominant N-acetyl Glucosamine (GlcNAc) side chain.

[0328] Embodiment III-61. The immunogenic composition of embodiment III-60, wherein the ferritin protein comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 21 and comprises a pAMF substitution at position I5.

[0329] Embodiment III-61A. The immunogenic composition of embodiment III-60, wherein the ferritin protein is at least 95% identical to SEQ ID NO: 21 and comprises a pAMF substitution at position I5.

[0330] Embodiment III-62. The immunogenic composition of embodiment III-60, wherein the ferritin protein comprises the amino acid sequence of SEQ ID NO: 23.

[0331] Embodiment III-62A. The immunogenic composition of embodiment III-60, wherein the ferritin protein consisting of the amino acid sequence of SEQ ID NO: 23.

[0332] Embodiment III-63. The immunogenic composition of any one of embodiments III-1 to III-62, wherein the composition comprises about 10% to about 50% of the first GAS antigen, about 10% to about 50%of the second GAS antigen, and about 10% to about 50% of the polypeptide-polysaccharide conjugate.

[0333] Embodiment III-64. The immunogenic composition of any one of embodiments III-1 to III-63, further comprising one or more adjuvants selected from alum, saponin, monophosphoryl lipid A (MPL), or combinations thereof.

[0334] Embodiment III-65. A method of inducing a protective immune response against a Group A Streptococcus (GAS) bacterium in a subject comprising administering the immunogenic composition of any one of embodiments III-1-56 to the subject.

[0335] Embodiment III-66. The method of embodiment III-65, wherein the subject is 18 years or older.

[0336] Embodiment III-67. The method of embodiment III-65, wherein the subject is less than 18 years old.

[0337] Embodiment III-68. The method of embodiment III-65, wherein the subject is between 5 years and 17 years old, between 6 months and 9 years old, or between 5 years and 9 years old.

[0338] Embodiment III-69. The method of any one of embodiments III-65 to III-68, wherein the GAS bacterium is of a serotype selected from M1, M2, M3, M4, M6, M11, M12, M22, M28, M75, and M89.

[0339] Embodiment III-70. The method of any one of embodiments III-65 to III-68, wherein the GAS bacterium is a serotype selected from M1, M3, M5, M9, M12, M18, M22, M25, M28, M71, M72, and M74.

[0340] Embodiment III-71. The method of any one of embodiments III-65 to III-68, wherein the GAS bacterium is a serotype selected from M1, M4, M6, M11, M12, M22, M44, M75, M77, M77, and M81.

[0341] Embodiment III-72. The method of any one of embodiments III-65 to III-68, wherein the GAS bacterium is a serotype selected from M1, M2, M3, M4, M6, M9, M12, M18, M22, M75, M77, M89, and M92.

[0342] Embodiment III-73. The method of any one of embodiments III-65 to III-68, wherein the GAS bacterium is a serotype selected from M1, M2, M3, M4, M5, M6, M9, M11, M12, M13, M28, M62, and M89.

[0343] Embodiment III-74. The method of any one of embodiments III-65 to III-68, wherein the GAS bacterium is a serotype selected from M1, M2, M3, M4, M6, M12, M22, M28, M49, M53, M68, M77, M80, M83, M87, M89, and M92.

[0344] Embodiment III-75. The method of any one of embodiments III-65 to III-74, wherein the immunogenic composition induces an antibody response in the subject against the Group A Streptococcus (GAS) bacterium and does not induce an antibody response in the subject against human tissue.

[0345] Embodiment III-76. The method of any one of embodiments III-65 to III-75, wherein the immunogenic composition induces a protective immune response against a Shigella bacterium in the subject, wherein the Shigella bacterium comprises a polysaccharide with a polyrhmanose backbone.

[0346] Embodiment III-77. A method of inducing a protective immune response against a Shigella bacterium in a subject comprising administering the immunogenic composition of any one of embodiments III-1-56 to the subject.

[0347] Embodiment III-78. Use of the immunogenic composition of any one of embodiments III-1 to III-54 for inducing a protective immune response against a Shigella bacterium in a subject.

[0348] Embodiment III-79. Use of the immunogenic composition of any one of embodiments III-1 to III-54 in the manufacture of a medicament for inducing a protective immune response against a Shigella bacterium in a subject.

[0349] Embodiment III-80. The method or use of any one of embodiments III-77 to III-79, wherein the subject is 18 years or older.

[0350] Embodiment III-81. The method or use of embodiment III-80, wherein the subject is less than 18 years old.

[0351] Embodiment III-82. The method or use of embodiment III-81, wherein the subject is between 5 years and 17 years old, between 6 months and 9 years old, or between 5 years and 9 years old.

[0352] Embodiment IV-1. An immunogenic composition comprising

a. a first Group A Streptococcus (GAS) polypeptide antigen;
b. a second GAS polypeptide antigen; and

c. at least one polypeptide-polysaccharide conjugate, wherein the conjugate polypeptide is a third GAS polypeptide antigen or a non-GAS carrier polypeptide and comprises at least one non-natural amino acid, and wherein the conjugate polysaccharide is a GAS polysaccharide or a variant thereof that lacks an immunodominant N-acetyl Glucosamine (GlcNAc) side chain.

[0353] Embodiment IV-2. The immunogenic composition of embodiment IV-1, wherein at least one of the GAS polypeptide antigens is a full length GAS antigen.

[0354] Embodiment IV-3. The immunogenic composition of embodiment IV-1, wherein at least one of the GAS polypeptide antigens is a peptide fragment of a full length GAS antigen.

[0355] Embodiment IV-4. The immunogenic composition of any one of embodiments IV-1 to IV-3, wherein the first and second GAS polypeptide antigens are independently selected from C5a peptidase, streptolysin O (SLO), Sib35, Sfb1, and SpyAD.

[0356] Embodiment IV-5. The immunogenic composition of any one of embodiments IV-1 to IV-3, wherein the first and second GAS polypeptide antigens are independently selected from C5a peptidase, streptolysin O (SLO), Sib35, and Sfb1.

[0357] Embodiment IV-6. The immunogenic composition of any one of embodiments IV-1 to IV-3, wherein the first and second GAS polypeptide antigens are C5a peptidase and SLO.

[0358] Embodiment IV-7. The immunogenic composition of any one of embodiments IV-1 to IV-6, wherein the C5a peptidase polypeptide antigen comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 29 or SEQ ID NO: 30.

[0359] Embodiment IV-8. The immunogenic composition of any one of embodiments IV-1 to IV-5, wherein the C5a peptidase polypeptide antigen is at least 95% identical to SEQ ID NO: 29 or SEQ ID NO: 30.

[0360] Embodiment IV-9. The immunogenic composition of embodiment IV-6, wherein the C5a peptidase polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 29 or SEQ ID NO: 30.

[0361] Embodiment IV-10. The immunogenic composition of any one of embodiments IV-1 to IV-6 wherein the first and second GAS polypeptide antigens are C5a peptidase and SLO, and the C5a peptidase polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 29 or SEQ ID NO: 30.

[0362] Embodiment IV-11. The immunogenic composition of any one of embodiments IV-1 to IV-6 wherein the first and second GAS polypeptide antigens are C5a peptidase and SLO, and the C5a peptidase polypeptide antigen consists of the amino acid sequence of SEQ ID NO: 29 or SEQ ID NO: 30.

[0363] Embodiment IV-12. The immunogenic composition of any one of embodiments IV-1 to IV-11, wherein the SLO polypeptide antigen comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 31 or SEQ ID NO: 32.

[0364] Embodiment IV-13. The immunogenic composition of any one of embodiments IV-1 to IV-11, wherein the SLO polypeptide antigen is at least 95% identical to SEQ ID NO: 31 or SEQ ID NO: 32.

[0365] Embodiment IV-14. The immunogenic composition of any one of embodiments IV-1 to IV-9 , wherein the first and second GAS polypeptide antigens are C5a peptidase and SLO, and the SLO polypeptide antigen comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53.

[0366] Embodiment IV-15. The immunogenic composition of any one of embodiments IV-1 to IV-9, wherein the first and second GAS polypeptide antigens are C5a peptidase and SLO, and the SLO polypeptide antigen is at least 95% identical to SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53.

[0367] Embodiment IV-16. The immunogenic composition of any one of embodiments IV-1 to IV-11, wherein the SLO polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 31 or SEQ ID NO: 32.

[0368] Embodiment IV-17. The immunogenic composition of embodiment IV-6, wherein the SLO polypeptide antigen consists of the amino acid sequence of SEQ ID NO: 31 or SEQ ID NO: 32.

[0369] Embodiment IV-18. The immunogenic composition of any one of embodiments IV-1 to IV-9, wherein the first and second GAS polypeptide antigens are C5a peptidase and SLO, and the SLO polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53.

[0370] Embodiment IV-19. The immunogenic composition of any one of embodiments IV-1 to IV-9, wherein the first and second GAS polypeptide antigens are C5a peptidase and SLO, and the SLO polypeptide antigen consists of the amino acid sequence of SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53.

[0371] Embodiment IV-20. The immunogenic composition of any one of embodiments IV-1 to IV-4, wherein the first and second GAS polypeptide antigens are C5a peptidase and SpyAD.

[0372] Embodiment IV-21. The immunogenic composition of any one of embodiments IV-1 to IV-6, 16-17, or 20, wherein the C5a peptidase polypeptide antigen comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 29 or SEQ ID NO: 30.

[0373] Embodiment IV-22. The immunogenic composition of embodiment IV-20 or IV-21, wherein the C5a peptidase polypeptide antigen is at least 95% identical to SEQ ID NO: 29 or SEQ ID NO: 30.

[0374] Embodiment IV-23. The immunogenic composition of any one of embodiments IV-1 to IV-6, 16-17 or 20, wherein the C5a peptidase polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 29 or SEQ ID NO: 30.

[0375] Embodiment IV-24. The immunogenic composition of any one of embodiments IV-1 to IV-6, 16-17, 20, or 23, wherein the C5a peptidase polypeptide antigen consists of the amino acid sequence of SEQ ID NO: 20 or SEQ ID NO: 30.

[0376] Embodiment IV-25. The immunogenic composition of any one of embodiments IV-1 to IV-24, wherein the non-GAS carrier polypeptide is ferritin.

[0377] Embodiment IV-26. The immunogenic composition of any one of embodiments IV-1 to IV-24, wherein the third GAS polypeptide antigen or non-GAS polypeptide antigen is selected from Streptococcus pyogenes Adhesion and Division (SpyAD) polypeptide, arginine deiminase (ADI), SEQ ID NO: 25, and Protein D.

[0378] Embodiment IV-27. The immunogenic composition of any one of embodiments IV-1 to IV-24, wherein the third GAS polypeptide antigen or non-GAS polypeptide antigen is selected from Streptococcus pyogenes Adhesion and Division (SpyAD) polypeptide, arginine deiminase (ADI), SEQ ID NO: 25, Protein D, and SLO.

[0379] Embodiment IV-28. The immunogenic composition of any one of embodiments IV-1 to IV-27, wherein the at least one nnAA is substituted for a lysine, a leucine, an arginine, or an isoleucine in the conjugate polypeptide.

[0380] Embodiment IV-29. The immunogenic composition of any one of embodiments IV-1 to IV-27, wherein the nnAA comprises a click chemistry reactive group.

[0381] Embodiment IV-30. The immunogenic composition of any one of embodiments IV-1 to IV-29, wherein the nnAA is selected from 2-amino-3-(4-azidophenyl)propanoic acid (pAF), 2-amino-4-azidobutanoic acid, 2-azido-3-phenylpropionic acid, 2-amino-3-azidopropanoic acid, 2-amino-3-(4-(azidomethyl)phenyl)propanoic acid (pAMF), 2-amino-3-(5-(azidomethyl)pyridin-2-yl)propanoic acid, 2-amino-3-(4-(azidomethyl)pyridin-2-yl)propanoic acid, 2-amino-3-(6-(azidomethyl)pyridin-3-yl)propanoic acid, and 2-amino-5-azidopentanoic acid.

[0382] Embodiment IV-31. The immunogenic composition of embodiment IV-29 or IV-30, wherein the nnAA is pAMF.

[0383] Embodiment IV-32. The immunogenic composition of any one of embodiments IV-26 to IV-31, wherein the conjugate polypeptide is a third GAS protein, which is a SpyAD polypeptide and comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 33.

[0384] Embodiment IV-33. The immunogenic composition of any one of embodiments IV-28 to IV-32, wherein the conjugate polypeptide is a third GAS protein, which is a SpyAD polypeptide and is at least 95% identical to SEQ ID NO: 33.

[0385] Embodiment IV-34. The immunogenic composition of any one of embodiments IV-26 to IV-33, wherein the SpyAD polypeptide comprises a pAMF substitution at positions K64, K287, K386, and K657 of SEQ ID NO: 33.

[0386] Embodiment IV-35. The immunogenic composition of any one of embodiments IV-26 to IV-31 or 34, wherein the SpyAD polypeptide comprises the amino acid sequence of SEQ ID NO: 33 or SEQ ID NO: 34.

[0387] Embodiment IV-36. The immunogenic composition of embodiment IV-34, wherein the SpyAD polypeptide consisting of the amino acid sequence of SEQ ID NO: 33 or SEQ ID NO: 34.

[0388] Embodiment IV-37. The immunogenic composition of any one of embodiments IV-26 to IV-31, wherein the conjugate polypeptide is a third GAS polypeptide, which is an ADI polypeptide and comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 35 or SEQ ID NO: 36.

[0389] Embodiment IV-38. The immunogenic composition of any one of embodiments IV-26 to IV-31, wherein the conjugate polypeptide is a third GAS polypeptide, which is an ADI polypeptide and is at least 95% identical to SEQ ID NO: 35 or SEQ ID NO: 36.

[0390] Embodiment IV-39. The immunogenic composition of any one of embodiments IV-26 to IV-31 or 37-38, wherein the ADI polypeptide comprises a pAMF substitution at positions K15, K193, and K316 of SEQ ID NO: 35 or SEQ ID NO: 36.

[0391] Embodiment IV-40. The immunogenic composition of embodiment IV-39, wherein the ADI polypeptide comprises the amino acid sequence of SEQ ID NO: 27, SEQ ID NO: 37 or SEQ ID NO: 38.

[0392] Embodiment IV-41. The immunogenic composition of any one of embodiments IV-26 to IV-31 or 39, wherein the ADI polypeptide consist of the amino acid sequence of SEQ ID NO: 27, SEQ ID NO: 37 or SEQ ID NO: 38.

[0393] Embodiment IV-42. The immunogenic composition of any one of embodiments IV-27 to IV-31, wherein the conjugate polypeptide is a third GAS polypeptide, which is a SLO polypeptide and comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 31, SEQ ID NO: 32, or SEQ ID NO: 53.

[0394] Embodiment IV-43. The immunogenic composition of any one of embodiments IV-27 to IV-31, wherein the conjugate polypeptide is a third GAS polypeptide, which is a SLO polypeptide and is at least 95% identical to SEQ ID NO: 31, SEQ ID NO: 32, or SEQ ID NO: 53.

[0395] Embodiment IV-44. The immunogenic composition of embodiment IV-42 or IV-43, wherein the SLO polypeptide comprises 3 or 4 pAMF substitutions at positions selected from K98, K112, R151, K189, K272, K323, K357, K375, K407, or K464.

[0396] Embodiment IV-45. The immunogenic composition of embodiment IV-44, wherein the SLO polypeptide comprises the amino acid sequence of SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, or SEQ ID NO: 64.

[0397] Embodiment IV-46. The immunogenic composition of embodiment IV-44, wherein the SLO polypeptide consists of the amino acid sequence of SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, or SEQ ID NO: 64.

[0398] Embodiment IV-47. The immunogenic composition of embodiment IV-42 or IV-43, wherein the SLO polypeptide comprises 5, 6, 7, or 8 pAMF substitutions at positions selected from K98, K112, R151, K189, K272, K323, K357, K375, K407, or K464

[0399] Embodiment IV-48. The immunogenic composition of embodiment IV-47, wherein the SLO polypeptide comprises the amino acid sequence of SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, or SEQ ID NO: 76.

[0400] Embodiment IV-49. The immunogenic composition of embodiment IV-47, wherein the SLO polypeptide consists of the amino acid sequence of SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, or SEQ ID NO: 76.

[0401] Embodiment IV-50. The immunogenic composition of any one of embodiments IV-28 to IV-31, wherein the conjugate polypeptide is a ferritin polypeptide and comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 21.

[0402] Embodiment IV-51. The immunogenic composition of any one of embodiments IV-28 to IV-31, wherein the conjugate polypeptide is a ferritin polypeptide and is at least 95% identical to SEQ ID NO: 21.

[0403] Embodiment IV-52. The immunogenic composition of embodiment IV-50 or IV-51, wherein the ferritin polypeptide comprises a pAMF substitution at position I5 of SEQ ID NO: 21.

[0404] Embodiment IV-53. The immunogenic composition of embodiment IV-52, wherein the ferritin polypeptide comprises the amino acid sequence of SEQ ID NO: 23.

[0405] Embodiment IV-54. The immunogenic composition of embodiment IV-52, wherein the ferritin polypeptide consists of the amino acid sequence of SEQ ID NO: 23.

[0406] Embodiment IV-55. The immunogenic composition of any one of embodiments IV-1 to IV-54, wherein the conjugate polysaccharide lacks the immunodominant GlcNAc side chain.

[0407] Embodiment IV-56. The immunogenic composition of any one of embodiments IV-1 to IV-56, wherein the conjugate polysaccharide has an average molecular weight of about 5 kDa to about 7 kDa.

[0408] Embodiment IV-57. The immunogenic composition of any one of embodiments IV-1 to IV-56, wherein the conjugate polysaccharide has an average molecular weight of about 10 kDa to about 45 kDa.

[0409] Embodiment IV-58. The immunogenic composition of any one of embodiments IV-1 to IV-55, wherein the conjugate polysaccharide is a tetramer, a hexamer, an octamer, or a decamer polysaccharide.

[0410] Embodiment IV-59. An immunogenic composition comprising

a. a Group A Streptococcus (GAS) C5a peptidase polypeptide antigen;

b. a GAS streptolysin O (SLO) polypeptide antigen; and

c. a polypeptide-polysaccharide conjugate comprising a Streptococcus pyogenes Adhesion and Division (SpyAD) conjugate polypeptide and a GAS conjugate polysaccharide or a variant thereof that lacks an immunodominant N-acetyl Glucosamine (GlcNAc) side chain.

[0411] Embodiment IV-60. The immunogenic composition of embodiment IV-59, wherein the SpyAD protein comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 33 and comprises a pAMF substitution at positions K64, K287, K386, and K657.

[0412] Embodiment IV-61. The immunogenic composition of embodiment IV-59, wherein the SpyAD protein is at least 95% identical to SEQ ID NO: 33 and comprises a pAMF substitution at positions K64, K287, K386, and K657.

[0413] Embodiment IV-62. The immunogenic composition of embodiments IV-59 to IV-61, wherein the SpyAD protein comprises the amino acid sequence of SEQ ID NO: 34.

[0414] Embodiment IV-63. The immunogenic composition of embodiments IV-59 to IV-61, wherein the SpyAD protein consists of the amino acid sequence of SEQ ID NO: 34.

[0415] Embodiment IV-64. The immunogenic composition of any one of embodiments IV-59 to IV-63, wherein the SLO polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53.

[0416] Embodiment IV-65. The immunogenic composition of any one of embodiments IV-59 to IV-64, wherein the SLO polypeptide antigen consists of the amino acid sequence of SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53.

[0417] Embodiment IV-66. The immunogenic composition of embodiment IV-1 or IV-59, comprising a C5a peptidase polypeptide antigen of SEQ ID NO: 30; a SLO polypeptide antigen of SEQ ID NO: 53; and a polypeptide-polysaccharide conjugate comprising a SpyAD conjugate polypeptide of SEQ ID NO: 34 and a GAS conjugate polysaccharide.

[0418] Embodiment IV-67. The immunogenic composition of embodiment IV-1 or IV-59, comprising a C5a peptidase polypeptide antigen of SEQ ID NO: 30; a SLO polypeptide antigen of SEQ ID NO: 52; and a polypeptide-polysaccharide conjugate comprising a SpyAD conjugate polypeptide of SEQ ID NO: 34 and a GAS conjugate polysaccharide.

[0419] Embodiment IV-68. The immunogenic composition of embodiment IV-1 or IV-59, comprising a C5a peptidase polypeptide antigen of SEQ ID NO: 30; a SLO polypeptide antigen of SEQ ID NO: 32; and a polypeptide-polysaccharide conjugate comprising a SpyAD conjugate polypeptide of SEQ ID NO: 34 and a GAS conjugate polysaccharide.

[0420] Embodiment IV-69. The immunogenic composition of embodiment IV-1 or IV-59, comprising a C5a peptidase polypeptide antigen of SEQ ID NO: 29; a SLO polypeptide antigen of SEQ ID NO: 53; and a polypeptide-polysaccharide conjugate comprising a SpyAD conjugate polypeptide of SEQ ID NO: 34 and a GAS conjugate polysaccharide.

[0421] Embodiment IV-70. An immunogenic composition comprising

a. a Group A Streptococcus (GAS) C5a peptidase polypeptide antigen;

b. a GAS streptolysin O (SLO) polypeptide antigen; and

c. a polypeptide-polysaccharide conjugate comprising an arginine deiminase (ADI) conjugate polypeptide and a GAS conjugate polysaccharide or a variant thereof that lacks an immunodominant N-acetyl Glucosamine (GlcNAc) side chain.

[0422] Embodiment IV-71. The immunogenic composition of embodiment IV-70, wherein the ADI polypeptide comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 35 or SEQ ID NO: 36 and comprises a pAMF substitution at positions K15, K193, and K316.

[0423] Embodiment IV-72. The immunogenic composition of embodiment IV-70, wherein the ADI polypeptide is at least 95% identical to SEQ ID NO: 35 or SEQ ID NO: 36 and comprises a pAMF substitution at positions K15, K193, and K316.

[0424] Embodiment IV-73. The immunogenic composition of embodiment IV-72, wherein the ADI polypeptide comprises the amino acid sequence of SEQ ID NO: 37 or SEQ ID NO: 38.

[0425] Embodiment IV-74. The immunogenic composition of any one of embodiment IV-72, wherein the ADI polypeptide consists of the amino acid sequence of SEQ ID NO: 37 or SEQ ID NO: 38.

[0426] Embodiment IV-75. An immunogenic composition comprising

a. a Group A Streptococcus (GAS) C5a peptidase polypeptide antigen;

b. a GAS streptolysin O (SLO) polypeptide antigen; and

c. a polypeptide-polysaccharide conjugate comprising a ferritin polypeptide and a GAS conjugate polysaccharide or a variant thereof that lacks an immunodominant N-acetyl Glucosamine (GlcNAc) side chain.

[0427] Embodiment IV-76. The immunogenic composition of embodiment IV-75, wherein the ferritin protein comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 21 and comprises a pAMF substitution at position I5.

[0428] Embodiment IV-77. The immunogenic composition of embodiment IV-75, wherein the ferritin protein is at least 95% identical to SEQ ID NO: 21 and comprises a pAMF substitution at position I5.

[0429] Embodiment IV-78. The immunogenic composition of embodiment IV-75, wherein the ferritin protein comprises the amino acid sequence of SEQ ID NO: 23.

[0430] Embodiment IV-79. The immunogenic composition of embodiment IV-75, wherein the ferritin protein consists of the amino acid sequence of SEQ ID NO: 23.

[0431] Embodiment IV-80. The immunogenic composition of any one of embodiments IV-1 to IV-79, wherein the composition comprises about 10% to about 50% of the first GAS antigen, about 10% to about 50% of the second GAS antigen, and about 10% to about 50% of the polypeptide-polysaccharide conjugate.

[0432] Embodiment IV-81. The immunogenic composition of any one of embodiments IV-1 to IV-80, further comprising one or more adjuvants selected from alum, saponin, monophosphoryl lipid A (MPL), or combinations thereof.

[0433] Embodiment IV-82. The immunogenic composition of any one of embodiments IV-1 to IV-81, wherein the polypeptide-polysaccharide conjugate has an average molecular mass of about 10 kDa to about 5000 kDa.

[0434] Embodiment IV-83. A method of inducing a protective immune response against a Group A Streptococcus (GAS) bacterium in a subject comprising administering the immunogenic composition of any one of embodiments IV-1 to IV-82 to the subject.

[0435] Embodiment IV-84. The method of embodiment IV-83, wherein the subject is 18 years or older.

[0436] Embodiment IV-85. The method of embodiment IV-65, wherein the subject is less than 18 years old.

[0437] Embodiment IV-86. The method of embodiment IV-65, wherein the subject is between 5 years and 17 years old, between 6 months and 9 years old, or between 5 years and 9 years old.

[0438] Embodiment IV-87. The method of any one of embodiments IV-83 to IV-86, wherein the GAS bacterium is of a serotype selected from M1, M2, M3, M4, M6, M11, M12, M22, M28, M75, and M89.

[0439] Embodiment IV-88. The method of any one of embodiments IV-83 to IV-86, wherein the GAS bacterium is a serotype selected from M1, M3, M5, M9, M12, M18, M22, M25, M28, M71, M72, and M74.

[0440] Embodiment IV-89. The method of any one of embodiments IV-83 to IV-86, wherein the GAS bacterium is a serotype selected from M1, M4, M6, M11, M12, M22, M44, M75, M77, M77, and M81.

[0441] Embodiment IV-90. The method of any one of embodiments IV-83 to IV-86, wherein the GAS bacterium is a serotype selected from M1, M2, M3, M4, M6, M9, M12, M18, M22, M75, M77, M89, and M92.

[0442] Embodiment IV-91. The method of any one of embodiments IV-83 to IV-86, wherein the GAS bacterium is a serotype selected from M1, M2, M3, M4, M5, M6, M9, M11, M12, M13, M28, M62, and M89.

[0443] Embodiment IV-92. The method of any one of embodiments IV-83 to IV-86, wherein the GAS bacterium is a serotype selected from M1, M2, M3, M4, M6, M12, M22, M28, M49, M53, M68, M77, M80, M83, M87, M89, and M92.

[0444] Embodiment IV-93. The method of any one of embodiments IV-83 to IV-92, wherein the immunogenic composition induces an antibody response in the subject against the Group A Streptococcus (GAS) bacterium and does not induce an antibody response in the subject against human tissue.

[0445] Embodiment IV-94. The method of any one of embodiments IV-83 to IV-93, wherein the immunogenic composition induces a protective immune response against a Shigella

bacterium in the subject, wherein the Shigella bacterium comprises a polysaccharide with a polyrhmanose backbone.

[0446] Embodiment IV-95. A method of inducing a protective immune response against a Shigella bacterium in a subject comprising administering the immunogenic composition of any one of embodiments IV-1 to IV-82 to the subject.

[0447] Embodiment IV-96. Use of the immunogenic composition of any one of embodiments IV-1 to IV-82 for inducing a protective immune response against a Shigella bacterium in a subject.

[0448] Embodiment IV-97. Use of the immunogenic composition of any one of embodiments IV-1 to IV-82 in the manufacture of a medicament for inducing a protective immune response against a Shigella bacterium in a subject.

[0449] Embodiment IV-98. Use of the immunogenic composition of any one of embodiments IV-1 to IV-82 in the manufacture of a medicament for inducing a protective immune response against a GAS bacterium in a subject.

[0450] Embodiment IV-99. Use of the immunogenic composition of any one of embodiments IV-1 to IV-82 for inducing a protective immune response against a GAS bacterium in a subject.

[0451] Embodiment IV-100. The method or use of any one of embodiments IV-93 to IV-99, wherein the subject is 18 years or older.

[0452] Embodiment IV-101. The method or use of embodiment IV-100, wherein the subject is less than 18 years old.

[0453] Embodiment IV-102. The method or use of embodiment IV-100, wherein the subject is between 5 years and 17 years old, between 6 months and 9 years old, or between 5 years and 9 years old.

INCORPORATION BY REFERENCE

[0454] All references, articles, publications, patents, patent publications, and patent applications cited herein are incorporated by reference in their entireties for all purposes. However, mention of any reference, article, publication, patent, patent publication, and patent application cited herein is not, and should not be taken as, an acknowledgment or any form of suggestion that they constitute valid prior art or form part of the common general knowledge in any country in the world.

EXAMPLES

Example 1: Expression and Purification of GAS Polysaccharides

[0455] Experiments were performed to express and purify GAS polysaccharides from GAS bacterial cultures.

[0456] 500 mL starter cultures were prepared from the appropriate recombinant GAS colony, allowing the bacteria to grow for several hours before inoculating a large 1.25 L culture for overnight growth at 37 °C without shaking. The bacteria were pelleted, washing once with PBS. 5 mL of cold, 48% aqueous hydrofluoric acid was added to the cell pellet in a 50 mL conical tube using polystyrene tips, carefully pipetting up and down using a serological pipette to disrupt the pellet. Using a small magnetic stir bar, the mixture was stirred for 48 hours at 4 °C. In a fume hood, with the conical tubes on ice, 45 mL (10x volume of 48% HF) of ice cold Milli-Q® (ultrapure) water was added to the bacterial suspension in preparation for dialysis.

[0457] For dialysis, 4L plastic beakers for dialysis were filled with deionized water. A stir bar was added to each, and the beakers were left to cool, covered, in the cold room. 1000 MWCO Regenerated Cellulose dialysis tubing was cut for each bacteria culture (~17 cm max for 4L beakers). 3 cuts of tubing is needed for six 1.25L cultures. Tubes were washed with DI water and rinsed with Milli-Q® (ultrapure) water before submerging in the beakers containing cold Milli-Q® (ultrapure) water. In a fume hood, the tubing was placed in the beakers before carefully being filled with the diluted bacterial suspension and sealed with a clip. The samples were dialyzed as follows: Day 1: 4 °C with stirring; Day 2: water change, 4 °C, with stirring; Day 3: water change, room temperature, with stirring.

[0458] The contents of the dialysis tubing were transferred to centrifuge tubes and spun at 14,000 rpm for 20 min at 4 °C. 50 mL conical tubes were rinsed with Milli-Q® (ultrapure) water 3x to remove any soluble plastic residues. Up to 30 mL of the supernatant containing polysaccharide was decanted into each tube and frozen in dry ice for 1 hour. The frozen samples were then lyophilized, and the residue was resuspended in 8 mL cold water, with gentle sonication if necessary. 1 mL of 10x proteinase K buffer (500 mM Tris HCl, 10 mM $CaCl_2$) and 12.5 mg of proteinase K per polysaccharide prep was added. The samples were incubated at 56 °C in a hybridization oven overnight, mixing occasionally. The reaction was terminated using 100 µL of 100 mM PMSF in ethanol (5 mM final) and was incubated for 1-2 hours at 37 °C, mixing periodically. The mixture was then dialyzed in DI water with 0.05% sodium azide, with stirring, for 24 hours to remove salts. The resulting crude polysaccharide solution was frozen and lyophilized.

[0459] The crude polysaccharide was purified by size exclusion chromatography using a 100 cm x 1.5 cm Biogel P4 column pre-equilibrated with 7% ethanol, equipped with a spiral collector set for 20.0 min per sample. The crude material was re-suspended in 500 µL of 7% ethanol and centrifuged at 4000 rpm for 5 minutes to remove any insoluble particulate matter. The sample was loaded onto the column, and the preload volume and four subsequent fractions were eluted with 500 µL of 7% ethanol. The column was eluted with 7% ethanol, collecting for about 20 hours. Fractions were analyzed using the phenol-sulfuric acid method to detect the presence of polysaccharide, and samples were read at 490 nm to quantify. Fractions that tested positive in the phenol-sulfuric acid test were pooled and lyophilized to give the final purified PS product.

Example 2: Expression and Purification of pAMF-modified Conjugate Polypeptide

[0460] Experiments were performed to express and purify pAMF-modified conjugate polypeptides from a cell free protein synthesis extract.

[0461] Conjugate polypeptides, for instance, SpyAD (SEQ ID NO: 11 or SEQ ID NO: 34), containing nnAAs (*e.g.*, pAMF) were expressed in a cell free protein synthesis (CFPS) reaction, using an extract (XtractCF$^+$) derived from *E. coli* engineered to produce an orthogonal tRNA for insertion of a nnAA at an amber stop codon. Sample protocols used for cloning, expression,

and purification of these modified conjugate polypeptides may be found, for example, in Kapoor *et al., Biochemistry*, 2018, 57(5), 516-519.

[0462] As an example, controlled large scale antigen expression can be performed using a DASbox mini bioreactor system for 10 h at 25°C with constant 650 rpm stirring, pH 7.2, dissolved oxygen 30%. After 10 h, reactions were harvested and spun down at 15,000 x *g* at 4°C for 30 min, passed through a 0.45 μm filter, filtrate loaded on a 5 ml HisTrap excel column (Cytiva) equilibrated and extensively washed with Buffer A [50 mM Tris, 150 mM M NaCl, 10 mM imidazole] until absorbance returned to baseline (FIG. 17 shows sample gels from the purification of select SLO variants). Proteins were eluted using a 50% Step gradient of Buffer B [50 mM Tris, 150 mM M NaCl, 500 mM Imidazole], and elution fractions pooled, concentrated and incubated with excess purified his6-tagged TEV protease overnight while dialyzing against Buffer A (FIG. 18 shows a sample gel following the TEV cleavage of SLO peptides). The dialyzed cleavage reaction was loaded back onto a pre-equilibrated HisTrap excel 5 ml column (Cytiva) and untagged proteins collected in the flow-through (FT) fractions. Thereafter, the FT was concentrated and loaded onto a Superdex 200 26/60 size exclusion chromatography (SEC) column pre-equilibrated with Buffer S [50 mM Tris pH 8.0, 150 mM NaCl] and purity of eluted fractions assessed by SDS-PAGE gel analysis (FIG. 19 shows the purity of select SLO variants following size exclusion chromatography). Purified SpyAD[4pAMF] was incubated with excess Dibenzocyclooctyne-PEG4-tetramethylrhodamine (DBCO-TAMRA) dye for 1h at room temperature to confirm pAMF sites by SDS-PAGE gel and fluorescence readout was recorded using a Syngene G-box gel imager.

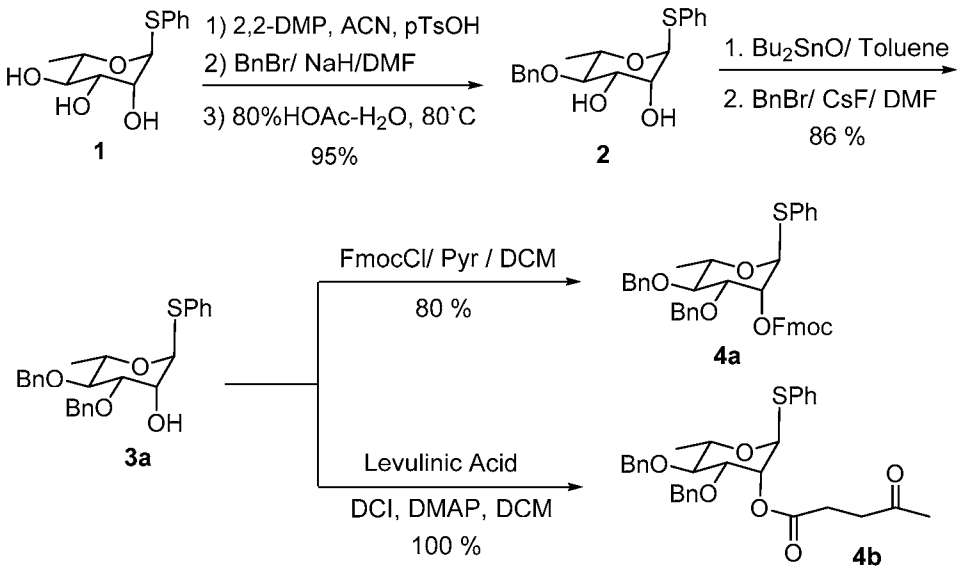
[0463] Polypeptides unmodified by nnAA were prepared using CFPS according to methods similar to the ones described above. FIG. 12 shows the modular architecture of GAS protein antigens and select immunogenic core fragments expressed in CFPS.

[0464] Single site mutagenesis studies were conducted on SpyAD (SEQ ID NO: 9), to understand how replacement of single amino acid residues with pAMF affected expression, as measured in both total protein and soluble protein. FIG. 1A shows the expression levels resulting from a lysine-scanning mutagenesis study. As shown, introduction of pAMF residues at single points in the SpyAD protein had variable effects on the total and soluble protein

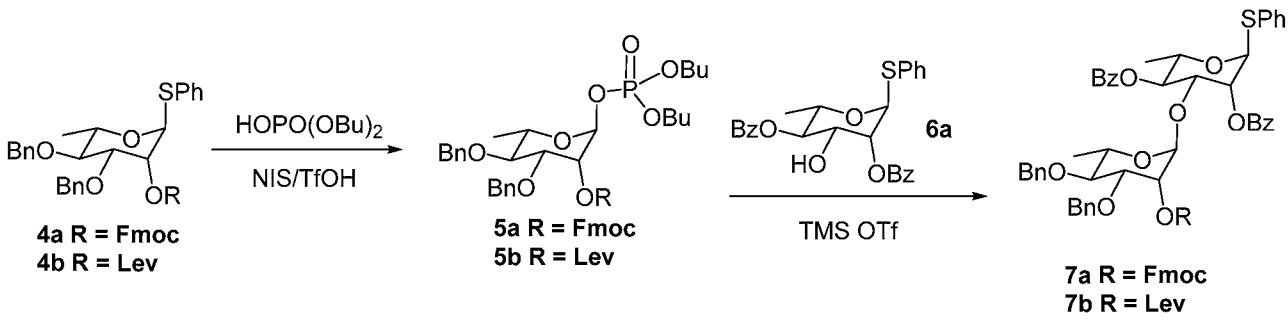
expression. FIG. 1B shows expression levels for antigens and antigen conjugates containing nnAAs. FIG. 13 shows greater than 95% purity of SLO variants, C5a peptidase, and SpyAD as measured by Safe Blue staining, and further shows incorporation of pAMF into SpyAD variant SpyAD[4pAMF] as confirmed by DBCO-TAMRA labeling.

Example 3: Synthesis and Purification of Rhamnose Polysaccharides Modified with Click Chemistry Reactive Groups

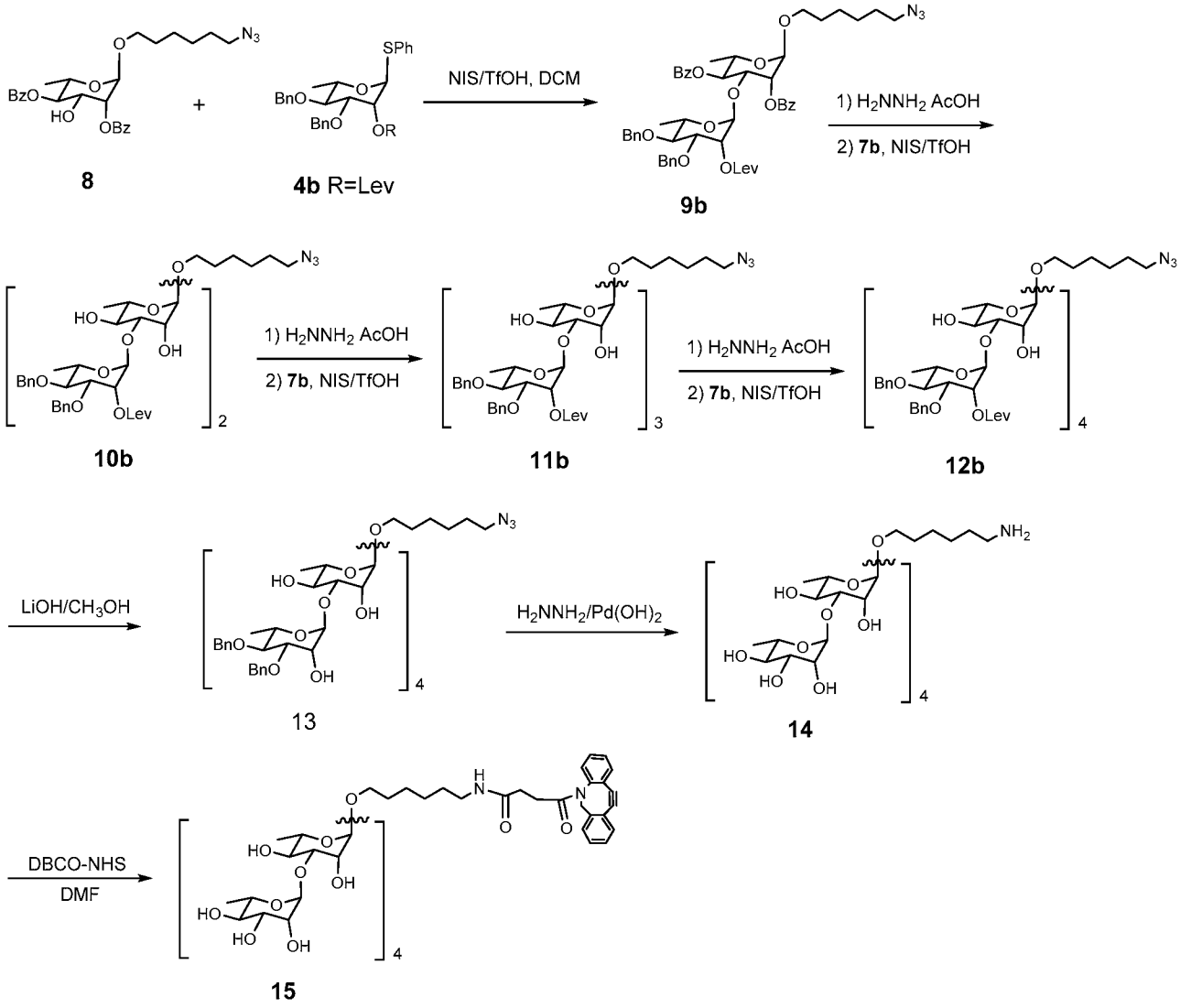
[0465] DBCO-modified polysaccharides were synthesized using a stepwise approach, allowing for tuning of oligomer length.



[0466] *Synthesis of Monosaccharide Intermediates 4a and 4b*: Monomer **1** was mono-benzylated to furnish **2**, which was further benzyl-protected to give intermediate **3a**. Treatment of **3a** with levulinic acid under esterification conditions provides levulinic (Lev)-ester monomer **4a**. Alternatively, Fmoc-protection of **3a** furnished monomer **4b**.

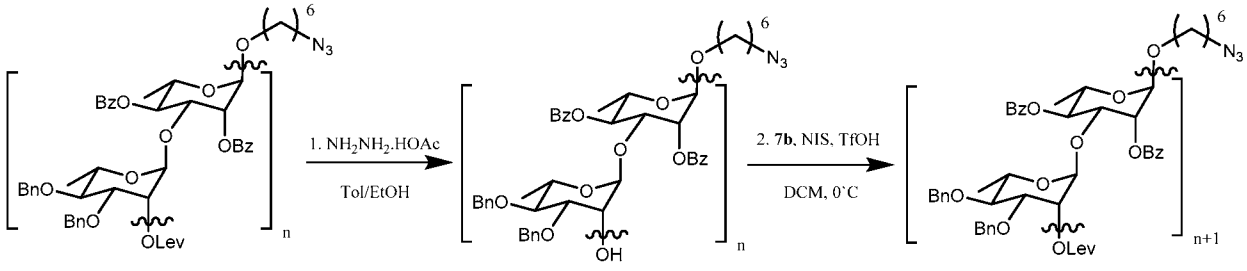


[0467] *Synthesis of Disaccharide Donors 7a and 7b*: Monosaccharide intermediates **4a/b** were converted to phosphate esters **5a/b**, which were subsequently treated with TMSOTf and **6a** to furnish disaccharide donors **7a/b**.



[0468] *Synthesis of Rhamnose Octasaccharide with a Click Chemistry Reactive Group*: Azide intermediate **8** was coupled to monosaccharide intermediate **4b** in the presence of NIS/TfOH to give dimer **9b**, which was then subjected to three sequential deprotection/coupling reaction sequences with disaccharide donor **7b** to give partially protected octamer intermediate **12b**. Treatment of **12b** with LiOH/methanol hydrolyzed the

levulinic ester to give **13**, and further treatment with $H_2NNH_2/Pd(OH)_2$ concomitantly reduced the pendent azide and reductively cleaved the remaining benzyl ethers to furnish **14.** Coupling of **14** with DBCO-NHS gave the final octasaccharide **15**.



[0469] Polysaccharides of varying lengths, for instance the dodecasaccharide and hexasaccharide, can be prepared by altering the number of deprotection/coupling sequences with donor **7b** as shown in the generic reaction scheme above.

[0470] Polysaccharides of the present disclosure can also be synthesized through solid-phase synthesis using the Fmoc-protected derivatives of the above intermediates, originating with monomer **4a**.

Example 4: Linkage of Purified PS to DBCO-PEG Linker

[0471] Purified polysaccharides, for instance those produced by the methods of Example 1, can be functionalized with a DBCO-PEG linker.

[0472] Generally, to a solution of polysaccharide in water (5.5 mM final concentration after all reagents are added), borate buffer (1M, pH 8.5) was added such that the final concentration of borate is 100 mM in the final volume. Water was then added to fill any extra reaction volume. 2.5 equivalents (with respect to the polysaccharide repeating unit) of 1-cyano-4-dimethylaminopyridinium tetra fluoroborate (CDAP; from 100 mg/mL solution in acetonitrile) was added with vigorous stirring. CDAP is stored at -20 °C and solution must be prepared immediately before use. Five minutes after the addition of CDAP (this timing is critical – any longer than 5 min results in reduced DBCO-PEG-Amine incorporation), 0.5 molar equivalents of dibenzocyclooctyne-amine linker (from DMSO stock solution, final concentration of DMSO is 5% v/v) was added. DBCO-PEG4-Amine linker is stored at -20 °C and must be prepared

immediately before use. After one hour of further reaction, glycine (2M, pH 8.35) was added 1:10 by volume to give a final concentration of 200 mM glycine to quench any unreacted cyanate esters. After 1 h of quenching, the derivatized polysaccharide was then purified via Zeba spin column. 2-3 mL of solution was added to each 10 mL Zeba column. The purified polysaccharide was analyzed on Bound/Free DBCO HPLC method to determine if residual DBCO-PEG4-Amine linker and DMAP were completely removed by column purification. The material can be further purified if necessary. The polysaccharide concentration was measured using an anthrone assay, and dibenzocyclooctyne concentration was measured using absorbance at 309 nm. These two values axcyte: were combined to give an estimate of the percentage of polysaccharide derivatized with a dibenzocyclooctyne functional group. Percent DBCO should be between 5-10% for CDAP reactions.

[0473] *DBCO-PEG4 Derivatization of GAC:* To a 6 mM solution of GAC in 100 mM Borate Buffer pH 8.5, three equivalents (to the polysaccharide repeating unit) of 1-cyano-4-dimethylaminopyridinium tetrafluoroborate (CDAP; from 100 mg/mL solution in acetonitrile) were added with vigorous stirring to facilitate cyanylation at reactive hydroxyl groups. 5 minutes after addition of CDAP, 2 molar equivalents of dibenzocyclooctyne-amine linker stock in DMSO was added such that the final DMSO concentration was 5% (v/v). After DBCO-derivatization, 200 mM glycine was added to the reaction to quench unreacted cyanate esters. The DBCO-derivatized polysaccharide was purified via zeba spin desalting column and the purity of the recovered material was assessed by reverse phase. A single peak in HPLC when absorbance was monitored at 309 nm confirmed complete removal of excess DBCO linker and other reaction byproducts. Finally, the polysaccharide concentration was measured using anthrone assay (see below) and dibenzocyclooctyne concentration was measured using absorbance at 309 nm. These two values were combined to give an estimate of the percentage of polysaccharide derivatized with a dibenzocyclooctyne functional group. For conjugation, % DBCO derivatization of the GAC was kept between 5-10%.

[0474] *Anthrone assay for total polysaccharide concentration:* A stock of 2 mg/ml of the anthrone reagent (Sigma-Aldrich, CAS#90-44-8) was prepared in cold sulfuric acid while a 1 mM stock of polysaccharide repeating unit (PSRU) comprising 2x rhamnose was prepared in water as a standard. In triplicate wells, 100 µl of PSRU stock (serially diluted into reference

standards) or the unknown samples (diluted 1:3) were plated (96-well plate) followed by addition of 200 µl / well of the anthrone reagent stock. All reactions were thoroughly mixed and sealed with a plate cover for incubation at 95°C for 10 min. The plate was briefly placed on ice to cool to ambient temperature before absorbance is measured at 620 nm using a UV/VIS plate reader. To determine concentration of unknown samples, PSRU standard concentrations and absorbances were used to generate a least-square fit regression.

Example 5: Conjugation of Carrier Protein to DBCO-derivatized Polysaccharides

[0475] Generally, carrier proteins ferritin (SEQ ID NO: 23), SpyAD (SEQ ID NO: 34), ADI (SEQ ID NO: 27 and SEQ ID NO: 38), SEQ ID NO: 25, and Protein D containing nnAAs (*e.g.*, pAMF) were conjugated to the purified DBCO-derivatized polysaccharides of Example 3 and Example 4 by reacting the cyclooctyne moiety of the DBCO group with the azide moiety of the nnAA side-chain incorporated into the carrier protein. Sample protocols for the conjugation reaction between the DBCO and azide groups may be found, for example, in Zimmerman *et al., Bioconjugate Chemistry*, 2014, 25(2), 351-361 and Kapoor *et al., Biochemistry*, 2018, 57(5), 516-519.

[0476] *Conjugation of pAMF-derivatized GAC to SpyAD:* SpyAD[4pAMF] (SEQ ID NO: 34) was mixed with DBCO-derivatized GAC at a 1:1 ratio [0.5 mg/ml each] to facilitate conjugation via click chemistry. Post-conjugation, the reaction mixture was dialyzed against a 50 kDa cutoff membrane to remove excess unreacted free polysaccharide. The recovered conjugates were analyzed by SEC (multi-angle light scattering) MALS and the concentration was estimated using an anthrone assay. FIG. 9 is a scheme outlining the derivatization of GAC with DBCO-$(PEG)_4$-NH_2 and subsequent conjugation to pAMF-derivatized SpyAD. FIG. 10 shows SEC MALS analysis of purified native GAC, purified native SpyAD[4pAMF], and purified conjugates. SEC-MALS analysis of purified native GAC estimates an average molecular mass of 7.2 kDa. SEC-MALS analysis of purified native SpyAD[4pAMF] estimates an average molecular mass of 87.3 kDa. Post click chemistry SEC-MALS analysis of purified conjugates estimates an average combined molecular mass of 153.4 kDa. Inset shows the safe blue stained SDS-PAGE analysis of SpyAD[4pAMF] pre- and post-conjugation, with no unreacted protein left after conjugation.

[0477] SEC MALS-UV-RI was performed with an Agilent HPLC 1100 degasser, temperature-controlled auto-sampler (4°C), column compartment (25°C) and UV-VIS diode array detector (Agilent, Santa Clara, CA) in line with a DAWN-HELEOS multi-angle laser light scattering detector and Optilab T-rEX differential refractive interferometer (Wyatt Technology, Santa Barbara, CA) coupled to three TOSOH columns in series: TSKgel Guard PWXL 6.0 mm ID x 4.0 cm long, 12 µm particle; TOSOH TSKgel 6000 PWXL 7.8 mm ID x 30 cm long, 13 µm particle; and a TSKgel 3000 PWXL 7.8 mm ID x 30 cm long, 7µm particle. A mobile phase consisting of 0.2 µm filtered 1x PBS with 5% (v/v) acetonitrile was used at a 0.5 mL/min flow rate and 50-100 µg sample was injected for analysis. Agilent Open Lab software was used to control the HPLC, and Wyatt Astra 7 software was used for data collection and molecular weight analysis.

Example 6: Antibody responses induced by GAS antigens and antigen-polysaccharide conjugates

[0478] Experiments were performed to assess the antibody responses produced purified GAS antigens and conjugates thereof. Briefly, rabbits were immunized intramuscularly with 50 µg of each indicated antigen or conjugate or vehicle control on days 0, 14, and 28. Animals were bled prior to immunization (pre-bleed), and on Days 7, 21, and 35 (terminal bleed) after immunizations. Serum was harvested from each blood sample and antibody titers against the immunizing antigens were assessed by ELISA.

[0479] For ELISA analysis of antibody titers, the indicated recombinant protein antigen was plated in sterile PBS at a concentration of 3 µg/well. High-binding, flat bottom 96-well plates were coated with 100 µL of prepared antigen, incubating overnight at room temperature without agitation. The plates were washed 3 times with 100 µL PBS + 0.05% Tween 20 using an Aquamax Microplate Washer, then blotted dry onto paper towels. Wells were blocked with 100 µL of Reagent Diluent 2 for 2 hours. 100 µL of PBS with 1% BSA can also be used. Plates covered with a lid and sealed can be stored in block solution at 4 °C for 3 days. Plates were then washed 3 times with 100 µL PBS + 0.05% Tween 20 as before.

[0480] Sera samples are prepared by diluting the sample into 1x Reagent Diluent 2 (typical dilution is 1:400 to $1:10^6$). 100 µL of the prepared sera was then added to the plates, followed by 2 hours of incubation. Pooled pre-immunization rabbit serum was used as a negative control,

and wells with no rabbit serum were used as a background control. Plates were washed 3 times with 100 µL PBS = 0.05% Tween 20 as before. Plates were then incubated with 100 µL of HRP-conjugated goat-anti rabbit IgG (H+L) secondary antibody (diluted 1:4000 into 1x Reagent Diluent 2) for 2 hours. Plates were then washed 3 times with 100 µL of PBS + 0.05% Tween 20 as before. TMB substrate was prepared following the manufacturer's instructions, and equal volumes of reagent A and reagent B were added at room temperature. 100 µL of prepared TMB substrate was added per well, and the plates were incubated protected from light for no more than 30 minutes. 50 µL of 2N sulfuric acid was added per well to stop the reaction. ELISA plates are read at 450 nm and 540 nm, subtracting the values at 540 nm from the values at 450 nm to correct for background. Titers were determined based on cut off: absorbance value of background controls + 3* standard deviation of background controls.

[0481] As shown in FIG. 2, immunization with ADI (SEQ ID NO: 38), SLO (SEQ ID NO: 32), C5a (SEQ ID NO: 30), SpyCEP, Sib35, SpyAD (SEQ ID NO: 33), and SpyAD-PS conjugates (conjugates of pAMF-substituted SEQ ID NO: 34) elicited a robust antibody response as compared to pre-bleed controls. Sfb1 was also tested but generated weak antibody titers. Each individual antigen identifier in the different panels represents a different rabbit in the experiment.

Example 7: GAS-antigen Elicited Antibody Binding to GAS Serotypes

[0482] Experiments were performed to assess the ability of GAS-antigen induced antibody responses to bind various GAS serotypes. Briefly, rabbits were immunized with 20 µg of SpyAD-PS conjugate or 50 µg of C5a peptidase on days 0, 14, and 28. Animals were bled prior to immunization (pre-immune serum), and on Days 7, 21, and 35 after immunization (immune serum). Serum was harvested from each blood sample.

[0483] All serum samples were heat inactivated at 55° C for 30 minutes. GAS of various serotypes were grown to OD_{600}=0.4, washed and frozen at $2x10^8$ cfu/mL. Frozen GAS stock was thawed, 10% goat serum was added, and the mixture was incubated at room temperature for 1 hour. For the binding assay, 100 µL blocked GAS (together with goat serum) was added to 1.5 mL micro centrifuge tubes. 2 µL pre-immune or immune serum was added to each tube. The samples were mixed well and incubated at room temperature for 1 hour. The samples were spun down at 3500 G for 5 minutes washed 1 time with 500 µL PBS. Samples were then

incubated with a fluorescent secondary anti-rabbit secondary antibody for. The samples were then transferred to FACS tubes in 500 µL PBS. Fluorescent signal intensity was measured by flow cytometry and calculated using FlowJo software, comparing the signal intensity of immune and pre-immune serum.

[0484] FIG. 3A shows the IgG-binding of sera post-immunization with SpyAD-conjugate (conjugate of SEQ ID NO: 34) vs. that of pre-immune sera to strains of M1 (1-21). FIG. 3B shows the IgG-binding of sera post-immunization with C5a Peptidase (SEQ ID NO: 30) vs. that of pre-immune sera to various M1 strains (1-21). The extent of IgG to SpyAD Conjugate and C5a Peptidase binding to all clinically relevant GAS strains is shown in FIG. 3C, with both showing strong binding to > 95% of clinically relevant GAS.

[0485] *Additional Evaluation of GAS Vaccine Antigens for Immunogenicity:* According to the protocols above, animals were immunized with 5 µg of either SLO, C5a peptidase, or SpyAD-GAC conjugate in 240 µL total volume succinate bugger adjuvanted with Adju-phos (Invivogen). The determine immunoglobulin g (IgG) titers elicited by vaccination, terminal bleed (day 35) rabbit antisera were evaluated by ELISA. For all three protein antigens (SpyAD, C5a peptidase, and SLO), the group of rabbits immunized with the recombinant protein showed significantly increased (3- to 4-$\log_{10}$fold) antibody titers against the target antigen compared to either pooled serum from the rabbits before immunization ("pre-immune (pooled)") or the other immunized rabbit groups (FIG. 3D, each point represents serum derived from one animal, run with 2-3 animals per group), confirming specificity of the response to each respective antigen. The anti-SpyAD protein titer generated by the SpyAD-GAC conjugate was not inferior to that of jthe SpyAD recombinant protein alone, suggesting that key B cell epitopes were not impacted by pAMF sites. Flow cytometry was used to evaluate the binding affinity of rabbit-derived IgG to the surface of eight live wild-type GAS strains of different M protein serotypes (m1-6, M12, M28). For the great preponderance of strains, the respective immunized serum yielded an increase in binding of 1000-400% over the baseline IgG binding of the pre-immune serum (FIG. 3E, numbers in the top right corner of each histogram show the mean percentage of increased signal of immunized rabbit serum over pre-immunized serum signal). For six of the eight strains, the GAS surface binding of the antisera raised against the SpyAD-GAC conjugate roughly doubled the level of IgG binding seen with antisera raised against SpyAD

alone. FIG. 3F shows rabbit IgG binding to SpyAD knockout GAS, confirming binding to native GAC.

Example 8: Neutrophil Killing Assay

[0486] Experiments were performed to determine the ability of immune sera from antigen and conjugate-immunized rabbits to elicit neutrophil killing of M1, M3, and M3 strains of GAS. Rabbits were immunized with the indicated antigens according to the protocol described in Example 7. For single antigens, rabbits were immunized with 50 µg of antigen. For conjugate antigens, rabbits were immunized with 20 µg of conjugate.

[0487] *Neutrophil isolation:* 25 mL blood was drawn from healthy volunteers, using 21 G butterfly needles, into vacutainer heparin tubes. Neutrophils were isolated from fresh blood using Polymorphprep following the manufacturer's protocol. All serum, including pre-immune serum, immune serum, and FBS, were heated at 65 °C for 30 minutes with occasional mixing before being used in the assay.

[0488] *Neutrophil Killing Assay Protocol*: Overnight cultures of GAS strains were inoculated in Todd Hewitt Broth (THB) at a ratio of 1:10. When the culture reaches OD_{600}=0.4, the culture was centrifuged and re-suspended in HBSS with 10% pre-immune or immune serum to $5x10^5$ colony forming units (CFU)/mL. GAS strains were kept in RPMI + serum for 30 minutes at room temperature. Prepped neutrophils were re-suspended in HBSS + 4% FBS + 4% Baby Rabbit complement (BRC) in a concentration of $5x10^6$ cells/mL. 100 µL neutrophils ($5x10^5$ cells) were seeded in a 96-well flat bottom plate, with each condition run in triplicate. 100 µL of prepared bacteria ($5x10^4$ cfu/well) were added to each well of neutrophils. The inoculated bacteria were plated on blood agar plate in serial dilutions to later count inoculum cfu. The 96 well plate was centrifuged at 500 G for 5 minutes and then incubated at 37 °C + 5% CO_2 for 30 minutes. Following 30 minutes of incubation, the samples were mixed well, plated on blood agar plates in serial dilutions, and incubated at 37 °C overnight. GAS colonies were counted and the % ratio of inoculum CFU versus recovered CFU is calculated as % survival. Increase in % kill compared to the pre-immune serum group was calculated by first calculating % killing (100-% survival) in both pre-immune and immune serum groups. Then, the % increase in the killing of the immune serum group was calculated compared to the % killing of the pre-immune serum group.

[0489] FIG. 4 shows the ability of serum resulting from immunization by antigens ADI 1625 (SEQ ID NO: 36), ADI conjugate mutant 1807 (conjugate of pAMF-substituted SEQ ID NO: 38), ADI conjugant mutant 1809 (conjugate of SEQ ID NO: 38), SLO 1627 (SEQ ID NO: 32), C5a peptidase 1630 (SEQ ID NO: 30), SpyAD 1642 (SEQ ID NO: 33), SpyAD-conjugate 1644, 1630+1627+1644, 1630+1627+1809, 1630+1627, and M1 to increase killing of GAS serotypes M1, M2, and M3 over pre-immune serum. The pAMF-substituted SpyAD conjugates were made using the peptide of SEQ ID NO: 12, and the ADI conjugates were made using the peptide of SEQ ID NO: 20.

Example 9: GAS antigens and conjugates elicit active immune responses that increase survival after GAS challenge

[0490] Mice were actively immunized prior to being challenged by subdermal and IP injection.

Table 5: Active Immunization Experiment Timeline - Subdermal and IP Challenges

Subdermal Challenge - Steps	IP Challenge - Steps
Day 1: 1st immunization	Day 1: 1st immunization
Day 14: 2nd immunization	Day 14: 2nd immunization
Day 28: 3rd immunization	Day 28: 3rd immunization
Day 42: infection challenge	Day 41: shave mice
Day 49: terminate experiment	Day 42: infection challenge
	Day 45: euthanize mice and collect lesions

[0491] *1st, 2nd, and 3rd Immunizations – Subdermal and IP Challenges:* Antigen/adjuvant mixtures were prepared by combining 50 µL alum (Alhydrogel) with 10 µg antigen(s) or 5 µg of conjugate and mixing rigorously to allow antigens to adsorb onto the alum. Each antigen/adjuvant mixture was drawn into 1 mL syringes fitted with 26 ½ gauge needles. Each mouse was anesthetized with inhaled isoflurane and injected with 100 µL of the prepared vaccine into the hind leg muscle.

[0492] *Preparation of Mice for Challenge – Subdermal Challenge*: Mice were anesthetized with isoflurane. The backs of the mice were shaved with an electric razor, with care taken not

to nick the skin. Hair depilation cream was applied to the shaved backs and was allowed to sit for a few minutes before thoroughly wiping them clean with damp paper towels. The mice were patted dry and allowed to recover from isoflurane treatment.

[0493] *Preparation of Materials for Challenge – Subdermal and IP Challenges*: M1 89155 strain GAS was grown to mid-logarithmic phase. The cell concentration was adjusted with sterile phosphate buffered saline, serially diluting and plating bacteria onto agar to confirm bacterial dose. For Subdermal Challenge, the targeted CFU per 10 µL per mouse was $1x10^6$, and the bacteria was drawn into 500 µL Hamilton syringes fitted with 26 ½ gauge needles. For IP Challenge on day 35, the targeted CFU per 100 µL per mouse is $1x10^7$, and the bacteria was drawn into 1 mL syringes fitted with 26 ½ gauge needles. The mice were anesthetized with inhaled isoflurane and then injected with 200 µL of M1 89155 bacteria into the peritoneal cavity. The mice were allowed to recover from the anesthetic in normal air. Survival of the mice was tracked over the course of 1 week, checking multiple times per day.

[0494] *Subdermal Challenge and Lesion Collection*: For subdermal challenges on day 35, the mice were anesthetized with inhaled isoflurane and then injected with 10 µL of M1 89155 bacteria into the shaved backs using a repeat dispenser for the Hamilton syringe. Lesion sizes were tracked daily over the course of 3 days by photographing isoflurane-anesthetized mice alongside a ruler. Prior to lesion collection on day 3, sterile 2 mL screw cap tubes with 1.0 mm silica beads and 1 mL PBS for each skin lesion were prepared. The weights of each tube were recorded. On day 3, when lesions were fully developed, the mice were euthanized with CO_2 and cervical dislocation. Using clean surgical instruments, each skin lesion was cut out and placed into the pre-weighed tubes. Tube weights are recorded for tissue mass calculations. The tubes were placed into a MagnaLyser bead beater, and the tubes were beat at 6000 rpm for 60 s. The tubes were then placed onto ice to cool for 60 s before repeating the beating cycle. Samples were serially diluted, and the lysate is placed onto agar to quantify bacterial burden.

[0495] Two parallel experiments were performed in which in one set of animals were bled throughout the experiment in order to test for antigen-specific antibody titers post-vaccination. In the other arm, the mice were not bled during the course of the experiment. Both set of animals were challenged similarly in the end to perform the lesion size & CFU/mg analysis. FIG. 5A shows the results of bled-arm active immunization experiments as measured by lesion

size and lesion CFU/mg, demonstrating that the conjugates of the present disclosure perform better than polysaccharides alone. Similarly, FIG. 5B show the results of the no-bleed-arm active immunization experiments as measured by lesion size and lesion CFU/mg, wherein the GAC conjugates of SpyAD (conjugates of pAMF-substituted SEQ ID NO: 34) and ADI (ADImut2 conjugates use pAMF-substituted SEQ ID NO: 27, and ADImut5 conjugates use pAMF-substituted SEQ ID NO: 38) conveyed better protection than the corresponding protein alone. The best protection was conferred by C5a peptidase (SEQ ID NO: 4). FIG. 5C shows the first, second, and third antibody titers after each immunization. FIG. 5D shows survival data for mice that have undergone active immunization and were subsequently subjected to IP challenge. The only group to show some protection was the group administered a 6-antigen combination with 30 μg total protein.

[0496] *Active Immunization with Antigen or Antigen Conjugate Combinations:* As above, mice were actively immunized with one or more antigen or antigen conjugate prior to being challenged by IP injection.

Table 6: Active Immunization Experiment Timeline - Subdermal and IP Challenges

IP Challenge - Steps
Day 1: 1st immunization
Day 14: 2nd immunization
Day 28: 3rd immunization
Day 42: IP challenge

[0497] Mice (n=10 per group) were immunized with one of 8 treatments: mock immunization (PBS + alum); 5 μg of SpyAD-GAC conjugate (conjugate of pAMF-substituted SEQ ID NO: 34); 5 μg of ADImut5-GAC conjugate (conjugate of pAMF-substituted SEQ ID NO: 38); 10 μg of C5a peptidase (SEQ ID NO: 30); 10 μg streptolysin O (SEQ ID NO: 32); a combination of 10 μg of C5a peptidase (SEQ ID NO: 30) and 10 μg streptolysin O (SEQ ID NO: 32); a combination of 10 μg of C5a peptidase(SEQ ID NO: 30), 10 μg streptolysin O (SEQ ID NO: 32), and 5 μg of SpyAD-GAC conjugate (conjugate of pAMF-substituted SEQ ID NO: 34); or a combination of 10 μg of C5a peptidase (SEQ ID NO: 30), 10 μg streptolysin O (SEQ ID NO: 32), and 5 μg of ADImut5-GAC conjugate (conjugate of pAMF-substituted SEQ ID

NO: 38). The SpyAD conjugates were made using the peptide of SEQ ID NO: 12, and the ADImut5 conjugates were made using the peptide of SEQ ID NO: 20.

[0498] FIG. 5E shows the survival of mice actively immunized with single antigens/antigen conjugates or two antigens/antigen conjugates (combination) after IP challenge. Mice immunized with SLO along had the greatest survival, but no individual or dual antigen combination provided 100% protection six days after challenge. Notably, when mice were actively immunized with the triple combinations prior to IP challenge, 100 percent survival was observed after 6 days. These data are shown in FIG. 5F.

Example 10: GAS antigens and conjugates elicit passive immune responses that increase survival after GAS challenge

[0499] Mice were passively immunized prior to being challenged by IP injection.

[0500] Experiments were performed to assess the protection conferred by anti-sera isolated from GAS antigen and GAS conjugate-immunized mice when transferred to mice that were subsequently challenged with GAS. Briefly, rabbits were vaccinated with 5 μg of ADI antigen (SEQ ID NO: 36), C5a antigen (SEQ ID NO: 30), Sfb1 antigen (SEQ ID NO: 28), SpyAD antigen (SEQ ID NO: 33), ADI:GAC conjugates (conjugates of pAMF-substituted SEQ ID NO: 38), or SpyAD:GAC conjugates (conjugates of pAMF-substituted SEQ ID NO: 34) at weeks 0, 3 and 6. Serum was collected from each animal on week 8.

[0501] *Passive Immunization - IP Challenge*: Thawed rabbit serum (approximately 200 uL per mouse), transferred on ice, was drawn into a 1 mL syringes fitted with 30 gauge needles. M1 89155 strain GAS, grown to mid-logarithmic phase, was diluted with sterile phosphate buffered saline to adjust cell concentration. The targeted CFU per 100 μL per mouse was $1x10^7$, and the bacteria was drawn into 1 mL syringes fitted with 26 ½ gauge needles. The rabbit serum was warmed in syringes to room temperature, and the mice were anesthetized with inhaled isoflurane. 200 μL of the rabbit serum was retro-orbitally transferred into each anesthetized mouse. Five minutes after serum transfer, 200 μL of the M1 81955 bacteria was injected into the peritoneal cavity, and the mice were allowed to recover from the isoflurane in normal air. Survival of the mice was tracked over the course of 1 week, checking multiple times per day.

[0502] Survival of mice that have undergone passive immunization and were subjected to subsequent GAS challenge are shown in FIG. 6A and FIG. 6B. All groups treated with antigens and antigen conjugates (pAMF conjugates) of SLO 1627, ADI-conjugate Mut5 1809, SpyAD conjugate 1644, C5a peptidase 1630, SLO+C5a peptidase, SLO+C5a+ADI-conjugate, and SLO+C5a+SpyAD-conjugate showed improved survival over the pre-immune immunized cohort.

Example 11: GAS antigens and conjugates elicit passive immune responses that increase survival after GAS challenge

[0503] Mice were passively immunized prior to being challenged by subdermal injection.

Table 7: Passive Immunization Experiment Timeline - Subdermal Challenge

Subdermal Challenge - Steps
Day -1: prepare mice
Day 0: prepare materials, serum transfer, and challenge
Day 3: lesion collection

[0504] *Preparation of Mice for Challenge – Subdermal Challenge*: Mice were anesthetized with isoflurane. The backs of the mice were shaved with an electric razor, with care taken not to nick the skin. Hair depilation cream was applied to the shaved backs and is allowed to sit for a few minutes before thoroughly wiping them clean with damp paper towels. The mice were patted dry and allowed to recover from isoflurane treatment.

[0505] *Preparation of Materials, Serum Transfer, and Challenge – Subdermal Challenge*: M1 89155 strain GAS was grown to mid-logarithmic phase. The cell concentration was adjusted with sterile phosphate buffered saline, serially diluting and plating bacteria onto agar to confirm bacterial dose. For Subdermal Challenge, the targeted CFU per 10 µL per mouse was $1x10^6$, and the bacteria was drawn into 500 µL Hamilton syringes fitted with 26 ½ gauge needles. Rabbit serum was thawed and transferred on ice before being drawn into 1 mL syringes fitted with 30 gauge needles. The rabbit serum was warmed in syringes to room temperature, and the mice were anesthetized with inhaled isoflurane. 200 µL of the rabbit serum is retro-orbitally transferred into each anesthetized mouse. Five minutes after serum transfer, 10 µL of the M1 81955 bacteria was injected into the shaved backs of the mice using a repeat dispenser,

and the mice were allowed to recover from the isoflurane in normal air. Lesion sizes were tracked daily over the course of 3 days by photographing isoflurane-anesthetized mice alongside a ruler.

[0506] *Lesion Collection for bacterial burden analyses*: Prior to lesion collection on day 3, sterile 2 mL screw cap tubes with 1.0 mm silica beads and 1 mL PBS for each skin lesion were prepared. The weights of each tube were recorded. On day 3, when lesions are fully developed, the mice were euthanized with CO_2 and cervical dislocation. Using clean surgical instruments, each skin lesion was cut out and placed into the pre-weighed tubes. Tube weights were recorded for tissue mass calculations. The tubes were placed into a MagnaLyser bead beater, and the tubes were beat at 6000 rpm for 60 s. The tubes were then placed onto ice to cool for 60 s before repeating the beating cycle. Samples are serially diluted, and the lysate was placed onto agar to quantify bacterial burden.

[0507] Lesion size and bacterial burden data of mice that have undergone passive immunization and were subjected to subdermal challenge with GAS are shown in FIG. 7. Mice (n=10 treated with antigens and antigen conjugates ADI (SEQ ID NO: 36), ADI-conjugate (SEQ ID NO: 38), SLO (SEQ ID NO: 32), C5a peptidase (SEQ ID NO: 30), sfb1 (SEQ ID NO: 28), SpyAD (SEQ ID NO: 33), SpyAD-conjugate (conjugate of pAMF-substituted SEQ ID NO: 34) exhibited reduced lesion size after 48h compared to the pre-immune cohort, and bacterial burden in most arms was significantly lower after 72 hours, with the SpyAD conjugate performing the best at reducing bacterial burden.

Example 12: Human Phase I and Phase IIA Clinical Trial Design

[0508] Initial Phase I clinical studies will be a randomized, placebo-controlled, ascending dose study in healthy adults 18–29 years of age (N=96) (Table 3). Objectives of this initial clinical study will be safety, dose response, and immunogenicity (IgG antibody). Since many individuals at this age range will have pre-existing exposure and immunity to GAS, baseline immunity will be fully evaluated to understand the impact of pre-existing immunity on vaccine responses. IgG response to each vaccine component and OPK antibody titer against a diverse panel of contemporary GAS isolates of different M serotypes will be evaluated.

Table 8: Phase I Clinical Trial Groups

Phase I Clinical Trial Design				
Group	**N**	**Treatment**	**Dose**	**Schedule**
1A	24	GAS vaccine	Low	Day 0, 28
1B	8	Placebo		Day 0, 28
2A	24	GAS vaccine	Mid	Day 0, 28
2B	8	Placebo		Day 0, 28
3A	24	GAS vaccine	High	Day 0, 28
3B	8	Placebo		Day 0, 28

[0509] The Phase 2A clinical study will be a randomized, placebo controlled, multi-center study to evaluate the vaccine in successive cohorts of individuals from 10 to 17 years of age, followed by children 5 to 9 years old (N=96) (Table 4). The objectives of this study will be safety, immunogenicity (IgG antibody responses and opsonophagocytic activity of serum), and an evaluation of preliminary efficacy (incidence of GAS pharyngitis). Each patient will be monitored for 12 months to determine the incidence of strep pharyngitis in the treatment groups.

Table 9: Phase IIA Clinical Trial Groups

Phase IIA Clinical Trial Design				
N	**Age (yr)**	**Treatment**	**Dose**	**Schedule**
36	10-17	GAS vaccine	Determined by PI	Day 0, 28
12	10-17	Placebo		Day 0, 28
36	5-9	GAS vaccine	Mid	Day 0, 28
12	5-9	Placebo		Day 0, 28

Example 13: GAS conjugates induce generation of anti-sera that binds to *S. flexneri 2a* OPS in a cross-reactivity experiment

[0510] Experiments were performed to assess the binding of anti-sera produced by purified GAS conjugates to *S. flexneri 2a* O-antigen polysaccharide (OPS).

[0511] *OPS Purification:* OPS was harvested directly from lipopolysaccharide (LPS) in *Shigella* cell biomass transformed with pSEC10-wzzB plasmid to overexpress wzzB, resulting in increased OPS chain length and conditioned growth media of fermentation (supplemented with amino acids), or shake flask (STm D65) cultures, by reducing the culture pH to 3.5-3.7 with glacial acetic acid, and incubating at 100°C for 4 h in glass bottles submerged in a boiling water bath. Post-hydrolysis supernatants were separated from insoluble material by centrifugation at 10k x g at 4°C for 30 minutes using a GS3 Rotor in a Sorvall RC5 refrigerated centrifuge. The supernatant fraction was brought to 1 M NaCl and filtered by tangential flow microfiltration through a 0.2 μm hollow-fiber filter at 4.5 psi transmembrane pressure (TMP), passing the full volume through, followed by flushing with an equivalent volume of 1 M NaCl. The 0.2 μm-cleared 1 M NaCl permeate was then concentrated 10-fold on a 30 kDa Hydrosart TFF membrane at 14 psi TMP and diafiltered against 35 diavolumes of 1 M NaCl, followed by 10 diavolumes of 50 mM Tris pH 7.

[0512] The retentate fraction in 20 mM Tris pH 7, 50 mM NaCl was then passed through 3 x 3 mL Sartobind NanoQ anion exchange membranes, linked in series, using an AKTA Purifier at 10 mL/min in 20 mM Tris pH 7, 50 mM NaCl. The flow-through fraction was brought to 25% (v/v) ammonium sulfate and incubated overnight at 4°C. Precipitated material was removed by centrifugation at 10k x g / 4°C for 30 min using a GS3 rotor in a Sorvall RC5 refrigerated centrifuge followed by filtration through a 0.45 μm Stericup vacuum filter unit (Millipore, MA). Filtrates were then concentrated 10-fold by TFF with a Slice 200 TFF device using a 10 kDa Hydrosart membrane at 7.5 psi TMP, and diafiltered against 10 diavolumes of de-ionized water. TFF retentates were lyophilized and stored at -20°C until use.

[0513] *Immunization:* Briefly, rabbits were immunized intramuscularly with ADI-mut2, ADI-mut5, and SpyAD conjugates according to the protocols of Example 6.

[0514] *Serum IgG ELISA:* Serum was harvested from each terminal blood draw, and serum IgG antibody titers against *S. flexneri 2a* OPS were assessed by ELISA. A working solution for the 2a OPS antigen was prepared as follows: 5.0 μg/ml of purified 2a OPS was diluted in carbonate coating buffer pH 9.6. Subsequently, Immulon 2HB "U" bottom microtiter plates (Thermo Labsystems #3655) were coated by adding 100 μl of the working solution to each well of a plate. Plates were then incubated at 37°C for 3 h. Following this incubation, plates

were washed six times with PBS-Tween (0.05%) with a two-minute soaking period between washes. Then the plates were blocked overnight at 4° C with 1X PBS containing 10% non-fat dry milk (NFDM) at 250 µl/well. After blocking, the plates were washed again as stated above.

[0515] The test samples (rabbit sera) and pre-bleed controls were diluted in PBS-Tween 10% NFDM and were added to the plates. The specimens and controls were tested in duplicate in a series of dilutions performed on each plate. Plates were incubated for 1 h at 37° C and then washed with PBS-Tween as described above. Next, Horseradish Peroxidase (HRP)-labeled anti-Rabbit IgG (Invitrogen #65-6120) was diluted to 1:2000 in PBS-Tween 10% non-fat dry milk (NFDM). All wells received 100 µl of the appropriate antibody solution and plates were incubated for 1 h at 37°C. Plates were again washed and 100 µl of TMB Microwell Peroxidase Substrate (SeraCare #5120-0047) was added to each well. Plates were incubated at room temperature for 15 minutes in darkness with agitation. The colorimetric reaction was stopped by adding 100 µl of 1M phosphoric acid to all wells. Absorbance values at 450nm were immediately measured using a Multiskan FC™ Microplate Reader.

[0516] FIG. 8 shows the result of the titer analysis. Notably, sera derived from all three GAS conjugates, ADI-mut2 (conjugates of pAMF-substituted SEQ ID NO: 27), ADI-mut5 (conjugates of pAMF-substituted SEQ ID NO: 38), and SpyAD (conjugates of pAMF-substituted SEQ ID NO: 34), exhibited up to approximately 60-fold increase in binding to *S. flexneri 2a* OPS at a 1:10 dilution versus pre-bleed control.

Example 14: GAS conjugates demonstrate bactericidal activity against *S. flexneri* strains in a serum bactericidal assay (SBA)

[0517] Experiments were performed, to determine the ability of immune sera from GAS conjugate-immunized rabbits to elicit neutrophil killing of *S. flexneri* strains. Rabbits were immunized with the indicated conjugates according to the protocol described in Example 7 prior to performing the bactericidal assay. Briefly, the serum bactericidal assay (SBA) and digital image analysis of colonies was performed according to methods previously described (Nahm, et al. mSphere, 2018, 2018 June 13;3(3), pii: e00146-18, and SBA Protocol linked to therein).

[0518] Table 9 shows the bactericidal ability of sera resulting from immunization by SpyAD conjugate 1645 (conjugate of pAMF-substituted SEQ ID NO: 34) and ADI conjugant mutants 1807, 1808, 1809, and 1810 (conjugates of pAMF-substituted SEQ ID NO: 38) against *S. flexneri 2a, 3a, and 6*. Values shown are SBA (serum bactericidal activity) titers, and all five conjugates demonstrated bactericidal activity versus pre-immune control. No killing was observed for *S. sonnei*, and titers in this arm are therefore reported as half of the lowest dilution tested. PnQC19, a human control serum, was used to normalize Shigella SBA titers as previously described (Nahm, et al. mSphere, 2018, 2018 June 13;3(3), pii: e00146-18). Titers shown for *S. flexneri 6* are the average of raw titers (2 experiments). There is no assigned PnQC19 value for *S. flexneri 6*.

Table 10: Serum Bactericidal Activity of Rabbit GAS antibodies

Conjugate	*S. flexneri 2a*	*S. flexneri* 3a	*S. flexneri* 6	*S. sonnei*
pre-immune serum	nd	nd	233	nd
1645	1301	1333	980	30
1807	384	2496	553	30
1808	671	703	394	30
1809	487	1325	1401	30
1810	1654	652	532	30
PnQC19	28000	19000	6915	1100

Example 15: Immunization with SpyAD-GAC does not incite detectable levels of antibodies cross-reactive to human heart epitopes

[0519] Varying amounts of normal adult human heart tissue lysate (Novus Biologicals cat# NB820-59217) with SDS-containing denaturing loading dye were separated by SDS-PAGE using 4-12% Bis-Tris gels before transfer onto a PVDF membrane using the manufacturer's protocol on iBlot (Thermo Fisher). Finally, the blot was blocked at ambient temperature for 1 h, followed by probing with rabbit antisera generated against each of the GAS antigens (diluted 1:1000). After three 30 min washes, HRP conjugated anti-rabbit (Jackson ImmunoResearch Laboratories, Inc., Cat # 211-035-109) secondary antibody (diluted 1:10,000) was added and

chemiluminescence recorded on an Syngene G-Box F3 image scanner after incubation of the blot with the pico substrate (Thermo Fisher Scientific Cat # 34580). All blot blocking, washes, and antibody / serum dilutions were done in TBS + 5% BSA.

[0520] Western blot analysis of the SLO, C5a peptidase and SpyAD-GAC rabbit immune sera on normal human heart lysates separated by polyacrylamide gel electrophoresis was performed. Antisera raised against the intact GAS M1 protein using the same rabbit immunization protocol served as a control. As shown in **FIG. 11**, while anti-M1 antisera reacted to very high molecular weight components of the lysate, no cross-reactivity to the human heart tissue was seen for the SLO (SEQ ID NO: 8), C5a peptidase (SEQ ID NO: 4) and SpyAD-GAC (conjugate of pAMF-substituted SEQ ID NO: 12) antisera, although they recognized the respective cognate GAS protein (or protein carrier in the case of SpyAD-GAC). This analysis is consistent with (a) the lack of sequence homology of SLO, C5a peptidase or SpyAD to human proteins, and (b) knowledge that GAC is comprised solely of repeating rhamnose, a sugar absent in humans, following the genetic deletion of its GlcNAc side chain, which represented a common mammalian sugar epitope.

Example 16: Truncated SLO peptides were expressed and assessed for their immunogenicity in both rabbit and murine models

[0521] Truncated SLO variants were expressed, for instance, according to the above methods. FIG. 15 shows a graphical representation of 10 such variant sequences (ΔC11, ΔC21, ΔC31, ΔC41, ΔC51, ΔC61, ΔC71, ΔC81, ΔC91, and ΔC101), corresponding to SEQ ID NO: 44 to SEQ ID NO: 53 (post-TEV cleavage). FIG. 16 shows the expression of these variants, in both total and soluble protein concentration with their corresponding gels. It is worth noting the differential truncation patterns observed for each variant. Some variants, like ΔC41 and ΔC51 has several closely eluting bands around 50 kDa, whereas variants like ΔC31 or ΔC81 don't show any discernible bands corresponding to their molecular masses.

[0522] FIG. 17 compares expression of several peptides, including truncated SLO variants after initial purification by HisTrap chromatography. Variants ΔC91 and ΔC101 expressed as single distinct bands corresponding to the correct molecular mass of the protein, along with some smaller truncation fragments. FIG. 18 and FIG. 19 compare SLO, and variants ΔC91 and ΔC101, during the steps of TEV cleavage and additional purification by HisTrap (FIG. 18) and size exclusion chromatography (FIG. 19).

[0523] *Generation of immunized rabbit serum:* Rabbits were intramuscularly immunized with alum + 50 μg of SLO, SLO ΔC91, or SLO ΔC101 through protocols similar to those described above. Animals were bled, and an ELISA titer against each respective antigen was performed for sera on days 0, 7, 21, and 35. FIG. 20 shows the ELISA titer for each of the three SLO variants.

[0524] *Immunization with a multivalent glycoconjugate vaccine provides significant protection against systemic and intradermal GAS challenge:* CD-1 mice were actively immunized with intramuscular injections of antigens at days 0, 14, and 28 to examine the contribution of enhanced bacterial clearance to the strong protective efficacy of a 3 component/4 GAS antigen formulation of SpyAD-GAC + SLO(ΔC101) + C5a peptidase. Mouse sera were collected from 9 immunized (and 10 mock immunized) animals at day 42 following the challenge, and the efficacy of these antisera tested for promoting *ex vivo* OPK killing of M1 GAS by freshly isolated human neutrophils (FIG. 21). When GAS were opsonized with post-immune SpyAD-GACPR290 + SLO(ΔC101) + C5a peptidase mouse antisera, ~20% of the original inoculum of M1 GAS was recovered following neutrophil exposure, compared to ~60% CFU recovery in control studies in 292 which GAS were opsonized with mock immune mouse sera (Figure 21, $P < 0.01$). Also, mice immunized with SpyAD-GACPR + SLO(ΔC101) + C5a peptidase or mock (PBS and adjuvant alone) control were challenged using intradermal M1 GAS infection. The size of necrotic lesions generated by the resulting acute inflammatory response did not differ significantly between the two groups (FIGS. 22A and 22B), but the recovered bacterial CFU/gm was reduced more than two-fold in the debrided tissue at the site of infection in the SpyAD-GACPR + SLO(ΔC101) + C5a peptidase immunized group (FIG. 22C, $P = 0.0012$).

[0525] *Red blood cell hemolysis assay to assess the presence of functional antibodies against SLO variants:* Antisera against SLO truncated variants were tested for their ability to block hemolytic activity of the corresponding SLO when added to red blood cells. FIG. 23 shows the results when 0.1 μg/mL of recombinant SLO was added to wells with red blood cells and antisera. Full-length SLO and SLO(ΔC101) anti-sera block cell lysis, while SLO(ΔC91) anti-sera does not block lysis.

[0526] *Evaluation of vaccine antisera in GAS opsonophagocytic killing and blocking SLO activity:* The ability of the rabbit antisera raised against the GAS vaccine antigens (SLO, C5a

peptidase, SpyAD and SpyAD-GAC) to promote human neutrophil opsonophagocytic killing (OPK) was evaluated using GAS strains of five different M protein serotypes (M1-M5). This assay was performed with 30 min pre-opsonization with the respective heat-inactivated antisera or pre-immune sera control, then 30 min exposure to freshly isolated human neutrophils at multiplicity of infection (MOI) = 0.1 bacteria per neutrophil in the presence of 2% baby rabbit complement. All antisera induced a statistically significant increase in short-term OPK of GAS (FIG. 24A,). The assay uses a 100 to 1,000-fold greater GAS inoculum than published OPK assays employing the human promyelocytic leukemia HL-60 cell line at MOI = 0.00140 or MOI = 0.000141, to more accurately recapitulate the immunization target of supporting primary human innate immune cell function. Antisera raised against the SpyAD protein and SpyAD-GAC performed similarly. Moreover, a combination antiserum, composed of one-third of each antiserum raised against SLO, C5a peptidase, and SpyAD-GAC to maintain a consistent total serum concentration, performed similarly or better than the individual components, indicating no cross-253 interference in their OPK functions. As SLO is a secreted cytotoxin not anchored to the GAS surface, its contribution to enhanced neutrophil killing is accrued not from increased phagocytic uptake, but rather reducing SLO-mediated membrane damage and impairment of neutrophil antimicrobial functions. The anti-SLO rabbit immune serum, whether elicited by the SLO or SLO(ΔC101) antigens, significantly inhibited hemolytic activity of purified SLO against human red blood cells up to a dilution of 1:2,048 (FIG. 24B). Furthermore, anti-SLO or anti-SLO(ΔC101) immune serum equally preserved neutrophil oxidative burst function (superoxide generation) against GAS supernatant (SLO)-mediated suppression (FIG. 24C).

[0527] Additional serotypes were tested in an IgG binding assay run similarly to those methods described above. Mouse sera were collected from 9 immunized (and 10 mock immunized) animals on day 42 following the three dose immunization (intramuscular injections of antigens on days 0, 14 and 28), and the efficacy of these antisera tested for IgG binding to live GAS strains via flow cytometry. Serum was collected from female CD-1 mice that were immunized with 3 doses of either mock or combination SpyAD-GACP R + SLO(ΔC101) + C5a peptidase. FIG: 25A and 25B show the fluorescent intensities of murine IgG from mock antisera (light histograms) or multivalent combination antisera (dark histograms) bound to GAS surface antigens of multiple serotypes as quantified via flow

cytometry. Mean fluorescence intensities are summarized in column scatter plots, with each point representing individual mouse serum (circles are mock treatment mice, and square are treatment group mice). Methicillin-resistant Staphylococcus aureus USA300 was added as a control to show GAS-specificity of IgG binding.

Example 17: Truncated SLO(ΔC101) polypeptides with 3 or 4 nnAAs were expressed and conjugated

[0528] Truncated SLO(ΔC101) variants containing nnAAs were expressed, for instance, according to the above methods. The variants contain 3 or 4 pAMF residues, corresponding to SEQ ID NO: 55 to SEQ ID NO: 64. SEQ ID NO: 54 contains all of the nnAA-containing residues. FIG. 26 shows the expression levels of these 10 variants, in both total and soluble protein concentration, with their corresponding gels. Purification proceeded as described previously. In short, The CFPS was harvested, spun down and filtered before loading onto a pre-equilibrated hisTRAP excel 1 ml column at 0.75 ml/min and eluted in a single step of 250 mM imidazole in a 6 ml final volume. 5 ul of each step fraction was analyzed using SDS-PAGE. FIG. 27A shows the gels of the variants after initial purification by hisTRAP. The hisTRAP elution fractions were then diluted 3x in 50 mM Tris pH8 for bring the final salt concentration to 50 mM NaCl before loading onto an AEX column. Both FT and elution fractions were analyzed using the SDS-PAGE analysis to detect presence of each of the variants. FIG. 27B shows gels of the variants after the second purification step. Table 11 shows the final concentration of the expressed variants after recovery from 2-2.5 mL of solution.

Table 11: Final variant concentrations after purification

Protein name	Concentration (mg/ml)
SLO(ΔC101)-var1	0.8
SLO(ΔC101)-var2	1.0
SLO(ΔC101)-var3	0.6
SLO(ΔC101)-var4	1.0
SLO(ΔC101)-var5	1.2
SLO(ΔC101)-var6	2.7
SLO(ΔC101)-var7	2.3
SLO(ΔC101)-var8	1.0
SLO(ΔC101)-var9	2.2
SLO(ΔC101)-var10	1.6

[0529] The SLO (ΔC101) variants were conjugated to DBCO-derivatized GAC as described above. FIG. 28 shows specific conjugation conditions for each variant. The conjugation reactions were analyzed by SDS page, as shown in FIG. 29. The first lane of each gel shows protein pre-conjugation. The final conjugate sizes were determined by SEC-MALS, the results of which are shown in FIG. 30.

Example 18: Truncated SLO(ΔC101) polypeptides with 3 or 4 nnAAs were assessed for their immunogenicity in murine models and for their hemolytic titer

[0530] The 3- and 4-pAMF SLO(ΔC101) variants, and their polysaccharide conjugates, of Example 17 are assessed in murine models by methods as described above in Example 9. Mice are immunized on days 0, 7, and 14, followed by a terminal bleed on day 21 post-sacrifice.

[0531] 5 μg each of the polysaccharide conjugates of pAMF-substituted SLO(ΔC101)-var1, SLO(ΔC101)-var5, SLO(ΔC101)-var6, and SLO(ΔC101)-var10 of Example 17 (corresponding to SEQ ID NO: 55, 59, 60, and 64) were used to immunize mice. The antibody titers against SLO(ΔC101) measured after the terminal bleed are shown in FIG. 31A. The same variants and their GAC conjugates were also evaluated in a hemolysis assay according to the protocols of Example 16. The results are shown in FIG. 31B. FIG. 31C also shows antibody

titers measured after the terminal bleed, however this ELISA utilized a long polysaccharide (produced by the methods of Example 19) as the coating antigen, rather than SLO(ΔC101). Together, these data show that the conjugates of pAMF-substituted variants SLO(ΔC101)-var1, SLO(ΔC101)-var5, SLO(ΔC101)-var6, and SLO(ΔC101)-var10 produce antibodies against both the PS and peptide components, individually.

Example 19: Expression, purification, and conjugation of higher molecular weight (long) polysaccharides

[0532] Experiments were performed to extract and purify GAS polysaccharides from GAS bacterial cultures.

[0533] *Base Hydrolysis:* A prepared GAS cell pellet was re-suspended in 50 mM NaCl solution. Using a serological pipette, 50mL NaCl was added and vortexed until re-suspended. 10N sodium hydroxide & 1M sodium borohydride were added to reach a final concentration of 4N NaOH and 25mM $NaBH_4$. The re-suspended pellet was split between centrifuge bottles with a final target volume of 160mL per 1L fermentation volume. When splitting the solution, constant swirling was used to ensure homogeneity. Bottles were placed on a shaker in the pre-heated incubator at 65°C for 2 hours. After incubation, the hydrolysis solution was centrifuged for 30 min at 14,000 x g at 25°C to pellet any cell debris, making sure to let the hydrolysis sample cool down to room temperature prior to centrifugation. The supernatant was collected once centrifugation stopped, in order not to disturb the pellet, and the supernatant was neutralized to pH 6.5 ± 0.3. When neutralizing, the bottle was placed on a 4°C ice bath, using 37% HCl to adjust pH, with 1M NaOH used for further adjustment if necessary. The sample is then incubated at 4°C overnight.

[0534] *Filtration:* After incubation, a white precipitate forms. The solution was centrifuged for 30 min at 10,000xg. A bulky white pellet is formed, containing host cell proteins (HCPs). The clear supernatant was collected. The pH was adjusted to 3.0 using 37% HCl, and the solution was incubated for 1 hour at RT. Using a Clarisolve Filter µPod -40MS, the sample was filtered. For example: at a pump flow rate of 23mL/min, water was flushed through the filter until primed, and the valve was then opened and flushed with 120mL volume, and equilibrated using 15mM NaCl for a volume of 120 mL. The extract was run through the filter

at the same pump flow rate of 23mL/min, and the clear permeate coming from the filter was collected. The filter was washed with 60mL of 15mM NaCl, and flushed out the tubes and filter content. The filter was discarded. The sample pH was readjusted to 6.5 ± 0.3. Tangential flow filtration (TFF-10k) was then conducted to remove salts, concentrated (ultrafiltered), then buffer exchanged (diafiltered) in 10mM NaCl.

[0535] *Mutanolysin Treatment*: The solution was prepared for mutanolysin treatment by adding 1M $MgCl_2$ to reach a final conc. of 1mM $MgCl_2$, and 200 mM sodium phosphate (10x) to reach a final concentration of 20mM sodium phosphate at pH 6.8. Mutanolysin solution (5000 IU/mL) to reach 120 IU/mL. The sample was incubated at 37°C overnight with shaking.

[0536] *Proteinase-K Treatment*: The sample was then treated with Proteinase-K by adding Proteinase-K solution to achieve a final concentration of 40 IU/mL (Proteinase-K at 45u/mg). The mixture was incubated at 50°C overnight while mixing gently.

[0537] *Precipitation and Filtration*: To precipitate enzymes, nucleic acids and HCPs in the sample, CTAB in 20mM sodium phosphate buffer at pH 6.8 was added, shaking for 1 hour at 30 °C. The solutions used were all pre-warmed: the PS sample, 5% CTAB stock solution and 200mM Na Phosphate pH 6.8. The PS solution was mixed (magnetic stir bar) while being heated, and the heated stir plate was set up with an internal thermometer in order to monitor temperature in the solution at all times. 200mM sodium phosphate (pH 6.8) solution was added to the PS solution, to reach a final concentration of 20mM sodium phosphate. 5% CTAB was added to the PS solution to reach a 1% CTAB concentration. The solution was allowed to mix for 1 hour. Using a 40MS filter, a depth filtration is conducted over the precipitating solution. For example, the system was flushed with 200mL MilliQ H20 at a pump flow rate of 23mL/min, and once water started to come out from the vent, it was closed so the solution is forced to come out from the outlet (priming). The system was then flushed with 75mL of a solution of 20mM sodium phosophate (pH 6.8) and 15mM NaCl. The sample was filtered at 23mL/min, and flushed with 60mL of 20mM sodium phosphate (pH 6.8) and15mM NaCl solution at 20 mL/min. The permeate was collected until air bubbles eluted. The filter was discarded.

[0538] The PS solution and 274 mM KI was warmed to 30°C. Enough 274mM KI was added to the mixing PS solution to achieve a final concentration of 27.4mM KI. The mixture was incubated at 30°C withmixing for 1 hour. The solution was centrifuged post incubation at 30°C, 10,000xg for 30 minutes. The supernatant was collected and the pellet was discarded, be cautious as the pellet breaks up easily. The supernatant was then vacuum filtered through a 0.45µm filter.

[0539] The sample was first concentrated by TFF-10k, followed by diafiltration with 9DVs of 350mM NaCl and finally 2 DVs with MilliQ water. For example, the TFF system volume is around 35mL, with pump flow set at 200mL/min, and the diafiltration is conducted with 9 DV (50mL) of buffer TMP: 7-8 psi

[0540] The polysaccharide solution was then purified by hydrophobic interaction chromatography using HiPrep™ Butyl Fast Flow 16/10 pre-equilibrated with 3M sodium chloride and 50mM sodium phosphate pH 6.8. Sodium chloride and phosphate buffer were added to the polysaccharide solution in order to reach 3M sodium chloride and 50mM potassium phosphate (pH 6.8). The polysaccharide solution was passed over HIC resin and was operated in flow through mode. The resin is washed with the same equilibration buffer and both the flow-through and wash were collected for further processing.

[0541] A final TFF 10kDa/ 3kDa was conducted in order to remove high NaCl content in the PS sample by diafiltration against 9DV of 15mM NaCl or WFI. The purified PS solution was then 0.22 um filtered.

[0542] FIG. 36A, FIG. 36B, and FIG. 36C show NMR analysis of purified polysaccharide produced by Synthetic Example 1, originating from a GAS bacterial strain expressing PS lacking GlcNAc (see PCT/US2012/049604). The NMR confirms the presence of polyrhamnose and the absence of GlcNAc. For sample preparation, the purified polysaccharide solution was lyophilized and exchanged into D_2O with TSP added as an internal standard. A proton NMR spectrum was obtained at 50 °C on a 400 MHz instrument, with signal averaging over 40 scans. 0.2 Hz of line broadening was applied. The purification protocol was also able to produce purified polysaccharide free of M-protein, as evidenced by the western blot of FIG. 37.

[0543] *Derivitization and Conjugation of Long Polysaccharides:* The higher-molecular weight (long) polysaccharides are derivatized with DBCO-PEG as previously described in the above methods (Example 4). The DBCO-derivatized PS is then conjugated to the carrier proteins described herein according to the methods of Example 4. For instance, SpyAD[4pAMF] (SEQ ID NO: 34) was conjugated to the long polysaccharide and analyzed after 3.5 hours and post-dialysis. The SEC-MALS analysis of these conjugates is shown in FIG. 32. After 3.5 hours, conjugates greater than 1 MDa were observed. Post-dialysis, the isolated conjugate size distribution showed a peak at approximately 186 kDa.

Example 20: Truncated SLO(ΔC101) polypeptides with 5, 6, 7, or 8 nnAAs were expressed, purified, and conjugated

[0544] Truncated SLO(ΔC101) variants containing nnAAs are expressed, for instance, according to the above methods. The variants contain 5, 6, 7, or 8 pAMF residues, corresponding to SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, and SEQ ID NO: 76. FIG. 33A shows the expression levels of these 12 pAMF variants (5pAMF-8pAMF), as well as for the variants of example 17, in both total and soluble protein concentration. FIG. 33B shows the corresponding gels for the expression (SEQ ID NOs: 65-76 correspond to var1-var12). Purification proceeded as described previously. In short, The CFPS was harvested, spun down and filtered before loading onto a pre-equilibrated hisTRAP excel 1 ml column at 0.75 ml/min and eluted in a single step of 250 mM imidazole in a 6 ml final volume. 5 µl of each step fraction was analyzed using SDS-PAGE safe blue staining. Thereafter, the elutions were 3x diluted and loaded onto the CaptoQ column for final purification and analyzed using Safe Blue staining and DBCO TAMRA labeling. The FT fractions were concentrated and stored at -80C in 200 µl aliquots.

[0545] *Conjugation of SLO(ΔC101) polypeptides with 5, 6, 7, or 8 nnAAs:* The polypeptides containing 5, 6, 7, or 8 nnAAs are conjugated to GAS polysaccharides using the methods described above, including in Example 4. In this way, the pAMF-containing SLO (ΔC101) variants of SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 68, and SEQ ID NO: 69 were conjugated to long DBCO-derivatized GAC. The conjugation reactions were analyzed by SDS page, as shown in FIG. 34. The first lane for each variant shows the protein pre-conjugation.

The final conjugate sizes were determined by SEC-MALS, the results of which are shown in FIG. 35.

Sequence identifier descriptions

Seq ID	Name/description	Amino acid sequence
1	C5a [90-1035 fragment] WT w/ leader	MHHHHHHGSGENLYFQGKTADTPVTSKATIRDLNDPSQVKTLQE KAGKGAGTVVAVIDAGFDKNHEAWRLTDKTKARYQSKEDLEKAK KEHGITYGEWVNDKVAYYHDYSKDGKTAVDQEHGTHVSGILSGN APSETKEPYRLEGAMPEAQLLLMRVEIVNGLADYARNYAQAIRD AVNLGAKVINMSFGNAALAYANLPDETKKAFDYAKSKGVSIVTS AGNDSSFGGKTRLPLADHPDYGVVGTPAAADSTLTVASYSPDKQ LTETATVKTADKQDKEMPVLSTNRFEPNKAYDYAYANRGMKEDD FKDVKGKIALIERGDIDFKDKIANAKKAGAVGVLIYDNQDKGFP IELPNVDQMPAAFISRKDGLLLKDNPKKTITFNATPKVLPTASG TKLSRFSSWGLTADGNIKPDIAAPGQDILSSVANNKYAKLSGTS MSAPLVAGIMGLLQKQYETQYPDMTPSERLDLAKKVLMSSATAL YDEDEKAYFSPRQQGAGAVDAKKASAATMYVTDKDNTSSKVHLN NVSDTFEVTVTVHNKSDKPQELYYQATVQTDKVDGKHFALAPKA LYETSWQKITIPANSSKQVTVPIDASRFSKDLLAQMKNGYFLEG FVRFKQDPTKEELMSIPYIGFRGDFGNLSALEKPIYDSKDGSSY YHEANSDAKDQLDGDGLQFYALKNNFTALTTESNPWTIIKAVKE GVENIEDIESSEITETIFAGTFAKQDDDSHYYIHRHANGKPYAA ISPNGDGNRDYVQFQGTFLRNAKNLVAEVLDKEGNVVWTSEVTE QVVKNYNNDLASTLGSTRFEKTRWDGKDKDGKVVANGTYTYRVR YTPISSGAKEQHTDFDVIVDNTTPEVATSATFSTEDRRLTLASK PKTSQPVYRERIAYTYMDEDLPTTEYISPNEDGTFTLPEEAETM EGATVPLKMSDFTYVVEDMAGNITYTPVTKLLEGHSNK

Seq ID	Name/description	Amino acid sequence
2	C5a [90-1035 fragment] WT w/o leader	KTADTPVTSKATIRDLNDPSQVKTLQEKAGKGAGTVVAVIDAGF DKNHEAWRLTDKTKARYQSKEDLEKAKKEHGITYGEWVNDKVAY YHDYSKDGKTAVDQEHGTHVSGILSGNAPSETKEPYRLEGAMPE AQLLLMRVEIVNGLADYARNYAQAIRDAVNLGAKVINMSFGNAA LAYANLPDETKKAFDYAKSKGVSIVTSAGNDSSFGGKTRLPLAD HPDYGVVGTPAAADSTLTVASYSPDKQLTETATVKTADKQDKEM PVLSTNRFEPNKAYDYAYANRGMKEDDFKDVKGKIALIERGDID FKDKIANAKKAGAVGVLIYDNQDKGFPIELPNVDQMPAAFISRK DGLLLKDNPKKTITFNATPKVLPTASGTKLSRFSSWGLTADGNI KPDIAAPGQDILSSVANNKYAKLSGTSMSAPLVAGIMGLLQKQY ETQYPDMTPSERLDLAKKVLMSSATALYDEDEKAYFSPRQQGAG AVDAKKASAATMYVTDKDNTSSKVHLNNVSDTFEVTVTVHNKSD KPQELYYQATVQTDKVDGKHFALAPKALYETSWQKITIPANSSK QVTVPIDASRFSKDLLAQMKNGYFLEGFVRFKQDPTKEELMSIP YIGFRGDFGNLSALEKPIYDSKDGSSYYHEANSDAKDQLDGDGL QFYALKNNFTALTTESNPWTIIKAVKEGVENIEDIESSEITETI FAGTFAKQDDDSHYYIHRHANGKPYAAISPNGDGNRDYVQFQGT FLRNAKNLVAEVLDKEGNVVWTSEVTEQVVKNYNNDLASTLGST RFEKTRWDGKDKDGKVVANGTYTYRVRYTPISSGAKEQHTDFDV IVDNTTPEVATSATFSTEDRRLTLASKPKTSQPVYRERIAYTYM DEDLPTTEYISPNEDGTFTLPEEAETMEGATVPLKMSDFTYVVE DMAGNITYTPVTKLLEGHSNK

Seq ID	Name/description	Amino acid sequence
3	C5a [90-1035 fragment] D131A/S513A w/ leader	MHHHHHHGSGENLYFQGKTADTPVTSKATIRDLNDPSQVKTLQE KAGKGAGTVVAVIAAGFDKNHEAWRLTDKTKARYQSKEDLEKAK KEHGITYGEWVNDKVAYYHDYSKDGKTAVDQEHGTHVSGILSGN APSETKEPYRLEGAMPEAQLLLMRVEIVNGLADYARNYAQAIRD AVNLGAKVINMSFGNAALAYANLPDETKKAFDYAKSKGVSIVTS AGNDSSFGGKTRLPLADHPDYGVVGTPAAADSTLTVASYSPDKQ LTETATVKTADKQDKEMPVLSTNRFEPNKAYDYAYANRGMKEDD FKDVKGKIALIERGDIDFKDKIANAKKAGAVGVLIYDNQDKGFP IELPNVDQMPAAFISRKDGLLLKDNPKKTITFNATPKVLPTASG TKLSRFSSWGLTADGNIKPDIAAPGQDILSSVANNKYAKLSGTA MSAPLVAGIMGLLQKQYETQYPDMTPSERLDLAKKVLMSSATAL YDEDEKAYFSPRQQGAGAVDAKKASAATMYVTDKDNTSSKVHLN NVSDTFEVTVTVHNKSDKPQELYYQATVQTDKVDGKHFALAPKA LYETSWQKITIPANSSKQVTVPIDASRFSKDLLAQMKNGYFLEG FVRFKQDPTKEELMSIPYIGFRGDFGNLSALEKPIYDSKDGSSY YHEANSDAKDQLDGDGLQFYALKNNFTALTTESNPWTIIKAVKE GVENIEDIESSEITETIFAGTFAKQDDDSHYYIHRHANGKPYAA ISPNGDGNRDYVQFQGTFLRNAKNLVAEVLDKEGNVVWTSEVTE QVVKNYNNDLASTLGSTRFEKTRWDGKDKDGKVVANGTYTYRVR YTPISSGAKEQHTDFDVIVDNTTPEVATSATFSTEDRRLTLASK PKTSQPVYRERIAYTYMDEDLPTTEYISPNEDGTFTLPEEAETM EGATVPLKMSDFTYVVEDMAGNITYTPVTKLLEGHSNK

Seq ID	Name/description	Amino acid sequence
4	C5a [90-1035 fragment] D131A/S513A w/o leader	KTADTPVTSKATIRDLNDPSQVKTLQEKAGKGAGTVVAVIAAGF DKNHEAWRLTDKTKARYQSKEDLEKAKKEHGITYGEWVNDKVAY YHDYSKDGKTAVDQEHGTHVSGILSGNAPSETKEPYRLEGAMPE AQLLLMRVEIVNGLADYARNYAQAIRDAVNLGAKVINMSFGNAA LAYANLPDETKKAFDYAKSKGVSIVTSAGNDSSFGGKTRLPLAD HPDYGVVGTPAAADSTLTVASYSPDKQLTETATVKTADKQDKEM PVLSTNRFEPNKAYDYAYANRGMKEDDFKDVKGKIALIERGDID FKDKIANAKKAGAVGVLIYDNQDKGFPIELPNVDQMPAAFISRK DGLLLKDNPKKTITFNATPKVLPTASGTKLSRFSSWGLTADGNI KPDIAAPGQDILSSVANNKYAKLSGTAMSAPLVAGIMGLLQKQY ETQYPDMTPSERLDLAKKVLMSSATALYDEDEKAYFSPRQQGAG AVDAKKASAATMYVTDKDNTSSKVHLNNVSDTFEVTVTVHNKSD KPQELYYQATVQTDKVDGKHFALAPKALYETSWQKITIPANSSK QVTVPIDASRFSKDLLAQMKNGYFLEGFVRFKQDPTKEELMSIP YIGFRGDFGNLSALEKPIYDSKDGSSYYHEANSDAKDQLDGDGL QFYALKNNFTALTTESNPWTIIKAVKEGVENIEDIESSEITETI FAGTFAKQDDDSHYYIHRHANGKPYAAISPNGDGNRDYVQFQGT FLRNAKNLVAEVLDKEGNVVWTSEVTEQVVKNYNNDLASTLGST RFEKTRWDGKDKDGKVVANGTYTYRVRYTPISSGAKEQHTDFDV IVDNTTPEVATSATFSTEDRRLTLASKPKTSQPVYRERIAYTYM DEDLPTTEYISPNEDGTFTLPEEAETMEGATVPLKMSDFTYVVE DMAGNITYTPVTKLLEGHSNK

Seq ID	Name/description	Amino acid sequence
5	SLO [79-571 fragment] WT w/ His tag and TEV sequence	MHHHHHHGSGENLYFQGAPKEMPLESAEKEEKKSEDKKKSEEDH TEEINDKIYSLNYNELEVLAKNGETIENFVPKEGVKKADKFIVI ERKKKNINTTPVDISIIDSVTDRTYPAALQLANKGFTENKPDAV VTKRNPQKIHIDLPGMGDKATVEVNDPTYANVSTAIDNLVNQWH DNYSGGNTLPARTQYTESMVYSKSQIEAALNVNSKILDGTLGID FKSISKGEKKVMIAAYKQIFYTVSANLPNNPADVFDKSVTFKEL QRKGVSNEAPPLFVSNVAYGRTVFVKLETSSKSNDVEAAFSAAL KGTDVKTNGKYSDILENSSFTAVVLGGDAAEHNKVVTKDFDVIR NVIKDNATFSRKNPAYPISYTSVFLKNNKIAGVNNRTEYVETTS TEYTSGKINLSHQGAYVAQYEILWDEINYDDKGKEVITKRRWDN NWYSKTSPFSTVIPLGANSRNIRIMARECTGLAWEWWRKVIDER DVKLSKEINVNISGSTLSPYGSITYK
6	SLO [79-571 fragment] WT w/o leader	APKEMPLESAEKEEKKSEDKKKSEEDHTEEINDKIYSLNYNELE VLAKNGETIENFVPKEGVKKADKFIVIERKKKNINTTPVDISII DSVTDRTYPAALQLANKGFTENKPDAVVTKRNPQKIHIDLPGMG DKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYTE SMVYSKSQIEAALNVNSKILDGTLGIDFKSISKGEKKVMIAAYK QIFYTVSANLPNNPADVFDKSVTFKELQRKGVSNEAPPLFVSNV AYGRTVFVKLETSSKSNDVEAAFSAALKGTDVKTNGKYSDILEN SSFTAVVLGGDAAEHNKVVTKDFDVIRNVIKDNATFSRKNPAYP ISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSGKINLSHQGAYV AQYEILWDEINYDDKGKEVITKRRWDNNWYSKTSPFSTVIPLGA NSRNIRIMARECTGLAWEWWRKVIDERDVKLSKEINVNISGSTL SPYGSITYK

Seq ID	Name/description	Amino acid sequence
7	SLO [79-571 fragment] W535A w/ leader	MHHHHHHGSGENLYFQGAPKEMPLESAEKEEKKSEDKKKSEEDH TEEINDKIYSLNYNELEVLAKNGETIENFVPKEGVKKADKFIVI ERKKKNINTTPVDISIIDSVTDRTYPAALQLANKGFTENKPDAV VTKRNPQKIHIDLPGMGDKATVEVNDPTYANVSTAIDNLVNQWH DNYSGGNTLPARTQYTESMVYSKSQIEAALNVNSKILDGTLGID FKSISKGEKKVMIAAYKQIFYTVSANLPNNPADVFDKSVTFKEL QRKGVSNEAPPLFVSNVAYGRTVFVKLETSSKSNDVEAAFSAAL KGTDVKTNGKYSDILENSSFTAVVLGGDAAEHNKVVTKDFDVIR NVIKDNATFSRKNPAYPISYTSVFLKNNKIAGVNNRTEYVETTS TEYTSGKINLSHQGAYVAQYEILWDEINYDDKGKEVITKRRWDN NWYSKTSPFSTVIPLGANSRNIRIMARECTGLAAEWWRKVIDER DVKLSKEINVNISGSTLSPYGSITYK
8	SLO [79-571 fragment] W535A w/o leader	APKEMPLESAEKEEKKSEDKKKSEEDHTEEINDKIYSLNYNELE VLAKNGETIENFVPKEGVKKADKFIVIERKKKNINTTPVDISII DSVTDRTYPAALQLANKGFTENKPDAVVTKRNPQKIHIDLPGMG DKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYTE SMVYSKSQIEAALNVNSKILDGTLGIDFKSISKGEKKVMIAAYK QIFYTVSANLPNNPADVFDKSVTFKELQRKGVSNEAPPLFVSNV AYGRTVFVKLETSSKSNDVEAAFSAALKGTDVKTNGKYSDILEN SSFTAVVLGGDAAEHNKVVTKDFDVIRNVIKDNATFSRKNPAYP ISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSGKINLSHQGAYV AQYEILWDEINYDDKGKEVITKRRWDNNWYSKTSPFSTVIPLGA NSRNIRIMARECTGLAAEWWRKVIDERDVKLSKEINVNISGSTL SPYGSITYK

Seq ID	Name/description	Amino acid sequence
9	SpyAD [33-849 fragment] WT w/ leader	MHHHHHHGSGENLYFQGQVKADDRASGETKASNTHDDSLPKPET IQEAKATIDAVEKTLSQQKAELTELATALTKTTAEINHLKEQQD NEQKALTSAQEIYTNTLASSEETLLAQGAEHQRELTATETELHN AQADQHSKETALSEQKASISAETTRAQDLVEQVKTSEQNIAKLN AMISNPDAITKAAQTANDNTKALSSELEKAKADLENQKAKVKKQ LTEELAAQKAALAEKEAELSRLKSSAPSTQDSIVGNNTMKAPQG YPLEELKKLEASGYIGSASYNNYYKEHADQIIAKASPGNQLNQY QDIPADRNRFVDPDNLTPEVQNELAQFAAHMINSVRRQLGLPPV TVTAGSQEFARLLSTSYKKTHGNTRPSFVYGQPGVSGHYGVGPH DKTIIEDSAGASGLIRNDDNMYENIGAFNDVHTVNGIKRGIYDS IKYMLFTDHLHGNTYGHAINFLRVDKHNPNAPVYLGFSTSNVGS LNEHFVMFPESNIANHQRFNKTPIKAVGSTKDYAQRVGTVSDTI AAIKGKVSSLENRLSAIHQEADIMAAQAKVSQLQGKLASTLKQS DSLNLQVRQLNDTKGSLRTELLAAKAKQAQLEATRDQSLAKLAS LKAALHQTEALAEQAAARVTALVAKKAHLQYLRDFKLNPNRLQV IRERIDNTKQDLAKTTSSLLNAQEALAALQAKQSSLEATIATTE HQLTLLKTLANEKEYRHLDEDIATVPDLQVAPPLTGVKPLSYSK IDTTPLVQEMVKETKQLLEASARLAAENTSLVAEALVGQTSEMV ASNAIVSKITSSITQPSSKTSYGSGSSTTSNLISDVDESTQR

Seq ID	Name/description	Amino acid sequence
10	SpyAD [33-849 fragment] WT w/o leader	QVKADDRASGETKASNTHDDSLPKPETIQEAKATIDAVEKTLSQ QKAELTELATALTKTTAEINHLKEQQDNEQKALTSAQEIYTNTL ASSEETLLAQGAEHQRELTATETELHNAQADQHSKETALSEQKA SISAETTRAQDLVEQVKTSEQNIAKLNAMISNPDAITKAAQTAN DNTKALSSELEKAKADLENQKAKVKKQLTEELAAQKAALAEKEA ELSRLKSSAPSTQDSIVGNNTMKAPQGYPLEELKKLEASGYIGS ASYNNYYKEHADQIIAKASPGNQLNQYQDIPADRNRFVDPDNLT PEVQNELAQFAAHMINSVRRQLGLPPVTVTAGSQEFARLLSTSY KKTHGNTRPSFVYGQPGVSGHYGVGPHDKTIIEDSAGASGLIRN DDNMYENIGAFNDVHTVNGIKRGIYDSIKYMLFTDHLHGNTYGH AINFLRVDKHNPNAPVYLGFSTSNVGSLNEHFVMFPESNIANHQ RFNKTPIKAVGSTKDYAQRVGTVSDTIAAIKGKVSSLENRLSAI HQEADIMAAQAKVSQLQGKLASTLKQSDSLNLQVRQLNDTKGSL RTELLAAKAKQAQLEATRDQSLAKLASLKAALHQTEALAEQAAA RVTALVAKKAHLQYLRDFKLNPNRLQVIRERIDNTKQDLAKTTS SLLNAQEALAALQAKQSSLEATIATTEHQLTLLKTLANEKEYRH LDEDIATVPDLQVAPPLTGVKPLSYSKIDTTPLVQEMVKETKQL LEASARLAAENTSLVAEALVGQTSEMVASNAIVSKITSSITQPS SKTSYGSGSSTTSNLISDVDESTQR

Seq ID	Name/description	Amino acid sequence
11	SpyAD [33-849 fragment] nnAA (*e.g.*, pAMF)mutant w/ leader X = nnAA (*e.g.*, pAMF) positions K64/K287/K386/K657 – numbered according to full length sequence	MHHHHHHGSGENLYFQGQVKADDRASGETKASNTHDDSLPKPET IQEAXATIDAVEKTLSQQKAELTELATALTKTTAEINHLKEQQD NEQKALTSAQEIYTNTLASSEETLLAQGAEHQRELTATETELHN AQADQHSKETALSEQKASISAETTRAQDLVEQVKTSEQNIAKLN AMISNPDAITKAAQTANDNTKALSSELEKAKADLENQKAKVKKQ LTEELAAQKAALAEKEAELSRLKSSAPSTQDSIVGNNTMKAPQG YPLEELKXLEASGYIGSASYNNYYKEHADQIIAKASPGNQLNQY QDIPADRNRFVDPDNLTPEVQNELAQFAAHMINSVRRQLGLPPV TVTAGSQEFARLLSTSYKXTHGNTRPSFVYGQPGVSGHYGVGPH DKTIIEDSAGASGLIRNDDNMYENIGAFNDVHTVNGIKRGIYDS IKYMLFTDHLHGNTYGHAINFLRVDKHNPNAPVYLGFSTSNVGS LNEHFVMFPESNIANHQRFNKTPIKAVGSTKDYAQRVGTVSDTI AAIKGKVSSLENRLSAIHQEADIMAAQAKVSQLQGKLASTLKQS DSLNLQVRQLNDTKGSLRTELLAAKAKQAQLEATRDQSLAKLAS LKAALHQTEALAEQAAARVTALVAKXAHLQYLRDFKLNPNRLQV IRERIDNTKQDLAKTTSSLLNAQEALAALQAKQSSLEATIATTE HQLTLLKTLANEKEYRHLDEDIATVPDLQVAPPLTGVKPLSYSK IDTTPLVQEMVKETKQLLEASARLAAENTSLVAEALVGQTSEMV ASNAIVSKITSSITQPSSKTSYGSGSSTTSNLISDVDESTQR

Seq ID	Name/description	Amino acid sequence
12	SpyAD [33-849 fragment] nnAA (e.g., pAMF) mutant w/o leader X = nnAA (*e.g.*, pAMF) K64/K287/K386/K657 – numbered according to full-length sequence	QVKADDRASGETKASNTHDDSLPKPETIQEAXATIDAVEKTLSQ QKAELTELATALTKTTAEINHLKEQQDNEQKALTSAQEIYTNTL ASSEETLLAQGAEHQRELTATETELHNAQADQHSKETALSEQKA SISAETTRAQDLVEQVKTSEQNIAKLNAMISNPDAITKAAQTAN DNTKALSSELEKAKADLENQKAKVKKQLTEELAAQKAALAEKEA ELSRLKSSAPSTQDSIVGNNTMKAPQGYPLEELKXLEASGYIGS ASYNNYYKEHADQIIAKASPGNQLNQYQDIPADRNRFVDPDNLT PEVQNELAQFAAHMINSVRRQLGLPPVTVTAGSQEFARLLSTSY KXTHGNTRPSFVYGQPGVSGHYGVGPHDKTIIEDSAGASGLIRN DDNMYENIGAFNDVHTVNGIKRGIYDSIKYMLFTDHLHGNTYGH AINFLRVDKHNPNAPVYLGFSTSNVGSLNEHFVMFPESNIANHQ RFNKTPIKAVGSTKDYAQRVGTVSDTIAAIKGKVSSLENRLSAI HQEADIMAAQAKVSQLQGKLASTLKQSDSLNLQVRQLNDTKGSL RTELLAAKAKQAQLEATRDQSLAKLASLKAALHQTEALAEQAAA RVTALVAKXAHLQYLRDFKLNPNRLQVIRERIDNTKQDLAKTTS SLLNAQEALAALQAKQSSLEATIATTEHQLTLLKTLANEKEYRH LDEDIATVPDLQVAPPLTGVKPLSYSKIDTTPLVQEMVKETKQL LEASARLAAENTSLVAEALVGQTSEMVASNAIVSKITSSITQPS SKTSYGSGSSTTSNLISDVDESTQR
13	ADI [full length] WT w/ leader	MHHHHHHGSGENLYFQGTAQTPIHVYSEIGKLKKVLLHRPGKEI ENLMPDYLERLLFDDIPFLEDAQKEHDAFAQALRDEGIEVLYLE TLAAESLVTPEIREAFIDEYLSEANIRGRATKKAIRELLMAIED NQELIEKTMAGVQKSELPEIPASEKGLTDLVESNYPFAIDPMPN LYFTRDPFATIGTGVSLNHMFSETRNRETLYGKYIFTHHPIYGG GKVPMVYDRNETTRIEGGDELVLSKDVLAVGISQRTDAASIEKL LVNIFKQNLGFKKVLAFEFANNRKFMHLDTVFTMVDYDKFTIHP EIEGDLRVYSVTYDNEELHIVEEKGDLAELLAANLGVEKVDLIR CGGDNLVAAGREQWNDGSNTLTIAPGVVVVYNRNTITNAILESK GLKLIKIHGSELVRGRGGPRCMSMPFEREDI

Seq ID	Name/description	Amino acid sequence
14	ADI [full length] WT w/o leader	TAQTPIHVYSEIGKLKKVLLHRPGKEIENLMPDYLERLLFDDIP FLEDAQKEHDAFAQALRDEGIEVLYLETLAAESLVTPEIREAFI DEYLSEANIRGRATKKAIRELLMAIEDNQELIEKTMAGVQKSEL PEIPASEKGLTDLVESNYPFAIDPMPNLYFTRDPFATIGTGVSL NHMFSETRNRETLYGKYIFTHHPIYGGGKVPMVYDRNETTRIEG GDELVLSKDVLAVGISQRTDAASIEKLLVNIFKQNLGFKKVLAF EFANNRKFMHLDTVFTMVDYDKFTIHPEIEGDLRVYSVTYDNEE LHIVEEKGDLAELLAANLGVEKVDLIRCGGDNLVAAGREQWNDG SNTLTIAPGVVVVYNRNTITNAILESKGLKLIKIHGSELVRGRG GPRCMSMPFEREDI
15	ADI [full length] D277A w/ leader	MHHHHHHGSGENLYFQGTAQTPIHVYSEIGKLKKVLLHRPGKEI ENLMPDYLERLLFDDIPFLEDAQKEHDAFAQALRDEGIEVLYLE TLAAESLVTPEIREAFIDEYLSEANIRGRATKKAIRELLMAIED NQELIEKTMAGVQKSELPEIPASEKGLTDLVESNYPFAIDPMPN LYFTRDPFATIGTGVSLNHMFSETRNRETLYGKYIFTHHPIYGG GKVPMVYDRNETTRIEGGDELVLSKDVLAVGISQRTDAASIEKL LVNIFKQNLGFKKVLAFEFANNRKFMHLATVFTMVDYDKFTIHP EIEGDLRVYSVTYDNEELHIVEEKGDLAELLAANLGVEKVDLIR CGGDNLVAAGREQWNDGSNTLTIAPGVVVVYNRNTITNAILESK GLKLIKIHGSELVRGRGGPRCMSMPFEREDI
16	ADI [full length] D277A w/o leader	TAQTPIHVYSEIGKLKKVLLHRPGKEIENLMPDYLERLLFDDIP FLEDAQKEHDAFAQALRDEGIEVLYLETLAAESLVTPEIREAFI DEYLSEANIRGRATKKAIRELLMAIEDNQELIEKTMAGVQKSEL PEIPASEKGLTDLVESNYPFAIDPMPNLYFTRDPFATIGTGVSL NHMFSETRNRETLYGKYIFTHHPIYGGGKVPMVYDRNETTRIEG GDELVLSKDVLAVGISQRTDAASIEKLLVNIFKQNLGFKKVLAF EFANNRKFMHLATVFTMVDYDKFTIHPEIEGDLRVYSVTYDNEE LHIVEEKGDLAELLAANLGVEKVDLIRCGGDNLVAAGREQWNDG SNTLTIAPGVVVVYNRNTITNAILESKGLKLIKIHGSELVRGRG GPRCMSMPFEREDI

Seq ID	Name/description	Amino acid sequence
17	ADI [full length] nnAA (*e.g.*, pAMF) w/ leader X = nnAA (*e.g.*, pAMF) positions – K15, K193, K316	MHHHHHHGSGENLYFQGTAQTPIHVYSEIGXLKKVLLHRPGKEI ENLMPDYLERLLFDDIPFLEDAQKEHDAFAQALRDEGIEVLYLE TLAAESLVTPEIREAFIDEYLSEANIRGRATKKAIRELLMAIED NQELIEKTMAGVQKSELPEIPASEKGLTDLVESNYPFAIDPMPN LYFTRDPFATIGTGVSLNHMFSETRNRETLYGXYIFTHHPIYGG GKVPMVYDRNETTRIEGGDELVLSKDVLAVGISQRTDAASIEKL LVNIFKQNLGFKKVLAFEFANNRKFMHLDTVFTMVDYDKFTIHP EIEGDLRVYSVTYDNEELHIVEEXGDLAELLAANLGVEKVDLIR CGGDNLVAAGREQWNDGSNTLTIAPGVVVVYNRNTITNAILESK GLKLIKIHGSELVRGRGGPRCMSMPFEREDI
18	ADI [full length] nnAA (*e.g.*, pAMF) mutant w/o leader X = nnAA (*e.g.*, pAMF) positions – K15, K193, K316	TAQTPIHVYSEIGXLKKVLLHRPGKEIENLMPDYLERLLFDDIP FLEDAQKEHDAFAQALRDEGIEVLYLETLAAESLVTPEIREAFI DEYLSEANIRGRATKKAIRELLMAIEDNQELIEKTMAGVQKSEL PEIPASEKGLTDLVESNYPFAIDPMPNLYFTRDPFATIGTGVSL NHMFSETRNRETLYGXYIFTHHPIYGGGKVPMVYDRNETTRIEG GDELVLSKDVLAVGISQRTDAASIEKLLVNIFKQNLGFKKVLAF EFANNRKFMHLDTVFTMVDYDKFTIHPEIEGDLRVYSVTYDNEE LHIVEEXGDLAELLAANLGVEKVDLIRCGGDNLVAAGREQWNDG SNTLTIAPGVVVVYNRNTITNAILESKGLKLIKIHGSELVRGRG GPRCMSMPFEREDI
19	ADI (mut5) [full length] D277A nnAA (*e.g.*, pAMF) w/ leader X = nnAA (*e.g.*, pAMF) positions – K15, K193, K316	MHHHHHHGSGENLYFQGTAQTPIHVYSEIGXLKKVLLHRPGKEI ENLMPDYLERLLFDDIPFLEDAQKEHDAFAQALRDEGIEVLYLE TLAAESLVTPEIREAFIDEYLSEANIRGRATKKAIRELLMAIED NQELIEKTMAGVQKSELPEIPASEKGLTDLVESNYPFAIDPMPN LYFTRDPFATIGTGVSLNHMFSETRNRETLYGXYIFTHHPIYGG GKVPMVYDRNETTRIEGGDELVLSKDVLAVGISQRTDAASIEKL LVNIFKQNLGFKKVLAFEFANNRKFMHLATVFTMVDYDKFTIHP EIEGDLRVYSVTYDNEELHIVEEXGDLAELLAANLGVEKVDLIR CGGDNLVAAGREQWNDGSNTLTIAPGVVVVYNRNTITNAILESK GLKLIKIHGSELVRGRGGPRCMSMPFEREDI

Seq ID	Name/description	Amino acid sequence
20	ADI (mut5) [full length] D277A nnAA (*e.g.*, pAMF) w/o leader X = nnAA (*e.g.*, pAMF) positions – K15, K193, K316	TAQTPIHVYSEIGXLKKVLLHRPGKEIENLMPDYLERLLFDDIP FLEDAQKEHDAFAQALRDEGIEVLYLETLAAESLVTPEIREAFI DEYLSEANIRGRATKKAIRELLMAIEDNQELIEKTMAGVQKSEL PEIPASEKGLTDLVESNYPFAIDPMPNLYFTRDPFATIGTGVSL NHMFSETRNRETLYGXYIFTHHPIYGGGKVPMVYDRNETTRIEG GDELVLSKDVLAVGISQRTDAASIEKLLVNIFKQNLGFKKVLAF EFANNRKFMHLATVFTMVDYDKFTIHPEIEGDLRVYSVTYDNEE LHIVEEXGDLAELLAANLGVEKVDLIRCGGDNLVAAGREQWNDG SNTLTIAPGVVVVYNRNTITNAILESKGLKLIKIHGSELVRGRG GPRCMSMPFEREDI	
21	hFL [full length] WT w/ leader	MHHHHHHGSGSSQIRQNYSTDVEAAVNSLVNLYLQASYTYLSLG FYFDRDDVALEGVSHFFRELAEEKREGYERLLKMQNQRGGRALF QDIKKPAEDEWGKTPDAMKAAMALEKKLNQALLDLHALGSARTD PHLCDFLETHFLDEEVKLIKKMGDHLTNLHRLGGPEAGLGEYLF ERLTLKHD	
22	hFL [full length] WT w/o leader	SSQIRQNYSTDVEAAVNSLVNLYLQASYTYLSLGFYFDRDDVAL EGVSHFFRELAEEKREGYERLLKMQNQRGGRALFQDIKKPAEDE WGKTPDAMKAAMALEKKLNQALLDLHALGSARTDPHLCDFLETH FLDEEVKLIKKMGDHLTNLHRLGGPEAGLGEYLFERLTLKHD	
23	hFL [full length] nnAA (*e.g.*, pAMF) w/ leader X = nnAA (*e.g.*, pAMF) position – I5	MHHHHHHGSGSSQXRQNYSTDVEAAVNSLVNLYLQASYTYLSLG FYFDRDDVALEGVSHFFRELAEEKREGYERLLKMQNQRGGRALF QDIKKPAEDEWGKTPDAMKAAMALEKKLNQALLDLHALGSARTD PHLCDFLETHFLDEEVKLIKKMGDHLTNLHRLGGPEAGLGEYLF ERLTLKHD	

Seq ID	Name/description	Amino acid sequence
24	hFL [full length] nnAA (*e.g.*, pAMF) w/o leader X = nnAA (*e.g.*, pAMF) position – I5	SSQXRQNYSTDVEAAVNSLVNLYLQASYTYLSLGFYFDRDDVAL EGVSHFFRELAEEKREGYERLLKMQNQRGGRALFQDIKKPAEDE WGKTPDAMKAAMALEKKLNQALLDLHALGSARTDPHLCDFLETH FLDEEVKLIKKMGDHLTNLHRLGGPEAGLGEYLFERLTLKHD
25	eCRM-pAMF6 mutant	MGADDVVDSSKSFVMENFSSYHGTKPGYVDSIQXGIQKPKSGTQ GNYDDDWKEFYSTDNKYDAAGYSVDNENPLSGKAGGVVKVTYPG LTKVLALKVDNAETIKKELGLSLTEPLMEQVGTEEFIKRFGDGA SRVVLSLPFAEGSSSVEYINNWEQAKALSVELEINFETRGKRGQ DAMYEYMAQACAGNRVRNSVGSSLSCINLDWDVIRDXTKTKIES LKEHGPIKNKMSESPNKTVSEEKAXQYLEEFHQTALEHPELSEL XTVTGTNPVFAGANYAAWAVNVAQVIDSETADNLEKTTAALSIL PGIGSVMGIADGAVHHNTEEIVAQSIALSSLMVAQAIPLVGELV DIGFAAYNFVESIINLFQVVHNSYNRPAYSPGHXTQPFLHDGYA VSWNTVEDSIIRTGFQGESGHDIKITAENTPLPIAGVLLPTIPG KLDVNKSKTHISVNGRKIRMRCRAIDGDVTFCRPKSPVYVGNGV HANLHVAFHRSSSEKIHSNEISSDSIGVLGYQKTVDHTKVNSXL SLFFEIKS

Seq ID	Name/description	Amino acid sequence
26	ADI (mut2) [full length] D277A nnAA (*e.g.*, pAMF) w/ leader X = nnAA (*e.g.*, pAMF) positions – K123, K247, K316	MHHHHHHGSGENLYFQGTAQTPIHVYSEIGKLKKVLLHRPGKEI ENLMPDYLERLLFDDIPFLEDAQKEHDAFAQALRDEGIEVLYLE TLAAESLVTPEIREAFIDEYLSEANIRGRATKKAIRELLMAIED NQELIEXTMAGVQKSELPEIPASEKGLTDLVESNYPFAIDPMPN LYFTRDPFATIGTGVSLNHMFSETRNRETLYGKYIFTHHPIYGG GKVPMVYDRNETTRIEGGDELVLSKDVLAVGISQRTDAASIEXL LVNIFKQNLGFKKVLAFEFANNRKFMHLATVFTMVDYDKFTIHP EIEGDLRVYSVTYDNEELHIVEEXGDLAELLAANLGVEKVDLIR CGGDNLVAAGREQWNDGSNTLTIAPGVVVVYNRNTITNAILESK GLKLIKIHGSELVRGRGGPRCMSMPFEREDI
27	ADI (mut2) [full length] D277A nnAA (*e.g.*, pAMF) w/o leader, with G overhang from cleavage X = nnAA (*e.g.*, pAMF) positions – K123, K247, K316	GTAQTPIHVYSEIGKLKKVLLHRPGKEIENLMPDYLERLLFDDI PFLEDAQKEHDAFAQALRDEGIEVLYLETLAAESLVTPEIREAF IDEYLSEANIRGRATKKAIRELLMAIEDNQELIEXTMAGVQKSE LPEIPASEKGLTDLVESNYPFAIDPMPNLYFTRDPFATIGTGVS LNHMFSETRNRETLYGKYIFTHHPIYGGGKVPMVYDRNETTRIE GGDELVLSKDVLAVGISQRTDAASIEXLLVNIFKQNLGFKKVLA FEFANNRKFMHLATVFTMVDYDKFTIHPEIEGDLRVYSVTYDNE ELHIVEEXGDLAELLAANLGVEKVDLIRCGGDNLVAAGREQWND GSNTLTIAPGVVVVYNRNTITNAILESKGLKLIKIHGSELVRGR GGPRCMSMPFEREDI
28	Sfb1 repeat region with leader	MHHHHHHGSGVETEDTKEPGVLMGGQSESVEFTKDTQTGMSGQT TPQVETEDTKEPGVLMGGQSESVEFTKDTQTGMSGQTASQVETE DTKEPGVLMGGQSESVEFTKDTQTGMSGQTTPQVETEDTKEPGV LMGGQSESVEFTKDTQTGMSGFSET

Seq ID	Name/description	Amino acid sequence
29	C5a [90-1035 fragment] WT w/o leader, with G overhang from cleavage	GKTADTPVTSKATIRDLNDPSQVKTLQEKAGKGAGTVVAVIDAG FDKNHEAWRLTDKTKARYQSKEDLEKAKKEHGITYGEWVNDKVA YYHDYSKDGKTAVDQEHGTHVSGILSGNAPSETKEPYRLEGAMP EAQLLLMRVEIVNGLADYARNYAQAIRDAVNLGAKVINMSFGNA ALAYANLPDETKKAFDYAKSKGVSIVTSAGNDSSFGGKTRLPLA DHPDYGVVGTPAAADSTLTVASYSPDKQLTETATVKTADKQDKE MPVLSTNRFEPNKAYDYAYANRGMKEDDFKDVKGKIALIERGDI DFKDKIANAKKAGAVGVLIYDNQDKGFPIELPNVDQMPAAFISR KDGLLLKDNPKKTITFNATPKVLPTASGTKLSRFSSWGLTADGN IKPDIAAPGQDILSSVANNKYAKLSGTSMSAPLVAGIMGLLQKQ YETQYPDMTPSERLDLAKKVLMSSATALYDEDEKAYFSPRQQGA GAVDAKKASAATMYVTDKDNTSSKVHLNNVSDTFEVTVTVHNKS DKPQELYYQATVQTDKVDGKHFALAPKALYETSWQKITIPANSS KQVTVPIDASRFSKDLLAQMKNGYFLEGFVRFKQDPTKEELMSI PYIGFRGDFGNLSALEKPIYDSKDGSSYYHEANSDAKDQLDGDG LQFYALKNNFTALTTESNPWTIIKAVKEGVENIEDIESSEITET IFAGTFAKQDDDSHYYIHRHANGKPYAAISPNGDGNRDYVQFQG TFLRNAKNLVAEVLDKEGNVVWTSEVTEQVVKNYNNDLASTLGS TRFEKTRWDGKDKDGKVVANGTYTYRVRYTPISSGAKEQHTDFD VIVDNTTPEVATSATFSTEDRRLTLASKPKTSQPVYRERIAYTY MDEDLPTTEYISPNEDGTFTLPEEAETMEGATVPLKMSDFTYVV EDMAGNITYTPVTKLLEGHSNK

Seq ID	Name/description	Amino acid sequence
30	C5a [90-1035 fragment] D131A/S513A w/o leader, with G overhang from cleavage	GKTADTPVTSKATIRDLNDPSQVKTLQEKAGKGAGTVVAVIAAG FDKNHEAWRLTDKTKARYQSKEDLEKAKKEHGITYGEWVNDKVA YYHDYSKDGKTAVDQEHGTHVSGILSGNAPSETKEPYRLEGAMP EAQLLLMRVEIVNGLADYARNYAQAIRDAVNLGAKVINMSFGNA ALAYANLPDETKKAFDYAKSKGVSIVTSAGNDSSFGGKTRLPLA DHPDYGVVGTPAAADSTLTVASYSPDKQLTETATVKTADKQDKE MPVLSTNRFEPNKAYDYAYANRGMKEDDFKDVKGKIALIERGDI DFKDKIANAKKAGAVGVLIYDNQDKGFPIELPNVDQMPAAFISR KDGLLLKDNPKKTITFNATPKVLPTASGTKLSRFSSWGLTADGN IKPDIAAPGQDILSSVANNKYAKLSGTAMSAPLVAGIMGLLQKQ YETQYPDMTPSERLDLAKKVLMSSATALYDEDEKAYFSPRQQGA GAVDAKKASAATMYVTDKDNTSSKVHLNNVSDTFEVTVTVHNKS DKPQELYYQATVQTDKVDGKHFALAPKALYETSWQKITIPANSS KQVTVPIDASRFSKDLLAQMKNGYFLEGFVRFKQDPTKEELMSI PYIGFRGDFGNLSALEKPIYDSKDGSSYYHEANSDAKDQLDGDG LQFYALKNNFTALTTESNPWTIIKAVKEGVENIEDIESSEITET IFAGTFAKQDDDSHYYIHRHANGKPYAAISPNGDGNRDYVQFQG TFLRNAKNLVAEVLDKEGNVVWTSEVTEQVVKNYNNDLASTLGS TRFEKTRWDGKDKDGKVVANGTYTYRVRYTPISSGAKEQHTDFD VIVDNTTPEVATSATFSTEDRRLTLASKPKTSQPVYRERIAYTY MDEDLPTTEYISPNEDGTFTLPEEAETMEGATVPLKMSDFTYVV EDMAGNITYTPVTKLLEGHSNK

Seq ID	Name/description	Amino acid sequence
31	SLO [79-571 fragment] WT w/o leader, with G overhang from cleavage	GAPKEMPLESAEKEEKKSEDKKKSEEDHTEEINDKIYSLNYNEL EVLAKNGETIENFVPKEGVKKADKFIVIERKKKNINTTPVDISI IDSVTDRTYPAALQLANKGFTENKPDAVVTKRNPQKIHIDLPGM GDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYT ESMVYSKSQIEAALNVNSKILDGTLGIDFKSISKGEKKVMIAAY KQIFYTVSANLPNNPADVFDKSVTFKELQRKGVSNEAPPLFVSN VAYGRTVFVKLETSSKSNDVEAAFSAALKGTDVKTNGKYSDILE NSSFTAVVLGGDAAEHNKVVTKDFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSGKINLSHQGAY VAQYEILWDEINYDDKGKEVITKRRWDNNWYSKTSPFSTVIPLG ANSRNIRIMARECTGLAWEWWRKVIDERDVKLSKEINVNISGST LSPYGSITYK
32	SLO [79-571 fragment] W535A w/o leader, with G overhang from cleavage	GAPKEMPLESAEKEEKKSEDKKKSEEDHTEEINDKIYSLNYNEL EVLAKNGETIENFVPKEGVKKADKFIVIERKKKNINTTPVDISI IDSVTDRTYPAALQLANKGFTENKPDAVVTKRNPQKIHIDLPGM GDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYT ESMVYSKSQIEAALNVNSKILDGTLGIDFKSISKGEKKVMIAAY KQIFYTVSANLPNNPADVFDKSVTFKELQRKGVSNEAPPLFVSN VAYGRTVFVKLETSSKSNDVEAAFSAALKGTDVKTNGKYSDILE NSSFTAVVLGGDAAEHNKVVTKDFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSGKINLSHQGAY VAQYEILWDEINYDDKGKEVITKRRWDNNWYSKTSPFSTVIPLG ANSRNIRIMARECTGLAAEWWRKVIDERDVKLSKEINVNISGST LSPYGSITYK

Seq ID	Name/description	Amino acid sequence
33	SpyAD [33-849 fragment] WT w/o leader, with G overhang from cleavage	GQVKADDRASGETKASNTHDDSLPKPETIQEAKATIDAVEKTLS QQKAELTELATALTKTTAEINHLKEQQDNEQKALTSAQEIYTNT LASSEETLLAQGAEHQRELTATETELHNAQADQHSKETALSEQK ASISAETTRAQDLVEQVKTSEQNIAKLNAMISNPDAITKAAQTA NDNTKALSSELEKAKADLENQKAKVKKQLTEELAAQKAALAEKE AELSRLKSSAPSTQDSIVGNNTMKAPQGYPLEELKKLEASGYIG SASYNNYYKEHADQIIAKASPGNQLNQYQDIPADRNRFVDPDNL TPEVQNELAQFAAHMINSVRRQLGLPPVTVTAGSQEFARLLSTS YKKTHGNTRPSFVYGQPGVSGHYGVGPHDKTIIEDSAGASGLIR NDDNMYENIGAFNDVHTVNGIKRGIYDSIKYMLFTDHLHGNTYG HAINFLRVDKHNPNAPVYLGFSTSNVGSLNEHFVMFPESNIANH QRFNKTPIKAVGSTKDYAQRVGTVSDTIAAIKGKVSSLENRLSA IHQEADIMAAQAKVSQLQGKLASTLKQSDSLNLQVRQLNDTKGS LRTELLAAKAKQAQLEATRDQSLAKLASLKAALHQTEALAEQAA ARVTALVAKKAHLQYLRDFKLNPNRLQVIRERIDNTKQDLAKTT SSLLNAQEALAALQAKQSSLEATIATTEHQLTLLKTLANEKEYR HLDEDIATVPDLQVAPPLTGVKPLSYSKIDTTPLVQEMVKETKQ LLEASARLAAENTSLVAEALVGQTSEMVASNAIVSKITSSITQP SSKTSYGSGSSTTSNLISDVDESTQR

Seq ID	Name/description	Amino acid sequence
34	SpyAD [33-849 fragment] nnAA (*e.g.*, pAMF) w/o leader, with G overhang from cleavage X = nnAA (*e.g.*, pAMF) positions K64/K287/K386/K657 – numbered	GQVKADDRASGETKASNTHDDSLPKPETIQEAXATIDAVEKTLS QQKAELTELATALTKTTAEINHLKEQQDNEQKALTSAQEIYTNT LASSEETLLAQGAEHQRELTATETELHNAQADQHSKETALSEQK ASISAETTRAQDLVEQVKTSEQNIAKLNAMISNPDAITKAAQTA NDNTKALSSELEKAKADLENQKAKVKKQLTEELAAQKAALAEKE AELSRLKSSAPSTQDSIVGNNTMKAPQGYPLEELKXLEASGYIG SASYNNYYKEHADQIIAKASPGNQLNQYQDIPADRNRFVDPDNL TPEVQNELAQFAAHMINSVRRQLGLPPVTVTAGSQEFARLLSTS YKXTHGNTRPSFVYGQPGVSGHYGVGPHDKTIIEDSAGASGLIR NDDNMYENIGAFNDVHTVNGIKRGIYDSIKYMLFTDHLHGNTYG HAINFLRVDKHNPNAPVYLGFSTSNVGSLNEHFVMFPESNIANH QRFNKTPIKAVGSTKDYAQRVGTVSDTIAAIKGKVSSLENRLSA IHQEADIMAAQAKVSQLQGKLASTLKQSDSLNLQVRQLNDTKGS LRTELLAAKAKQAQLEATRDQSLAKLASLKAALHQTEALAEQAA ARVTALVAKXAHLQYLRDFKLNPNRLQVIRERIDNTKQDLAKTT SSLLNAQEALAALQAKQSSLEATIATTEHQLTLLKTLANEKEYR HLDEDIATVPDLQVAPPLTGVKPLSYSKIDTTPLVQEMVKETKQ LLEASARLAAENTSLVAEALVGQTSEMVASNAIVSKITSSITQP SSKTSYGSGSSTTSNLISDVDESTQR
35	ADI [full length] WT w/o leader, with G overhang from cleavage	GTAQTPIHVYSEIGKLKKVLLHRPGKEIENLMPDYLERLLFDDI PFLEDAQKEHDAFAQALRDEGIEVLYLETLAAESLVTPEIREAF IDEYLSEANIRGRATKKAIRELLMAIEDNQELIEKTMAGVQKSE LPEIPASEKGLTDLVESNYPFAIDPMPNLYFTRDPFATIGTGVS LNHMFSETRNRETLYGKYIFTHHPIYGGGKVPMVYDRNETTRIE GGDELVLSKDVLAVGISQRTDAASIEKLLVNIFKQNLGFKKVLA FEFANNRKFMHLDTVFTMVDYDKFTIHPEIEGDLRVYSVTYDNE ELHIVEEKGDLAELLAANLGVEKVDLIRCGGDNLVAAGREQWND GSNTLTIAPGVVVVYNRNTITNAILESKGLKLIKIHGSELVRGR GGPRCMSMPFEREDI

Seq ID	Name/description	Amino acid sequence
36	ADI [full length] D277A w/o leader, with G overhang from cleavage	GTAQTPIHVYSEIGKLKKVLLHRPGKEIENLMPDYLERLLFDDI PFLEDAQKEHDAFAQALRDEGIEVLYLETLAAESLVTPEIREAF IDEYLSEANIRGRATKKAIRELLMAIEDNQELIEKTMAGVQKSE LPEIPASEKGLTDLVESNYPFAIDPMPNLYFTRDPFATIGTGVS LNHMFSETRNRETLYGKYIFTHHPIYGGGKVPMVYDRNETTRIE GGDELVLSKDVLAVGISQRTDAASIEKLLVNIFKQNLGFKKVLA FEFANNRKFMHLATVFTMVDYDKFTIHPEIEGDLRVYSVTYDNE ELHIVEEKGDLAELLAANLGVEKVDLIRCGGDNLVAAGREQWND GSNTLTIAPGVVVVYNRNTITNAILESKGLKLIKIHGSELVRGR GGPRCMSMPFEREDI
37	ADI [full length] nnAA (*e.g.*, pAMF) w/o leader, with G overhang from cleavage X = nnAA (*e.g.*, pAMF) positions – K15, K193, K316	GTAQTPIHVYSEIGXLKKVLLHRPGKEIENLMPDYLERLLFDDI PFLEDAQKEHDAFAQALRDEGIEVLYLETLAAESLVTPEIREAF IDEYLSEANIRGRATKKAIRELLMAIEDNQELIEKTMAGVQKSE LPEIPASEKGLTDLVESNYPFAIDPMPNLYFTRDPFATIGTGVS LNHMFSETRNRETLYGXYIFTHHPIYGGGKVPMVYDRNETTRIE GGDELVLSKDVLAVGISQRTDAASIEKLLVNIFKQNLGFKKVLA FEFANNRKFMHLDTVFTMVDYDKFTIHPEIEGDLRVYSVTYDNE ELHIVEEXGDLAELLAANLGVEKVDLIRCGGDNLVAAGREQWND GSNTLTIAPGVVVVYNRNTITNAILESKGLKLIKIHGSELVRGR GGPRCMSMPFEREDI
38	ADI (mut5) [full length] D277A nnAA (*e.g.*, pAMF) w/o leader, with G overhang from cleavage X = nnAA (*e.g.*, pAMF) positions – K15, K193, K316	GTAQTPIHVYSEIGXLKKVLLHRPGKEIENLMPDYLERLLFDDI PFLEDAQKEHDAFAQALRDEGIEVLYLETLAAESLVTPEIREAF IDEYLSEANIRGRATKKAIRELLMAIEDNQELIEKTMAGVQKSE LPEIPASEKGLTDLVESNYPFAIDPMPNLYFTRDPFATIGTGVS LNHMFSETRNRETLYGXYIFTHHPIYGGGKVPMVYDRNETTRIE GGDELVLSKDVLAVGISQRTDAASIEKLLVNIFKQNLGFKKVLA FEFANNRKFMHLATVFTMVDYDKFTIHPEIEGDLRVYSVTYDNE ELHIVEEXGDLAELLAANLGVEKVDLIRCGGDNLVAAGREQWND GSNTLTIAPGVVVVYNRNTITNAILESKGLKLIKIHGSELVRGR GGPRCMSMPFEREDI

Seq ID	Name/description	Amino acid sequence
39	hFL [full length] WT w/ leader	MHHHHHHGSGSSQIRQNYSTDVEAAVNSLVNLYLQASYTYLSLG FYFDRDDVALEGVSHFFRELAEEKREGYERLLKMQNQRGGRALF QDIKKPAEDEWGKTPDAMKAAMALEKKLNQALLDLHALGSARTD PHLCDFLETHFLDEEVKLIKKMGDHLTNLHRLGGPEAGLGEYLF ERLTLKHD
40	hFL [full length] WT w/o leader	SSQIRQNYSTDVEAAVNSLVNLYLQASYTYLSLGFYFDRDDVAL EGVSHFFRELAEEKREGYERLLKMQNQRGGRALFQDIKKPAEDE WGKTPDAMKAAMALEKKLNQALLDLHALGSARTDPHLCDFLETH FLDEEVKLIKKMGDHLTNLHRLGGPEAGLGEYLFERLTLKHD
41	hFL [full length] nnAA (*e.g.*, pAMF) w/ leader X = nnAA (*e.g.*, pAMF) position – I5	MHHHHHHGSGSSQXRQNYSTDVEAAVNSLVNLYLQASYTYLSLG FYFDRDDVALEGVSHFFRELAEEKREGYERLLKMQNQRGGRALF QDIKKPAEDEWGKTPDAMKAAMALEKKLNQALLDLHALGSARTD PHLCDFLETHFLDEEVKLIKKMGDHLTNLHRLGGPEAGLGEYLF ERLTLKHD
42	hFL [full length] nnAA (*e.g.*, pAMF) w/o leader X = nnAA (*e.g.*, pAMF) position – I5	SSQXRQNYSTDVEAAVNSLVNLYLQASYTYLSLGFYFDRDDVAL EGVSHFFRELAEEKREGYERLLKMQNQRGGRALFQDIKKPAEDE WGKTPDAMKAAMALEKKLNQALLDLHALGSARTDPHLCDFLETH FLDEEVKLIKKMGDHLTNLHRLGGPEAGLGEYLFERLTLKHD

Seq ID	Name/description	Amino acid sequence
43	eCRM-pAMF6 mutant	GADDVVDSSKSFVMENFSSYHGTKPGYVDSIQXGIQKPKSGTQG NYDDDWKEFYSTDNKYDAAGYSVDNENPLSGKAGGVVKVTYPGL TKVLALKVDNAETIKKELGLSLTEPLMEQVGTEEFIKRFGDGAS RVVLSLPFAEGSSSVEYINNWEQAKALSVELEINFETRGKRGQD AMYEYMAQACAGNRVRNSVGSSLSCINLDWDVIRDXTKTKIESL KEHGPIKNKMSESPNKTVSEEKAXQYLEEFHQTALEHPELSELX TVTGTNPVFAGANYAAWAVNVAQVIDSETADNLEKTTAALSILP GIGSVMGIADGAVHHNTEEIVAQSIALSSLMVAQAIPLVGELVD IGFAAYNFVESIINLFQVVHNSYNRPAYSPGHXTQPFLHDGYAV SWNTVEDSIIRTGFQGESGHDIKITAENTPLPIAGVLLPTIPGK LDVNKSKTHISVNGRKIRMRCRAIDGDVTFCRPKSPVYVGNGVH ANLHVAFHRSSSEKIHSNEISSDSIGVLGYQKTVDHTKVNSXLS LFFEIKS
44	SLO(ΔC11) W535A w/o leader, with G overhang from cleavage	GAPKEMPLESAEKEEKKSEDKKKSEEDHTEEINDKIYSLNYNEL EVLAKNGETIENFVPKEGVKKADKFIVIERKKKNINTTPVDISI IDSVTDRTYPAALQLANKGFTENKPDAVVTKRNPQKIHIDLPGM GDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYT ESMVYSKSQIEAALNVNSKILDGTLGIDFKSISKGEKKVMIAAY KQIFYTVSANLPNNPADVFDKSVTFKELQRKGVSNEAPPLFVSN VAYGRTVFVKLETSSKSNDVEAAFSAALKGTDVKTNGKYSDILE NSSFTAVVLGGDAAEHNKVVTKDFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSGKINLSHQGAY VAQYEILWDEINYDDKGKEVITKRRWDNNWYSKTSPFSTVIPLG ANSRNIRIMARECTGLAAEWWRKVIDERDVKLSKEINVNISGS

Seq ID	Name/description	Amino acid sequence
45	SLO(ΔC21) fragment W535A w/o leader, with G overhang from cleavage	GAPKEMPLESAEKEEKKSEDKKKSEEDHTEEINDKIYSLNYNEL EVLAKNGETIENFVPKEGVKKADKFIVIERKKKNINTTPVDISI IDSVTDRTYPAALQLANKGFTENKPDAVVTKRNPQKIHIDLPGM GDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYT ESMVYSKSQIEAALNVNSKILDGTLGIDFKSISKGEKKVMIAAY KQIFYTVSANLPNNPADVFDKSVTFKELQRKGVSNEAPPLFVSN VAYGRTVFVKLETSSKSNDVEAAFSAALKGTDVKTNGKYSDILE NSSFTAVVLGGDAAEHNKVVTKDFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSGKINLSHQGAY VAQYEILWDEINYDDKGKEVITKRRWDNNWYSKTSPFSTVIPLG ANSRNIRIMARECTGLAAEWWRKVIDERDVKLS
46	SLO(ΔC31) fragment W535A w/o leader, with G overhang from cleavage	GAPKEMPLESAEKEEKKSEDKKKSEEDHTEEINDKIYSLNYNEL EVLAKNGETIENFVPKEGVKKADKFIVIERKKKNINTTPVDISI IDSVTDRTYPAALQLANKGFTENKPDAVVTKRNPQKIHIDLPGM GDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYT ESMVYSKSQIEAALNVNSKILDGTLGIDFKSISKGEKKVMIAAY KQIFYTVSANLPNNPADVFDKSVTFKELQRKGVSNEAPPLFVSN VAYGRTVFVKLETSSKSNDVEAAFSAALKGTDVKTNGKYSDILE NSSFTAVVLGGDAAEHNKVVTKDFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSGKINLSHQGAY VAQYEILWDEINYDDKGKEVITKRRWDNNWYSKTSPFSTVIPLG ANSRNIRIMARECTGLAAEWWRK

Seq ID	Name/description	Amino acid sequence
47	SLO(ΔC41) fragment W535A w/o leader, with G overhang from cleavage	GAPKEMPLESAEKEEKKSEDKKKSEEDHTEEINDKIYSLNYNEL EVLAKNGETIENFVPKEGVKKADKFIVIERKKKNINTTPVDISI IDSVTDRTYPAALQLANKGFTENKPDAVVTKRNPQKIHIDLPGM GDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYT ESMVYSKSQIEAALNVNSKILDGTLGIDFKSISKGEKKVMIAAY KQIFYTVSANLPNNPADVFDKSVTFKELQRKGVSNEAPPLFVSN VAYGRTVFVKLETSSKSNDVEAAFSAALKGTDVKTNGKYSDILE NSSFTAVVLGGDAAEHNKVVTKDFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSGKINLSHQGAY VAQYEILWDEINYDDKGKEVITKRRWDNNWYSKTSPFSTVIPLG ANSRNIRIMAREC
48	SLO(ΔC51) fragment W535A w/o leader, with G overhang from cleavage	GAPKEMPLESAEKEEKKSEDKKKSEEDHTEEINDKIYSLNYNEL EVLAKNGETIENFVPKEGVKKADKFIVIERKKKNINTTPVDISI IDSVTDRTYPAALQLANKGFTENKPDAVVTKRNPQKIHIDLPGM GDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYT ESMVYSKSQIEAALNVNSKILDGTLGIDFKSISKGEKKVMIAAY KQIFYTVSANLPNNPADVFDKSVTFKELQRKGVSNEAPPLFVSN VAYGRTVFVKLETSSKSNDVEAAFSAALKGTDVKTNGKYSDILE NSSFTAVVLGGDAAEHNKVVTKDFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSGKINLSHQGAY VAQYEILWDEINYDDKGKEVITKRRWDNNWYSKTSPFSTVIPLG ANS

Seq ID	Name/description	Amino acid sequence
49	SLO(ΔC61) fragment W535A w/o leader, with G overhang from cleavage	GAPKEMPLESAEKEEKKSEDKKKSEEDHTEEINDKIYSLNYNEL EVLAKNGETIENFVPKEGVKKADKFIVIERKKKNINTTPVDISI IDSVTDRTYPAALQLANKGFTENKPDAVVTKRNPQKIHIDLPGM GDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYT ESMVYSKSQIEAALNVNSKILDGTLGIDFKSISKGEKKVMIAAY KQIFYTVSANLPNNPADVFDKSVTFKELQRKGVSNEAPPLFVSN VAYGRTVFVKLETSSKSNDVEAAFSAALKGTDVKTNGKYSDILE NSSFTAVVLGGDAAEHNKVVTKDFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSGKINLSHQGAY VAQYEILWDEINYDDKGKEVITKRRWDNNWYSKTSPF
50	SLO(ΔC71) fragment W535A w/o leader, with G overhang from cleavage	GAPKEMPLESAEKEEKKSEDKKKSEEDHTEEINDKIYSLNYNEL EVLAKNGETIENFVPKEGVKKADKFIVIERKKKNINTTPVDISI IDSVTDRTYPAALQLANKGFTENKPDAVVTKRNPQKIHIDLPGM GDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYT ESMVYSKSQIEAALNVNSKILDGTLGIDFKSISKGEKKVMIAAY KQIFYTVSANLPNNPADVFDKSVTFKELQRKGVSNEAPPLFVSN VAYGRTVFVKLETSSKSNDVEAAFSAALKGTDVKTNGKYSDILE NSSFTAVVLGGDAAEHNKVVTKDFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSGKINLSHQGAY VAQYEILWDEINYDDKGKEVITKRRWD
51	SLO(ΔC81) fragment W535A w/o leader, with G overhang from cleavage	GAPKEMPLESAEKEEKKSEDKKKSEEDHTEEINDKIYSLNYNEL EVLAKNGETIENFVPKEGVKKADKFIVIERKKKNINTTPVDISI IDSVTDRTYPAALQLANKGFTENKPDAVVTKRNPQKIHIDLPGM GDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYT ESMVYSKSQIEAALNVNSKILDGTLGIDFKSISKGEKKVMIAAY KQIFYTVSANLPNNPADVFDKSVTFKELQRKGVSNEAPPLFVSN VAYGRTVFVKLETSSKSNDVEAAFSAALKGTDVKTNGKYSDILE NSSFTAVVLGGDAAEHNKVVTKDFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSGKINLSHQGAY VAQYEILWDEINYDDKG

Seq ID	Name/description	Amino acid sequence
52	SLO(ΔC91) fragment W535A w/o leader, with G overhang from cleavage	GAPKEMPLESAEKEEKKSEDKKKSEEDHTEEINDKIYSLNYNEL EVLAKNGETIENFVPKEGVKKADKFIVIERKKKNINTTPVDISI IDSVTDRTYPAALQLANKGFTENKPDAVVTKRNPQKIHIDLPGM GDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYT ESMVYSKSQIEAALNVNSKILDGTLGIDFKSISKGEKKVMIAAY KQIFYTVSANLPNNPADVFDKSVTFKELQRKGVSNEAPPLFVSN VAYGRTVFVKLETSSKSNDVEAAFSAALKGTDVKTNGKYSDILE NSSFTAVVLGGDAAEHNKVVTKDFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSGKINLSHQGAY VAQYEIL
53	SLO(ΔC101) fragment W535A w/o leader, with G overhang from cleavage	GAPKEMPLESAEKEEKKSEDKKKSEEDHTEEINDKIYSLNYNEL EVLAKNGETIENFVPKEGVKKADKFIVIERKKKNINTTPVDISI IDSVTDRTYPAALQLANKGFTENKPDAVVTKRNPQKIHIDLPGM GDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYT ESMVYSKSQIEAALNVNSKILDGTLGIDFKSISKGEKKVMIAAY KQIFYTVSANLPNNPADVFDKSVTFKELQRKGVSNEAPPLFVSN VAYGRTVFVKLETSSKSNDVEAAFSAALKGTDVKTNGKYSDILE NSSFTAVVLGGDAAEHNKVVTKDFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSGKINLSHQ

Seq ID	Name/description	Amino acid sequence
54	SLO(ΔC101) fragment W535A w/o leader, with G overhang from cleavage X = nnAA (*e.g.*, pAMF) or native amino acid (*e.g.*:K98, K112, R151, K189, K272, K323, K357, K375, K407, K464; or K or R.)	GAPKEMPLESAEKEEKKSED**X**KKSEEDHTEEIND**X**IYSLNYNEL EVLAKNGETIENFVPKEGVKKADKFIVIE**X**KKKNINTTPVDISI IDSVTDRTYPAALQLANKGFTEN**X**PDAVVTKRNPQKIHIDLPGM GDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYT ESMVYSKSQIEAALNVNS**X**ILDGTLGIDFKSISKGEKKVMIAAY KQIFYTVSANLPNNPADVFDKSVTF**X**ELQRKGVSNEAPPLFVSN VAYGRTVFVKLETSS**X**SNDVEAAFSAALKGTDV**X**TNGKYSDILE NSSFTAVVLGGDAAEHNKVVT**X**DFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSG**X**INLSHQ
55	SLO(ΔC101) fragment W535A – nnAA (*e.g.*, pAMF) 1 – w/o leader, with G overhang from cleavage (420995) X = nnAA (*e.g.*, pAMF) positions – K98, R151 , K272, K357	GAPKEMPLESAEKEEKKSED**X**KKSEEDHTEEINDKIYSLNYNEL EVLAKNGETIENFVPKEGVKKADKFIVIE**X**KKKNINTTPVDISI IDSVTDRTYPAALQLANKGFTENKPDAVVTKRNPQKIHIDLPGM GDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYT ESMVYSKSQIEAALNVNS**X**ILDGTLGIDFKSISKGEKKVMIAAY KQIFYTVSANLPNNPADVFDKSVTFKELQRKGVSNEAPPLFVSN VAYGRTVFVKLETSS**X**SNDVEAAFSAALKGTDVKTNGKYSDILE NSSFTAVVLGGDAAEHNKVVTKDFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSGKINLSHQ

Seq ID	Name/description	Amino acid sequence
56	SLO(ΔC101) fragment W535A – nnAA (*e.g.*, pAMF) 2 – w/o leader, with G overhang from cleavage (420996) X = nnAA (*e.g.*, pAMF) positions – K112, K189, K323, K375	GAPKEMPLESAEKEEKKSEDKKKSEEDHTEEIND**X**IYSLNYNEL EVLAKNGETIENFVPKEGVKKADKFIVIERKKKNINTTPVDISI IDSVTDRTYPAALQLANKGFTEN**X**PDAVVTKRNPQKIHIDLPGM GDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYT ESMVYSKSQIEAALNVNSKILDGTLGIDFKSISKGEKKVMIAAY KQIFYTVSANLPNNPADVFDKSVTF**X**ELQRKGVSNEAPPLFVSN VAYGRTVFVKLETSSKSNDVEAAFSAALKGTDV**X**TNGKYSDILE NSSFTAVVLGGDAAEHNKVVTKDFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSGKINLSHQ
57	SLO(ΔC101) fragment W535A – nnAA (*e.g.*, pAMF) 3 – w/o leader, with G overhang from cleavage (420997) X = nnAA (*e.g.*, pAMF) positions – R151, K272, K357, K407	GAPKEMPLESAEKEEKKSEDKKKSEEDHTEEINDKIYSLNYNEL EVLAKNGETIENFVPKEGVKKADKFIVIE**X**KKKNINTTPVDISI IDSVTDRTYPAALQLANKGFTENKPDAVVTKRNPQKIHIDLPGM GDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYT ESMVYSKSQIEAALNVNS**X**ILDGTLGIDFKSISKGEKKVMIAAY KQIFYTVSANLPNNPADVFDKSVTFKELQRKGVSNEAPPLFVSN VAYGRTVFVKLETSS**X**SNDVEAAFSAALKGTDVKTNGKYSDILE NSSFTAVVLGGDAAEHNKVVT**X**DFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSGKINLSHQ

Seq ID	Name/description	Amino acid sequence
58	SLO(ΔC101) fragment W535A – nnAA (*e.g.*, pAMF) 4 – w/o leader, with G overhang from cleavage (420998) X = nnAA (*e.g.*, pAMF) positions – R151, K272, K375, K464	GAPKEMPLESAEKEEKKSEDKKKSEEDHTEEINDKIYSLNYNEL EVLAKNGETIENFVPKEGVKKADKFIVIE**X**KKKNINTTPVDISI IDSVTDRTYPAALQLANKGFTENKPDAVVTKRNPQKIHIDLPGM GDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYT ESMVYSKSQIEAALNVNS**X**ILDGTLGIDFKSISKGEKKVMIAAY KQIFYTVSANLPNNPADVFDKSVTFKELQRKGVSNEAPPLFVSN VAYGRTVFVKLETSSKSNDVEAAFSAALKGTDV**X**TNGKYSDILE NSSFTAVVLGGDAAEHNKVVTKDFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSG**X**INLSHQ
59	SLO(ΔC101) fragment W535A – nnAA (*e.g.*, pAMF)5 – w/o leader, with G overhang from cleavage (420999) X = nnAA (*e.g.*, pAMF) positions – K112, K272, K357, K464	GAPKEMPLESAEKEEKKSEDKKKSEEDHTEEIND**X**IYSLNYNEL EVLAKNGETIENFVPKEGVKKADKFIVIERKKKNINTTPVDISI IDSVTDRTYPAALQLANKGFTENKPDAVVTKRNPQKIHIDLPGM GDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYT ESMVYSKSQIEAALNVNS**X**ILDGTLGIDFKSISKGEKKVMIAAY KQIFYTVSANLPNNPADVFDKSVTFKELQRKGVSNEAPPLFVSN VAYGRTVFVKLETSS**X**SNDVEAAFSAALKGTDVKTNGKYSDILE NSSFTAVVLGGDAAEHNKVVTKDFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSG**X**INLSHQ
60	SLO(ΔC101) fragment W535A – nnAA (*e.g.*, pAMF) 6 – w/o leader, with G overhang from cleavage (421000) X = nnAA (*e.g.*, pAMF) positions – K98, K189, K357	GAPKEMPLESAEKEEKKSED**X**KKSEEDHTEEINDKIYSLNYNEL EVLAKNGETIENFVPKEGVKKADKFIVIERKKKNINTTPVDISI IDSVTDRTYPAALQLANKGFTEN**X**PDAVVTKRNPQKIHIDLPGM GDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYT ESMVYSKSQIEAALNVNSKILDGTLGIDFKSISKGEKKVMIAAY KQIFYTVSANLPNNPADVFDKSVTFKELQRKGVSNEAPPLFVSN VAYGRTVFVKLETSS**X**SNDVEAAFSAALKGTDVKTNGKYSDILE NSSFTAVVLGGDAAEHNKVVTKDFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSGKINLSHQ

Seq ID	Name/description	Amino acid sequence
61	SLO(ΔC101) fragment W535A – nnAA (*e.g.*, pAMF) 7 – w/o leader, with G overhang from cleavage (421001) X = nnAA (*e.g.*, pAMF) positions – K112, K189, K323	GAPKEMPLESAEKEEKKSEDXKKSEEDHTEEINDXIYSLNYNEL EVLAKNGETIENFVPKEGVKKADKFIVIERKKKNINTTPVDISI IDSVTDRTYPAALQLANKGFTENXPDAVVTKRNPQKIHIDLPGM GDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYT ESMVYSKSQIEAALNVNSKILDGTLGIDFKSISKGEKKVMIAAY KQIFYTVSANLPNNPADVFDKSVTFXELQRKGVSNEAPPLFVSN VAYGRTVFVKLETSSKSNDVEAAFSAALKGTDVKTNGKYSDILE NSSFTAVVLGGDAAEHNKVVTKDFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSGKINLSHQ
62	SLO(ΔC101) fragment W535A – nnAA (*e.g.*, pAMF) 8 – w/o leader, with G overhang from cleavage (421002) X = nnAA (*e.g.*, pAMF) positions – K98, R151, K272	GAPKEMPLESAEKEEKKSEDXKKSEEDHTEEINDKIYSLNYNEL EVLAKNGETIENFVPKEGVKKADKFIVIEXKKKNINTTPVDISI IDSVTDRTYPAALQLANKGFTENKPDAVVTKRNPQKIHIDLPGM GDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYT ESMVYSKSQIEAALNVNSXILDGTLGIDFKSISKGEKKVMIAAY KQIFYTVSANLPNNPADVFDKSVTFKELQRKGVSNEAPPLFVSN VAYGRTVFVKLETSSKSNDVEAAFSAALKGTDVKTNGKYSDILE NSSFTAVVLGGDAAEHNKVVTKDFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSGKINLSHQ
63	SLO(ΔC101) fragment W535A – nnAA (*e.g.*, pAMF) 9 – w/o leader, with G overhang from cleavage (421003) X = nnAA (*e.g.*, pAMF) positions – K112, K272, K375	GAPKEMPLESAEKEEKKSEDKKKSEEDHTEEINDXIYSLNYNEL EVLAKNGETIENFVPKEGVKKADKFIVIERKKKNINTTPVDISI IDSVTDRTYPAALQLANKGFTENKPDAVVTKRNPQKIHIDLPGM GDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYT ESMVYSKSQIEAALNVNSXILDGTLGIDFKSISKGEKKVMIAAY KQIFYTVSANLPNNPADVFDKSVTFKELQRKGVSNEAPPLFVSN VAYGRTVFVKLETSSKSNDVEAAFSAALKGTDVXTNGKYSDILE NSSFTAVVLGGDAAEHNKVVTKDFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSGKINLSHQ

Seq ID	Name/description	Amino acid sequence
64	SLO(ΔC101) fragment W535A – nnAA (*e.g.*, pAMF) 10 – w/o leader, with G overhang from cleavage (421004) X = nnAA (*e.g.*, pAMF) positions – K112, K323, K407	GAPKEMPLESAEKEEKKSEDKKKSEEDHTEEIND**X**IYSLNYNEL EVLAKNGETIENFVPKEGVKKADKFIVIERKKKNINTTPVDISI IDSVTDRTYPAALQLANKGFTENKPDAVVTKRNPQKIHIDLPGM GDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYT ESMVYSKSQIEAALNVNSKILDGTLGIDFKSISKGEKKVMIAAY KQIFYTVSANLPNNPADVFDKSVTF**X**ELQRKGVSNEAPPLFVSN VAYGRTVFVKLETSSKSNDVEAAFSAALKGTDVKTNGKYSDILE NSSFTAVVLGGDAAEHNKVVT**X**DFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSGKINLSHQ
65	SLO(ΔC101) fragment W535A – nnAA (*e.g.*, pAMF) 10 – w/o leader, with G overhang from cleavage (426507) X = nnAA (*e.g.*, pAMF) positions – K98, R151, K272, K357, K407	GAPKEMPLESAEKEEKKSED**X**KKSEEDHTEEINDKIYSLNYNEL EVLAKNGETIENFVPKEGVKKADKFIVIE**X**KKKNINTTPVDISI IDSVTDRTYPAALQLANKGFTENKPDAVVTKRNPQKIHIDLPGM GDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYT ESMVYSKSQIEAALNVNS**X**ILDGTLGIDFKSISKGEKKVMIAAY KQIFYTVSANLPNNPADVFDKSVTFKELQRKGVSNEAPPLFVSN VAYGRTVFVKLETSS**X**SNDVEAAFSAALKGTDVKTNGKYSDILE NSSFTAVVLGGDAAEHNKVVT**X**DFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSGKINLSHQ
66	SLO(ΔC101) fragment W535A – nnAA (*e.g.*, pAMF) 10 – w/o leader, with G overhang from cleavage (426508) X = nnAA (*e.g.*, pAMF) positions – K112, K189, K323, K375, K464	GAPKEMPLESAEKEEKKSEDKKKSEEDHTEEIND**X**IYSLNYNEL EVLAKNGETIENFVPKEGVKKADKFIVIERKKKNINTTPVDISI IDSVTDRTYPAALQLANKGFTEN**X**PDAVVTKRNPQKIHIDLPGM GDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYT ESMVYSKSQIEAALNVNSKILDGTLGIDFKSISKGEKKVMIAAY KQIFYTVSANLPNNPADVFDKSVTF**X**ELQRKGVSNEAPPLFVSN VAYGRTVFVKLETSSKSNDVEAAFSAALKGTDV**X**TNGKYSDILE NSSFTAVVLGGDAAEHNKVVTKDFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSG**X**INLSHQ

Seq ID	Name/description	Amino acid sequence
67	SLO(ΔC101) fragment W535A – nnAA (*e.g.*, pAMF) 10 – w/o leader, with G overhang from cleavage (426509) X = nnAA (*e.g.*, pAMF) positions – K112, R151, K272, K357, K407	GAPKEMPLESAEKEEKKSEDKKKSEEDHTEEIND**X**IYSLNYNEL EVLAKNGETIENFVPKEGVKKADKFIVIE**X**KKKNINTTPVDISI IDSVTDRTYPAALQLANKGFTENKPDAVVTKRNPQKIHIDLPGM GDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYT ESMVYSKSQIEAALNVNS**X**ILDGTLGIDFKSISKGEKKVMIAAY KQIFYTVSANLPNNPADVFDKSVTFKELQRKGVSNEAPPLFVSN VAYGRTVFVKLETSS**X**SNDVEAAFSAALKGTDVKTNGKYSDILE NSSFTAVVLGGDAAEHNKVVT**X**DFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSGKINLSHQ
68	SLO(ΔC101) fragment W535A – nnAA (*e.g.*, pAMF) 10 – w/o leader, with G overhang from cleavage (426510) X = nnAA (*e.g.*, pAMF) positions – K98, R151, K272, K357, K407, K464	GAPKEMPLESAEKEEKKSED**X**KKSEEDHTEEINDKIYSLNYNEL EVLAKNGETIENFVPKEGVKKADKFIVIE**X**KKKNINTTPVDISI IDSVTDRTYPAALQLANKGFTENKPDAVVTKRNPQKIHIDLPGM GDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYT ESMVYSKSQIEAALNVNS**X**ILDGTLGIDFKSISKGEKKVMIAAY KQIFYTVSANLPNNPADVFDKSVTFKELQRKGVSNEAPPLFVSN VAYGRTVFVKLETSS**X**SNDVEAAFSAALKGTDVKTNGKYSDILE NSSFTAVVLGGDAAEHNKVVT**X**DFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSG**X**INLSHQ

Seq ID	Name/description	Amino acid sequence
69	SLO(ΔC101) fragment W535A – nnAA (*e.g.*, pAMF) 10 – w/o leader, with G overhang from cleavage (426511) X = nnAA (*e.g.*, pAMF) positions – K112, R151, K189, K323, K375, K464	GAPKEMPLESAEKEEKKSEDKKKSEEDHTEEIND**X**IYSLNYNEL EVLAKNGETIENFVPKEGVKKADKFIVIE**X**KKKNINTTPVDISI IDSVTDRTYPAALQLANKGFTEN**X**PDAVVTKRNPQKIHIDLPGM GDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYT ESMVYSKSQIEAALNVNSKILDGTLGIDFKSISKGEKKVMIAAY KQIFYTVSANLPNNPADVFDKSVTF**X**ELQRKGVSNEAPPLFVSN VAYGRTVFVKLETSSKSNDVEAAFSAALKGTDV**X**TNGKYSDILE NSSFTAVVLGGDAAEHNKVVTKDFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSG**X**INLSHQ
70	SLO(ΔC101) fragment W535A – nnAA (*e.g.*, pAMF) 10 – w/o leader, with G overhang from cleavage (426512) X = nnAA (*e.g.*, pAMF) positions – K98, K112, K189, K323, K375, K464	GAPKEMPLESAEKEEKKSED**X**KKSEEDHTEEIND**X**IYSLNYNEL EVLAKNGETIENFVPKEGVKKADKFIVIERKKKNINTTPVDISI IDSVTDRTYPAALQLANKGFTEN**X**PDAVVTKRNPQKIHIDLPGM GDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYT ESMVYSKSQIEAALNVNSKILDGTLGIDFKSISKGEKKVMIAAY KQIFYTVSANLPNNPADVFDKSVTF**X**ELQRKGVSNEAPPLFVSN VAYGRTVFVKLETSSKSNDVEAAFSAALKGTDV**X**TNGKYSDILE NSSFTAVVLGGDAAEHNKVVTKDFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSG**X**INLSHQ

Seq ID	Name/description	Amino acid sequence
71	SLO(ΔC101) fragment W535A – nnAA (*e.g.*, pAMF) 10 – w/o leader, with G overhang from cleavage (426513) X = nnAA (*e.g.*, pAMF) positions – K112, R151, K189, K272, K357, K407, K464	GAPKEMPLESAEKEEKKSEDKKKSEEDHTEEIND**X**IYSLNYNEL EVLAKNGETIENFVPKEGVKKADKFIVIE**X**KKKNINTTPVDISI IDSVTDRTYPAALQLANKGFTEN**X**PDAVVTKRNPQKIHIDLPGM GDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYT ESMVYSKSQIEAALNVNS**X**ILDGTLGIDFKSISKGEKKVMIAAY KQIFYTVSANLPNNPADVFDKSVTFKELQRKGVSNEAPPLFVSN VAYGRTVFVKLETSS**X**SNDVEAAFSAALKGTDVKTNGKYSDILE NSSFTAVVLGGDAAEHNKVVT**X**DFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSG**X**INLSHQ
72	SLO(ΔC101) fragment W535A – nnAA (*e.g.*, pAMF) 10 – w/o leader, with G overhang from cleavage (426514) X = nnAA (*e.g.*, pAMF) positions – K98, R151, K189, K323, K375, K407, K464	GAPKEMPLESAEKEEKKSED**X**KKSEEDHTEEINDKIYSLNYNEL EVLAKNGETIENFVPKEGVKKADKFIVIE**X**KKKNINTTPVDISI IDSVTDRTYPAALQLANKGFTEN**X**PDAVVTKRNPQKIHIDLPGM GDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYT ESMVYSKSQIEAALNVNSKILDGTLGIDFKSISKGEKKVMIAAY KQIFYTVSANLPNNPADVFDKSVTF**X**ELQRKGVSNEAPPLFVSN VAYGRTVFVKLETSSKSNDVEAAFSAALKGTDV**X**TNGKYSDILE NSSFTAVVLGGDAAEHNKVVT**X**DFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSG**X**INLSHQ

Seq ID	Name/description	Amino acid sequence
73	SLO(ΔC101) fragment W535A – nnAA (*e.g.*, pAMF) 10 – w/o leader, with G overhang from cleavage (426515) X = nnAA (*e.g.*, pAMF) positions – K112, K189, K272, K357, K375, K407, K464	GAPKEMPLESAEKEEKKSEDKKKSEEDHTEEIND**X**IYSLNYNEL EVLAKNGETIENFVPKEGVKKADKFIVIERKKKNINTTPVDISI IDSVTDRTYPAALQLANKGFTEN**X**PDAVVTKRNPQKIHIDLPGM GDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYT ESMVYSKSQIEAALNVNS**X**ILDGTLGIDFKSISKGEKKVMIAAY KQIFYTVSANLPNNPADVFDKSVTFKELQRKGVSNEAPPLFVSN VAYGRTVFVKLETSS**X**SNDVEAAFSAALKGTDV**X**TNGKYSDILE NSSFTAVVLGGDAAEHNKVVT**X**DFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSG**X**INLSHQ
74	SLO(ΔC101) fragment W535A – nnAA (*e.g.*, pAMF) 10 – w/o leader, with G overhang from cleavage (426516) X = nnAA (*e.g.*, pAMF) positions – K98, K112, R151, K189, K272, K323, K357, K375,	GAPKEMPLESAEKEEKKSED**X**KKSEEDHTEEIND**X**IYSLNYNEL EVLAKNGETIENFVPKEGVKKADKFIVIE**X**KKKNINTTPVDISI IDSVTDRTYPAALQLANKGFTEN**X**PDAVVTKRNPQKIHIDLPGM GDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYT ESMVYSKSQIEAALNVNS**X**ILDGTLGIDFKSISKGEKKVMIAAY KQIFYTVSANLPNNPADVFDKSVTF**X**ELQRKGVSNEAPPLFVSN VAYGRTVFVKLETSS**X**SNDVEAAFSAALKGTDV**X**TNGKYSDILE NSSFTAVVLGGDAAEHNKVVTKDFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSGKINLSHQ

Seq ID	Name/description	Amino acid sequence
75	SLO(ΔC101) fragment W535A – nnAA (*e.g.*, pAMF) 10 – w/o leader, with G overhang from cleavage (426510) X = nnAA (*e.g.*, pAMF) positions – K98, R151, K189, K272, K323, K357, K407, K464	GAPKEMPLESAEKEEKKSED**X**KKSEEDHTEEINDKIYSLNYNEL EVLAKNGETIENFVPKEGVKKADKFIVIE**X**KKKNINTTPVDISI IDSVTDRTYPAALQLANKGFTEN**X**PDAVVTKRNPQKIHIDLPGM GDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYT ESMVYSKSQIEAALNVNS**X**ILDGTLGIDFKSISKGEKKVMIAAY KQIFYTVSANLPNNPADVFDKSVTF**X**ELQRKGVSNEAPPLFVSN VAYGRTVFVKLETSS**X**SNDVEAAFSAALKGTDVKTNGKYSDILE NSSFTAVVLGGDAAEHNKVVT**X**DFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSG**X**INLSHQ
76	SLO(ΔC101) fragment W535A – nnAA (*e.g.*, pAMF) 10 – w/o leader, with G overhang from cleavage (426515) X = nnAA (*e.g.*, pAMF) positions – K112, K189, K272, K323, K357, K375, K407, K464	GAPKEMPLESAEKEEKKSEDKKKSEEDHTEEIND**X**IYSLNYNEL EVLAKNGETIENFVPKEGVKKADKFIVIERKKKNINTTPVDISI IDSVTDRTYPAALQLANKGFTEN**X**PDAVVTKRNPQKIHIDLPGM GDKATVEVNDPTYANVSTAIDNLVNQWHDNYSGGNTLPARTQYT ESMVYSKSQIEAALNVNS**X**ILDGTLGIDFKSISKGEKKVMIAAY KQIFYTVSANLPNNPADVFDKSVTF**X**ELQRKGVSNEAPPLFVSN VAYGRTVFVKLETSS**X**SNDVEAAFSAALKGTDV**X**TNGKYSDILE NSSFTAVVLGGDAAEHNKVVT**X**DFDVIRNVIKDNATFSRKNPAY PISYTSVFLKNNKIAGVNNRTEYVETTSTEYTSG**X**INLSHQ

CLAIMS

1. An immunogenic composition comprising
   a. a first Group A Streptococcus (GAS) polypeptide antigen;
   b. a second GAS polypeptide antigen; and
   c. at least one polypeptide-polysaccharide conjugate, wherein the conjugate polypeptide is a third GAS polypeptide antigen or a non-GAS carrier polypeptide and comprises at least one non-natural amino acid, and wherein the conjugate polysaccharide is a GAS polysaccharide or a variant thereof that lacks an immunodominant N-acetyl Glucosamine (GlcNAc) side chain.

2. The immunogenic composition of claim 1, wherein the first and second GAS polypeptide antigens are independently selected from C5a peptidase, streptolysin O (SLO), Sib35, Sfb1, and SpyAD.

3. The immunogenic composition of claim 1, wherein the first and second GAS polypeptide antigens are C5a peptidase and SLO.

4. The immunogenic composition of any one of claims 1-3 wherein the first and second GAS polypeptide antigens are C5a peptidase and SLO, and the C5a peptidase polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 29 or SEQ ID NO: 30.

5. The immunogenic composition of any one of claims 1-3 wherein the first and second GAS polypeptide antigens are C5a peptidase and SLO, and the C5a peptidase polypeptide antigen consists of the amino acid sequence of SEQ ID NO: 29 or SEQ ID NO: 30.

6. The immunogenic composition of any one of claims 1-5, wherein the SLO polypeptide antigen comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 31 or SEQ ID NO: 32.

7. The immunogenic composition of any one of claims1-4 , wherein the first and second GAS polypeptide antigens are C5a peptidase and SLO, and the SLO polypeptide

antigen comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53.

8. The immunogenic composition of any one of claims 1-5, wherein the SLO polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 31 or SEQ ID NO: 32.

9. The immunogenic composition of claim 3, wherein the SLO polypeptide antigen consists of the amino acid sequence of SEQ ID NO: 31 or SEQ ID NO: 32.

10. The immunogenic composition of any one of claims 1-4, wherein the first and second GAS polypeptide antigens are C5a peptidase and SLO, and the SLO polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53.

11. The immunogenic composition of any one of claims 1-4, wherein the first and second GAS polypeptide antigens are C5a peptidase and SLO, and the SLO polypeptide antigen consists of the amino acid sequence of SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53.

12. The immunogenic composition claims 1 or 4, wherein the first and second GAS polypeptide antigens are C5a peptidase and SpyAD.

13. The immunogenic composition of any one of claims 1-3, 8-9, or 12, wherein the C5a peptidase polypeptide antigen comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 29 or SEQ ID NO: 30.

14. The immunogenic composition of any one of claims 1-3, 8-9 or 12, wherein the C5a peptidase polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 29 or SEQ ID NO: 30.

15. The immunogenic composition of any one of claims 1-3, 8-9, 12, or 14, wherein the C5a peptidase polypeptide antigen consists of the amino acid sequence of SEQ ID NO: 20 or SEQ ID NO: 30.

16. The immunogenic composition of any one of claims 1-15, wherein the third GAS polypeptide antigen or non-GAS polypeptide antigen is selected from Streptococcus pyogenes Adhesion and Division (SpyAD) polypeptide, arginine deiminase (ADI), SEQ ID NO: 25, and Protein D.

17. The immunogenic composition of any one of claims 1-15, wherein the third GAS polypeptide antigen or non-GAS polypeptide antigen is selected from Streptococcus pyogenes Adhesion and Division (SpyAD) polypeptide, arginine deiminase (ADI), SEQ ID NO: 25, Protein D, and SLO.

18. The immunogenic composition of any one of claims 1-17, wherein the nnAA comprises a click chemistry reactive group.

19. The immunogenic composition of any one of claims 1-18, wherein the nnAA is selected from 2-amino-3-(4-azidophenyl)propanoic acid (pAF), 2-amino-4-azidobutanoic acid, 2-azido-3-phenylpropionic acid, 2-amino-3-azidopropanoic acid, 2-amino-3-(4-(azidomethyl)phenyl)propanoic acid (pAMF), 2-amino-3-(5-(azidomethyl)pyridin-2-yl)propanoic acid, 2-amino-3-(4-(azidomethyl)pyridin-2-yl)propanoic acid, 2-amino-3-(6-(azidomethyl)pyridin-3-yl)propanoic acid, and 2-amino-5-azidopentanoic acid.

20. The immunogenic composition of claim 18 or 19, wherein the nnAA is pAMF.

21. The immunogenic composition of any one of claims 16-20, wherein the conjugate polypeptide is a third GAS protein, which is a SpyAD polypeptide and comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 33.

22. The immunogenic composition of any one of claims 16-21, wherein the SpyAD polypeptide comprises a pAMF substitution at positions K64, K287, K386, and K657 of SEQ ID NO: 33.

23. The immunogenic composition of any one of claims 16-22, wherein the SpyAD polypeptide comprises the amino acid sequence of SEQ ID NO: 33 or SEQ ID NO: 34.

24. The immunogenic composition of claim 22, wherein the SpyAD polypeptide consisting of the amino acid sequence of SEQ ID NO: 33 or SEQ ID NO: 34.

25. The immunogenic composition of any one of claims 16-20, wherein the conjugate polypeptide is a third GAS polypeptide, which is an ADI polypeptide and comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 35 or SEQ ID NO: 36.

26. The immunogenic composition of any one of claims 16-20 or 25, wherein the ADI polypeptide comprises the amino acid sequence of SEQ ID NO: 27, SEQ ID NO: 37 or SEQ ID NO: 38.

27. The immunogenic composition of any one of claims 16-20 or 25, wherein the ADI polypeptide consist of the amino acid sequence of SEQ ID NO: 27, SEQ ID NO: 37 or SEQ ID NO: 38.

28. The immunogenic composition of any one of claims 17-20, wherein the conjugate polypeptide is a third GAS polypeptide, which is a SLO polypeptide and comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 31, SEQ ID NO: 32, or SEQ ID NO: 53.

29. The immunogenic composition of claim 28, wherein the SLO polypeptide comprises 3 or 4 pAMF substitutions at positions selected from K98, K112, R151, K189, K272, K323, K357, K375, K407, or K464.

30. The immunogenic composition of claim 29, wherein the SLO polypeptide comprises the amino acid sequence of SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, or SEQ ID NO: 64.

31. The immunogenic composition of claim 29, wherein the SLO polypeptide consists of the amino acid sequence of SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, or SEQ ID NO: 64.

32. The immunogenic composition of claim 28, wherein the SLO polypeptide comprises the amino acid sequence of SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, or SEQ ID NO: 76.

33. The immunogenic composition of claim 28, wherein the SLO polypeptide consists of the amino acid sequence of SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, or SEQ ID NO: 76.

34. The immunogenic composition of any one of claims 18-20, wherein the conjugate polypeptide is a ferritin polypeptide and comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 21.

35. The immunogenic composition of claim 34, wherein the ferritin polypeptide comprises a pAMF substitution at position I5 of SEQ ID NO: 21.

36. The immunogenic composition of any one of claims 1-35, wherein the conjugate polysaccharide lacks the immunodominant GlcNAc side chain.

37. The immunogenic composition of any one of claims 1-36, wherein the conjugate polysaccharide has an average molecular weight of about 10 kDa to about 45 kDa.

38. An immunogenic composition comprising

a. a Group A Streptococcus (GAS) C5a peptidase polypeptide antigen;

b. a GAS streptolysin O (SLO) polypeptide antigen; and

c. a polypeptide-polysaccharide conjugate comprising a Streptococcus pyogenes Adhesion and Division (SpyAD) conjugate polypeptide and a GAS conjugate polysaccharide or a variant thereof that lacks an immunodominant N-acetyl Glucosamine (GlcNAc) side chain.

39. The immunogenic composition of claim 38, wherein the SpyAD protein comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 33 and comprises a pAMF substitution at positions K64, K287, K386, and K657.

40. The immunogenic composition of claims 38 or 39, wherein the SLO polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53.

41. The immunogenic composition of claims 1 or 38, comprising a C5a peptidase polypeptide antigen of SEQ ID NO: 30; a SLO polypeptide antigen of SEQ ID NO: 53; and a polypeptide-polysaccharide conjugate comprising a SpyAD conjugate polypeptide of SEQ ID NO: 34 and a GAS conjugate polysaccharide.

42. The immunogenic composition of claims 1 or 38, comprising a C5a peptidase polypeptide antigen of SEQ ID NO: 30; a SLO polypeptide antigen of SEQ ID NO: 52; and a polypeptide-polysaccharide conjugate comprising a SpyAD conjugate polypeptide of SEQ ID NO: 34 and a GAS conjugate polysaccharide.

43. The immunogenic composition of claims 1 or 38, comprising a C5a peptidase polypeptide antigen of SEQ ID NO: 30; a SLO polypeptide antigen of SEQ ID NO: 32; and a polypeptide-polysaccharide conjugate comprising a SpyAD conjugate polypeptide of SEQ ID NO: 34 and a GAS conjugate polysaccharide.

44. The immunogenic composition of claims 1 or 38, comprising a C5a peptidase polypeptide antigen of SEQ ID NO: 29; a SLO polypeptide antigen of SEQ ID NO: 53; and a polypeptide-polysaccharide conjugate comprising a SpyAD conjugate polypeptide of SEQ ID NO: 34 and a GAS conjugate polysaccharide.

45. An immunogenic composition comprising

a. a Group A Streptococcus (GAS) C5a peptidase polypeptide antigen;

b. a GAS streptolysin O (SLO) polypeptide antigen; and

c. a polypeptide-polysaccharide conjugate comprising an arginine deiminase (ADI) conjugate polypeptide and a GAS conjugate polysaccharide or a variant thereof that lacks an immunodominant N-acetyl Glucosamine (GlcNAc) side chain.

46. The immunogenic composition of claim 45, wherein the ADI polypeptide comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 35 or SEQ ID NO: 36 and comprises a pAMF substitution at positions K15, K193, and K316.

47. An immunogenic composition comprising

a. a Group A Streptococcus (GAS) C5a peptidase polypeptide antigen;

b. a GAS streptolysin O (SLO) polypeptide antigen; and

c. a polypeptide-polysaccharide conjugate comprising a ferritin polypeptide and a GAS conjugate polysaccharide or a variant thereof that lacks an immunodominant N-acetyl Glucosamine (GlcNAc) side chain.

48. The immunogenic composition of claim 47, wherein the ferritin protein comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 21 and comprises a pAMF substitution at position I5.

49. The immunogenic composition of any one of claims 1-48, wherein the polypeptide-polysaccharide conjugate has an average molecular mass of about 10 kDa to about 5000 kDa.

50. A method of inducing a protective immune response against a Group A Streptococcus (GAS) bacterium in a subject comprising administering the immunogenic composition of any one of claims 1-49 to the subject.

51. The method of claim 50, wherein the immunogenic composition induces an antibody response in the subject against the Group A Streptococcus (GAS) bacterium and does not induce an antibody response in the subject against human tissue.

52. The method of claims 50 or 51, wherein the immunogenic composition induces a protective immune response against a Shigella bacterium in the subject, wherein the Shigella bacterium comprises a polysaccharide with a polyrhmanose backbone.

53. A method of inducing a protective immune response against a Shigella bacterium in a subject comprising administering the immunogenic composition of any one of claims 1-49 to the subject.

54. Use of the immunogenic composition of any one of claims 1-49 for inducing a protective immune response against a Shigella bacterium in a subject.

55. Use of the immunogenic composition of any one of claims 1-49 in the manufacture of a medicament for inducing a protective immune response against a Shigella bacterium in a subject.

56. Use of the immunogenic composition of any one of claims 1-49 in the manufacture of a medicament for inducing a protective immune response against a GAS bacterium in a subject.

57. Use of the immunogenic composition of any one of claims 1-82 for inducing a protective immune response against a GAS bacterium in a subject.

FIG. 1A

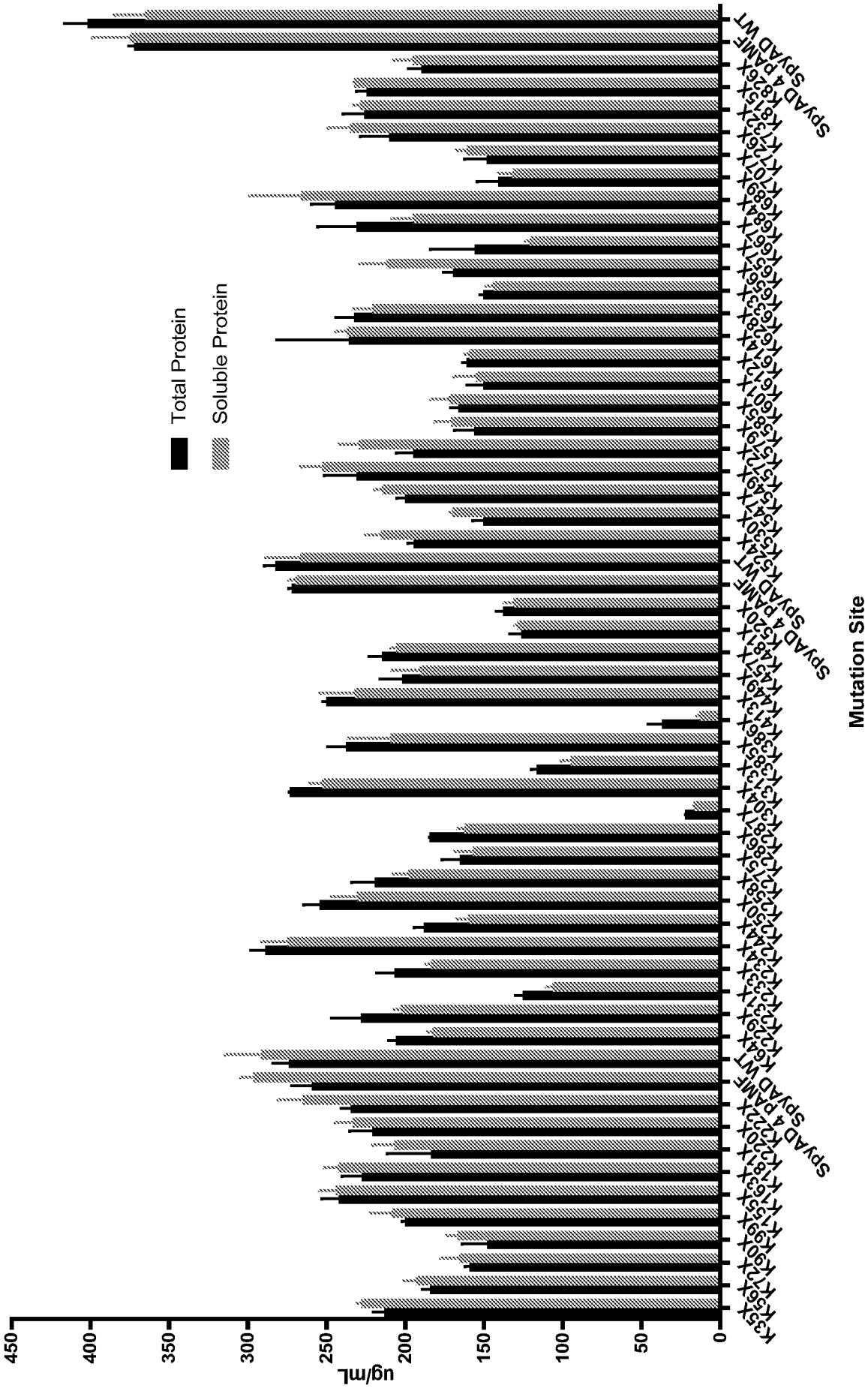
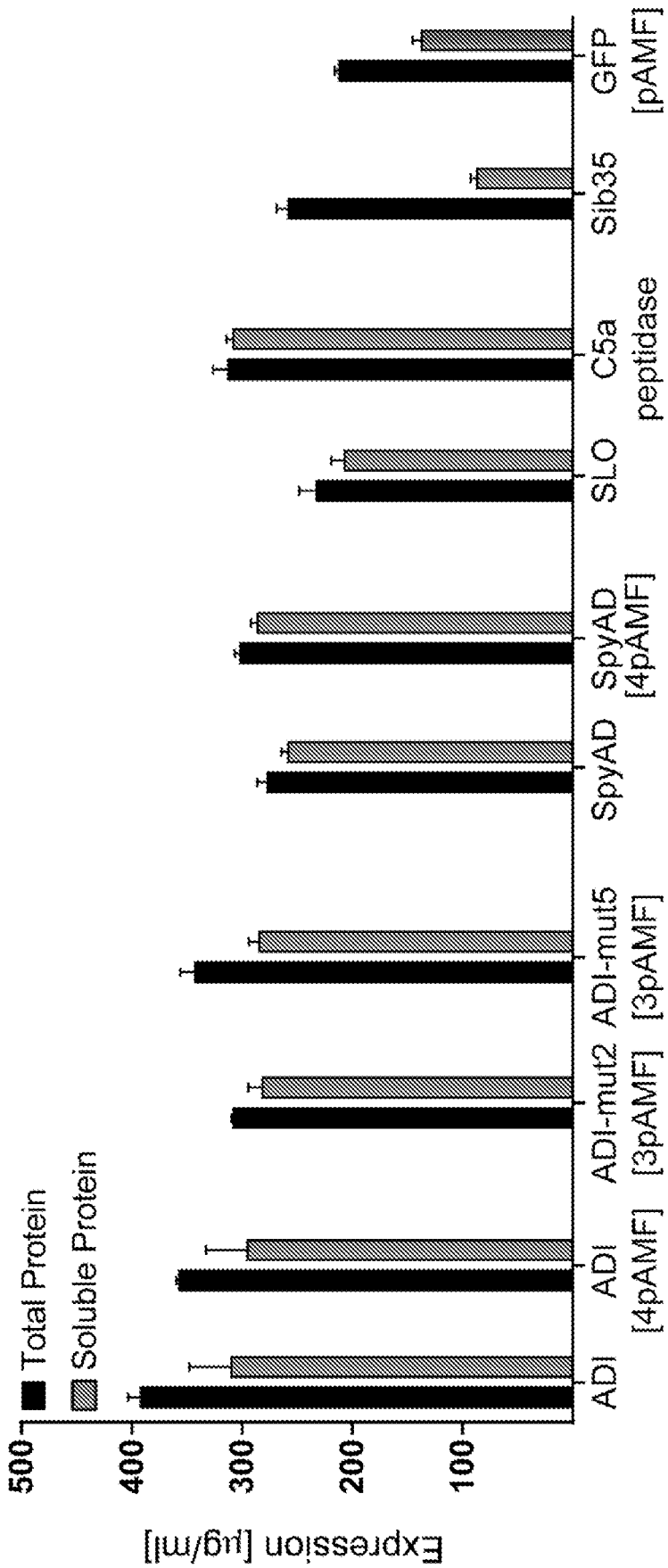

SpyAD Single Site Substitution Expression Analysis
Total Protein
Soluble Protein
ug/mL
450
400
350
300
250
200
150
100
50
0
Mutation Site
SpyAD WT
SpyAD P4AMF
SpyAD K26A
SpyAD K32A
SpyAD K51A
SpyAD K53A
SpyAD K54A
SpyAD K57A
SpyAD K58A
SpyAD K60A
SpyAD K61A
SpyAD K62A
SpyAD K63A
SpyAD K65A
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SpyAD K70A
SpyAD K72A
SpyAD K73A
SpyAD K81A
SpyAD K82A
SpyAD P4AMF
SpyAD WT
SpyAD K220A
SpyAD K222A
SpyAD K223A
SpyAD K224A
SpyAD K225A
SpyAD K227A
SpyAD K228A
SpyAD K229A
SpyAD K230A
SpyAD K233A
SpyAD K234A
SpyAD K235A
SpyAD K236A
SpyAD K238A
SpyAD K239A
SpyAD K241A
SpyAD K242A
SpyAD K243A
SpyAD K244A
SpyAD K247A
SpyAD K249A
SpyAD K251A
SpyAD K252A
SpyAD P4AMF
SpyAD WT

FIG. 1B


Total Protein
Soluble Protein
Expression [µg/ml]
500
400
300
200
100
ADI [4pAMF]
ADI [4pAMF]
ADI-mut2 [3pAMF]
ADI-mut5 [3pAMF]
SpyAD
SpyAD [4pAMF]
SLO
C5a peptidase
Sib35
GFP [pAMF]

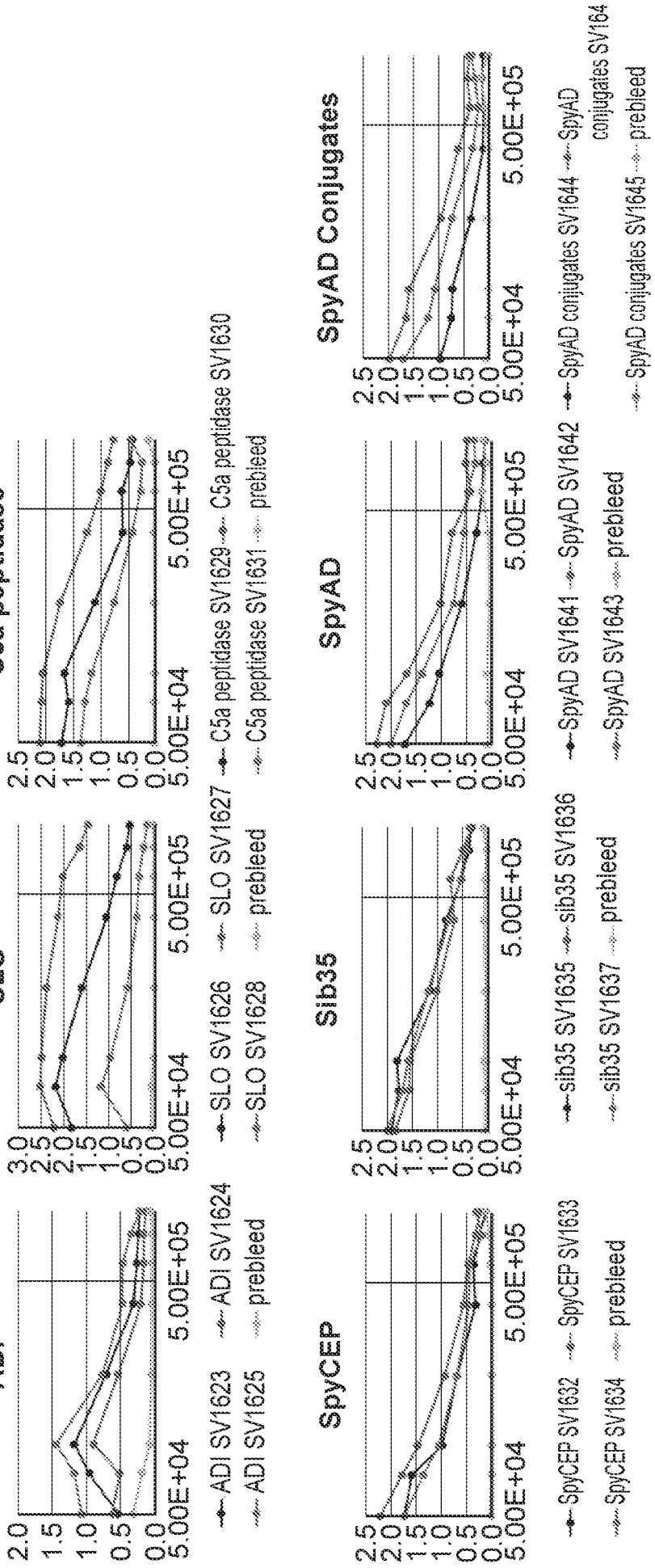

ADI
SLO
C5a peptidase
SpyCEP
Sib35
SpyAD
SpyAD Conjugates
ADI SV1623
ADI SV1624
ADI SV1625
prebleed
SLO SV1626
SLO SV1627
SLO SV1628
C5a peptidase SV1629
C5a peptidase SV1630
C5a peptidase SV1631
SpyCEP SV1632
SpyCEP SV1633
SpyCEP SV1634
sib35 SV1635
sib35 SV1636
sib35 SV1637
SpyAD SV1641
SpyAD SV1642
SpyAD SV1643
SpyAD conjugates SV1644
SpyAD conjugates SV1645
SpyAD conjugates SV164
5.00E+04
5.00E+05

FIG. 2

FIG. 3A

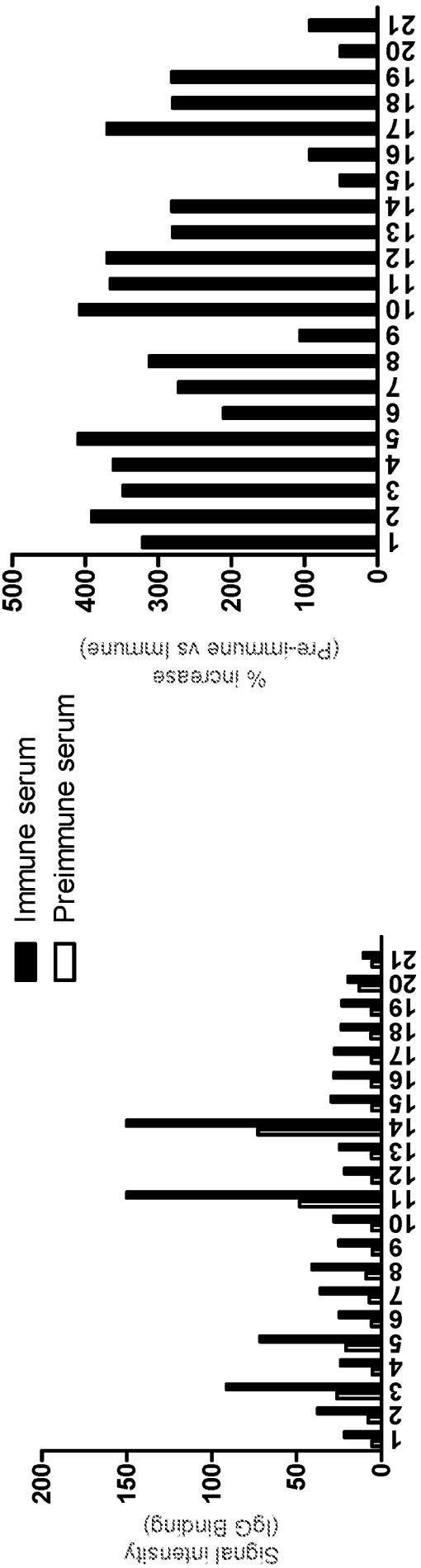
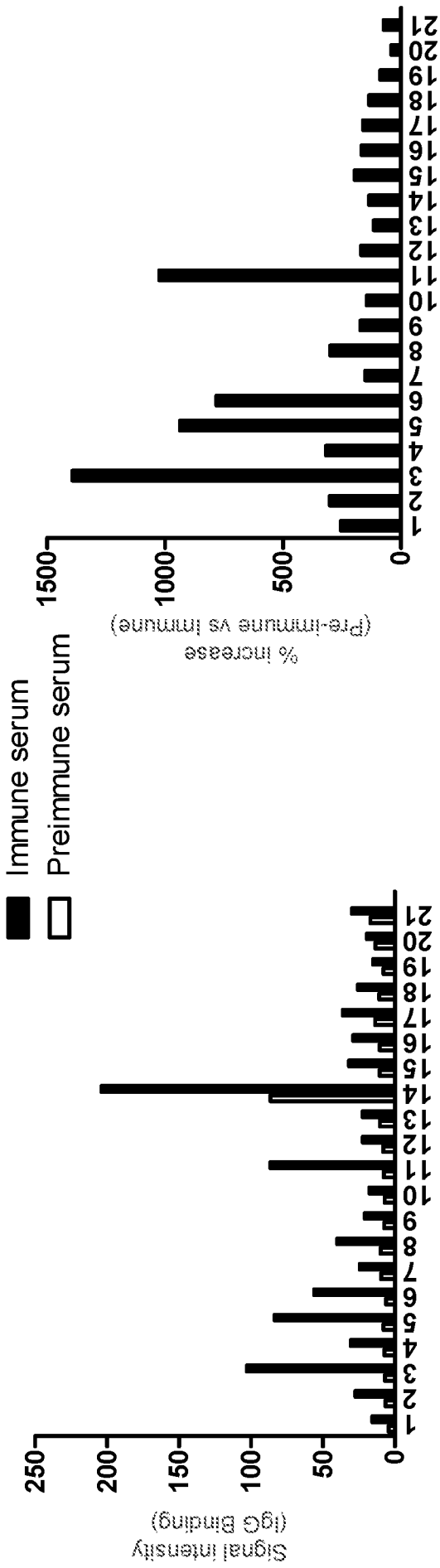
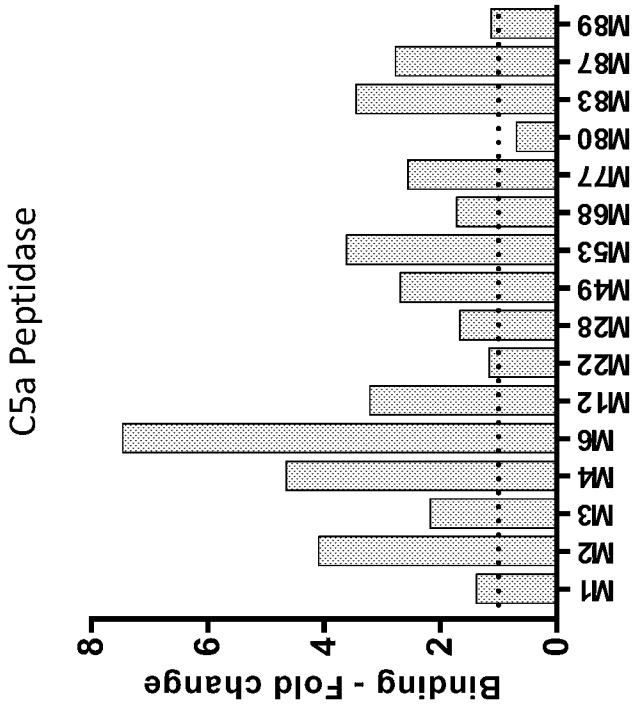

Signal intensity
(IgG Binding)
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Immune serum
Preimmune serum
% increase
(Pre-immune vs Immune)
500
400
300
200
100
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

FIG. 3B


Signal intensity
(IgG Binding)
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Immune serum
Preimmune serum
% increase
(Pre-immune vs Immune)
1500
1000
500
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21


C5a Peptidase
Binding - Fold change
0
2
4
6
8
M1
M2
M3
M4
M6
M12
M22
M28
M49
M53
M68
M77
M80
M83
M87
M89

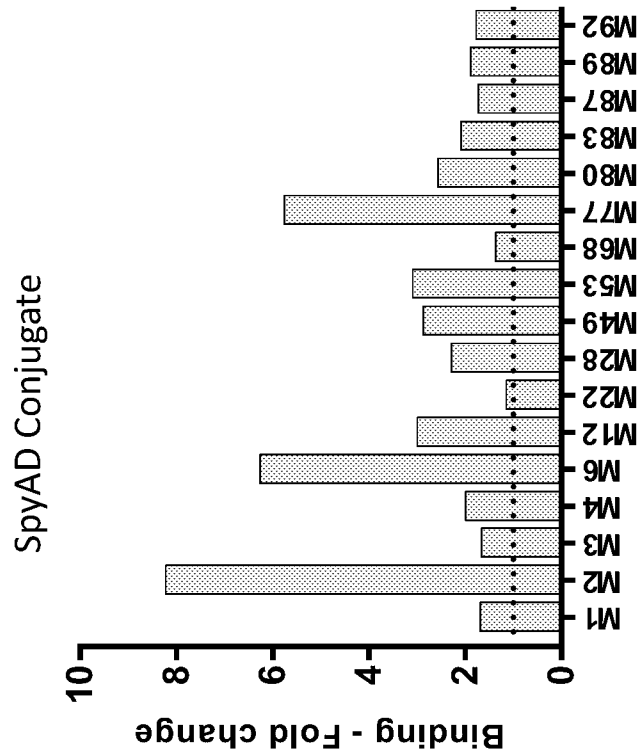

SpyAD Conjugate
Binding - Fold change
0
2
4
6
8
10
M1
M2
M3
M4
M6
M12
M22
M28
M49
M53
M68
M77
M80
M83
M87
M89
M92

FIG. 3C

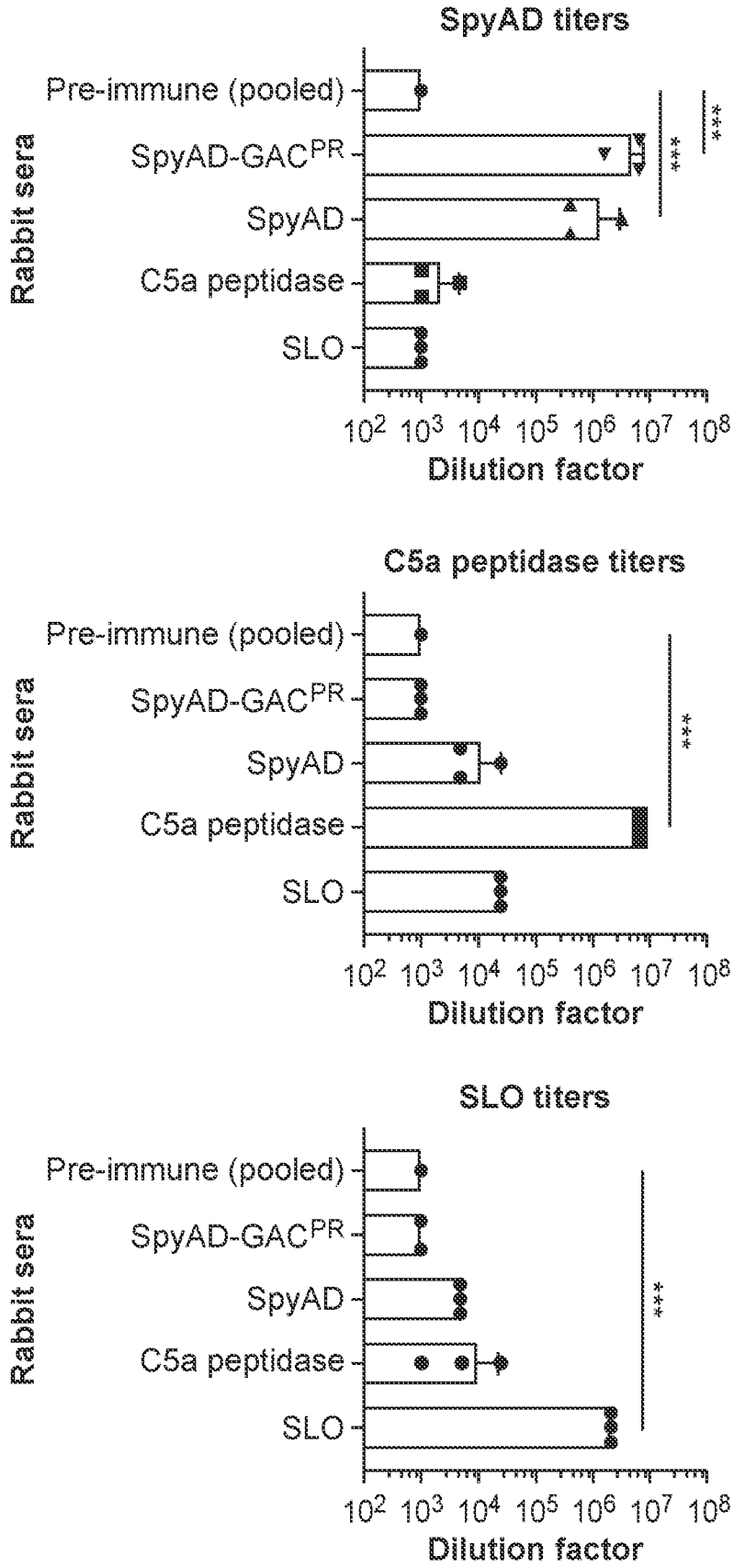

SpyAD titers
Pre-immune (pooled)
SpyAD-GAC^PR
SpyAD
C5a peptidase
SLO
Rabbit sera
***
***
10^2 10^3 10^4 10^5 10^6 10^7 10^8
Dilution factor
C5a peptidase titers
Pre-immune (pooled)
SpyAD-GAC^PR
SpyAD
C5a peptidase
SLO
Rabbit sera
***
10^2 10^3 10^4 10^5 10^6 10^7 10^8
Dilution factor
SLO titers
Pre-immune (pooled)
SpyAD-GAC^PR
SpyAD
C5a peptidase
SLO
Rabbit sera
***
10^2 10^3 10^4 10^5 10^6 10^7 10^8
Dilution factor

FIG. 3D

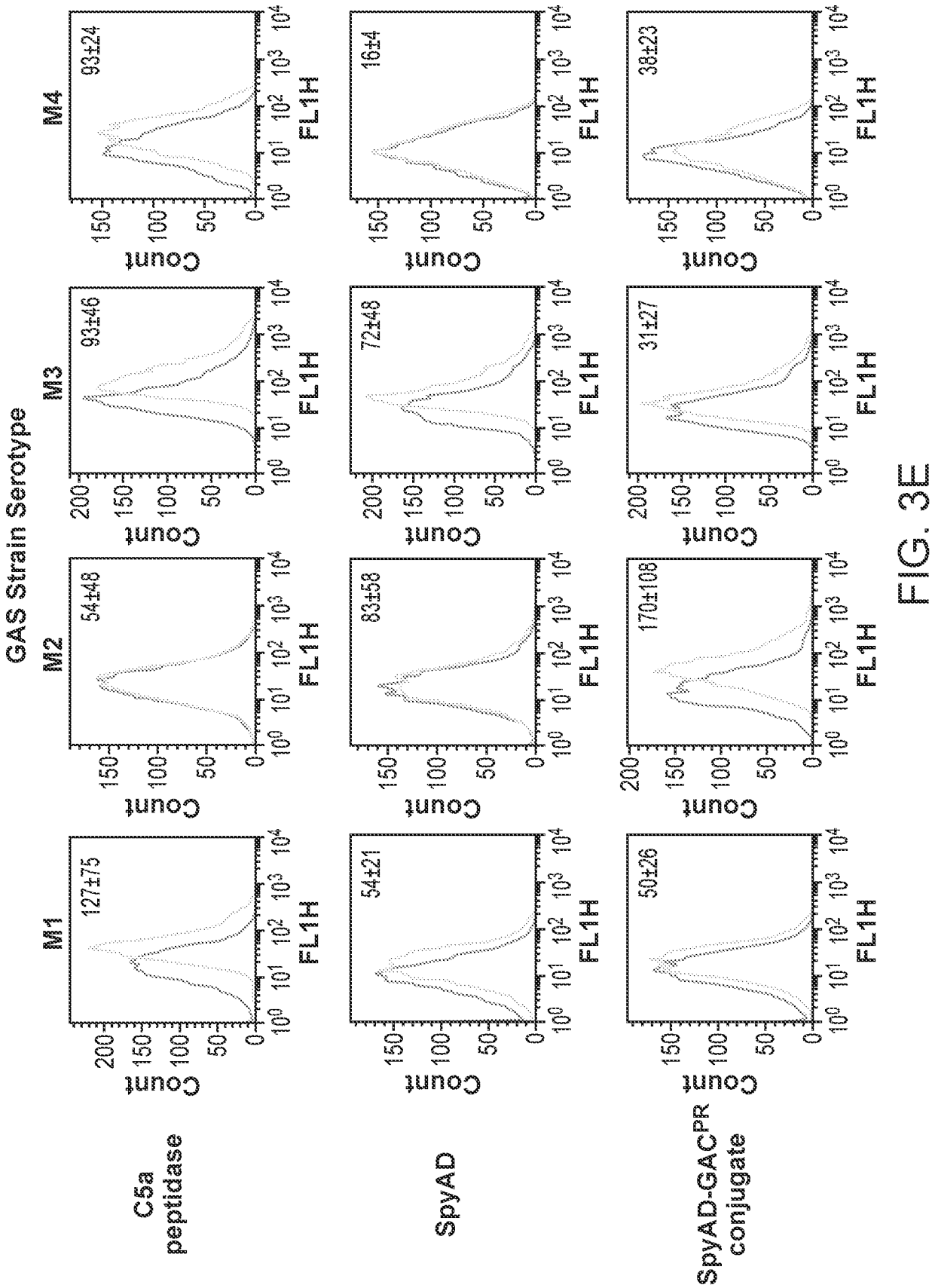

GAS Strain Serotype
M1
M2
M3
M4
C5a peptidase
SpyAD
SpyAD-GAC^PR conjugate
127±75
54±48
93±46
93±24
54±21
83±58
72±48
16±4
50±26
170±108
31±27
38±23
Count
FL1H

FIG. 3E

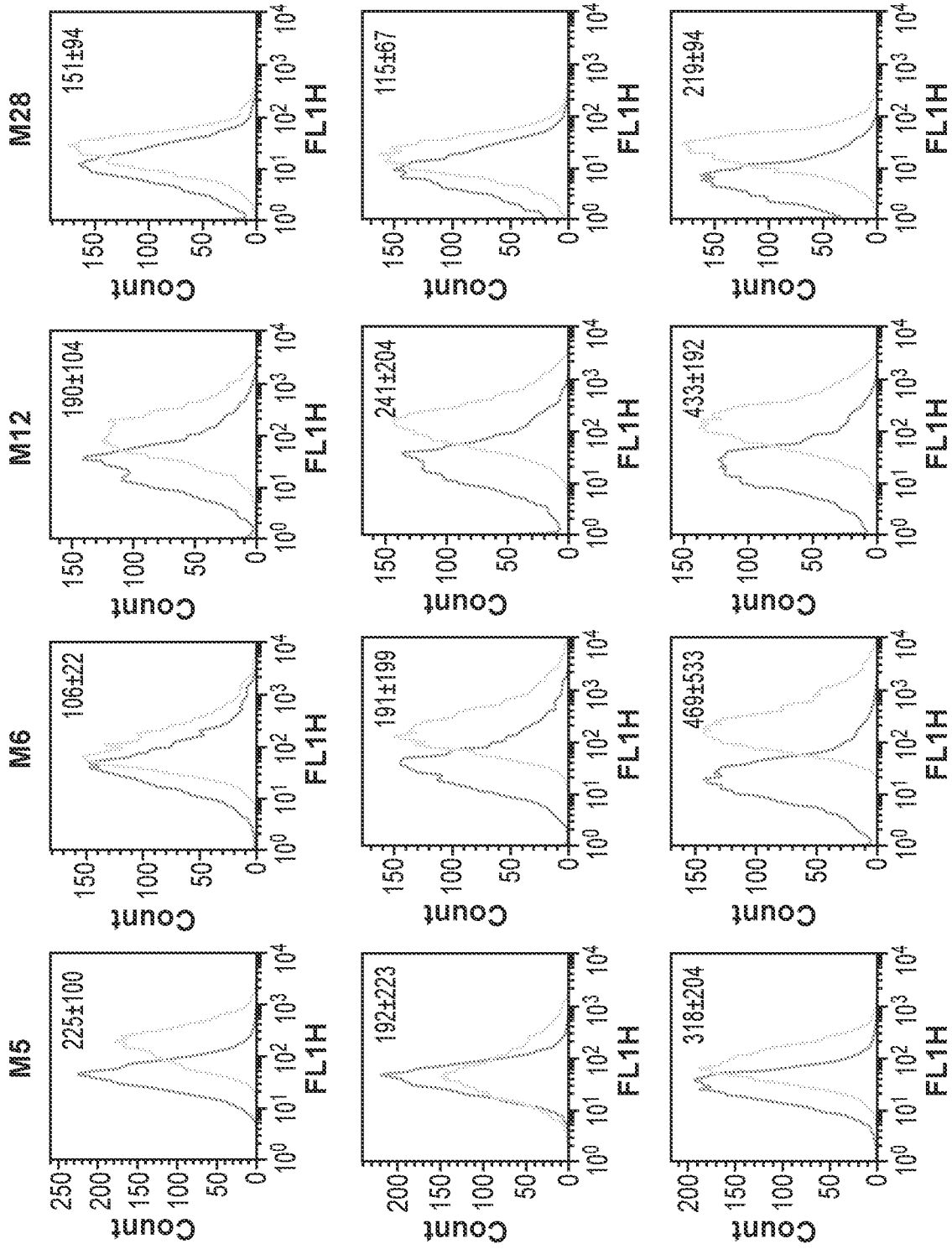

M5
M6
M12
M28
225±100
106±22
190±104
151±94
192±223
191±199
241±204
115±67
318±204
469±533
433±192
219±94
Count
FL1H

FIG. 3E (Cont.)

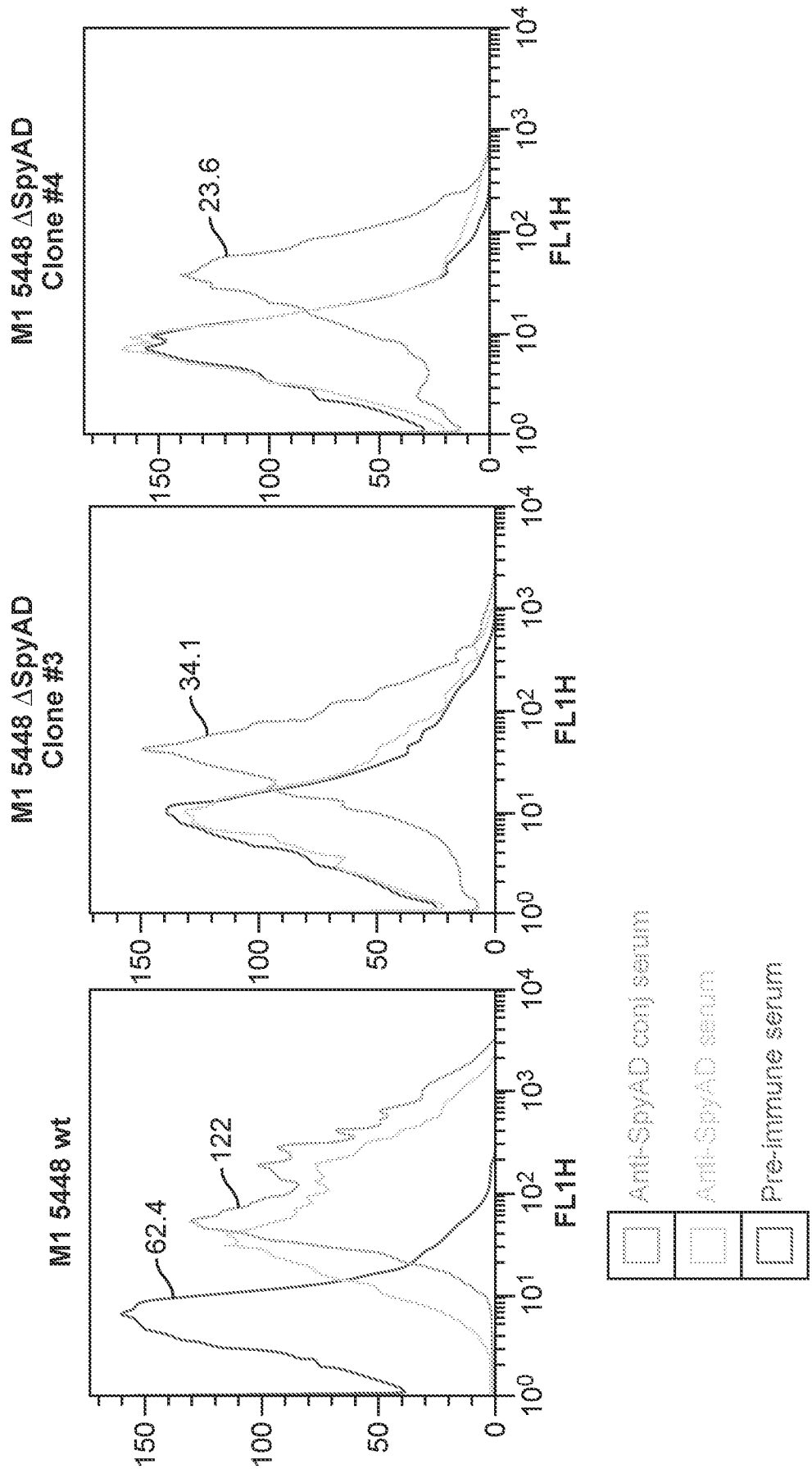

M1 5448 wt
M1 5448 ΔSpyAD Clone #3
M1 5448 ΔSpyAD Clone #4
62.4
122
34.1
23.6
FL1H
Anti-SpyAD conj serum
Anti-SpyAD serum
Pre-immune serum

FIG. 3F

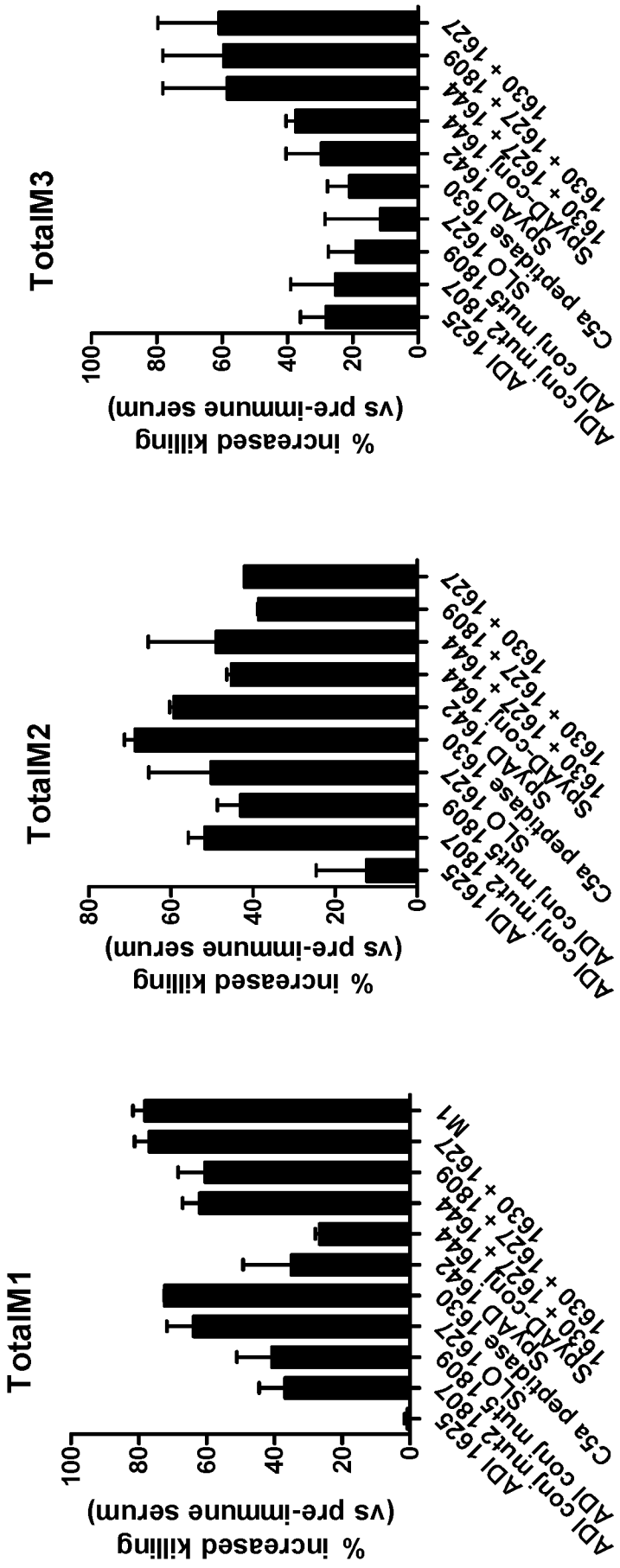

TotalM1
% increased killing
(vs pre-immune serum)
0
20
40
60
80
100
ADI 1625
ADI conj mut2 1807
ADI conj mut5 1809
Csa peptidase SLO 1627
SpyAD 1630
SpyAD-conj 1642
1630 + 1627 + 1644
1630 + 1627 + 1644 + 1809
1630 + 1627
M1
TotalM2
% increased killing
(vs pre-immune serum)
0
20
40
60
80
ADI 1625
ADI conj mut2 1807
ADI conj mut5 1809
Csa peptidase SLO 1627
SpyAD 1630
SpyAD-conj 1642
1630 + 1627 + 1644
1630 + 1627 + 1644 + 1809
1630 + 1627
TotalM3
% increased killing
(vs pre-immune serum)
0
20
40
60
80
100
ADI 1625
ADI conj mut2 1807
ADI conj mut5 1809
Csa peptidase SLO 1627
SpyAD 1630
SpyAD-conj 1642
1630 + 1627 + 1644
1630 + 1627 + 1644 + 1809
1630 + 1627

FIG. 4

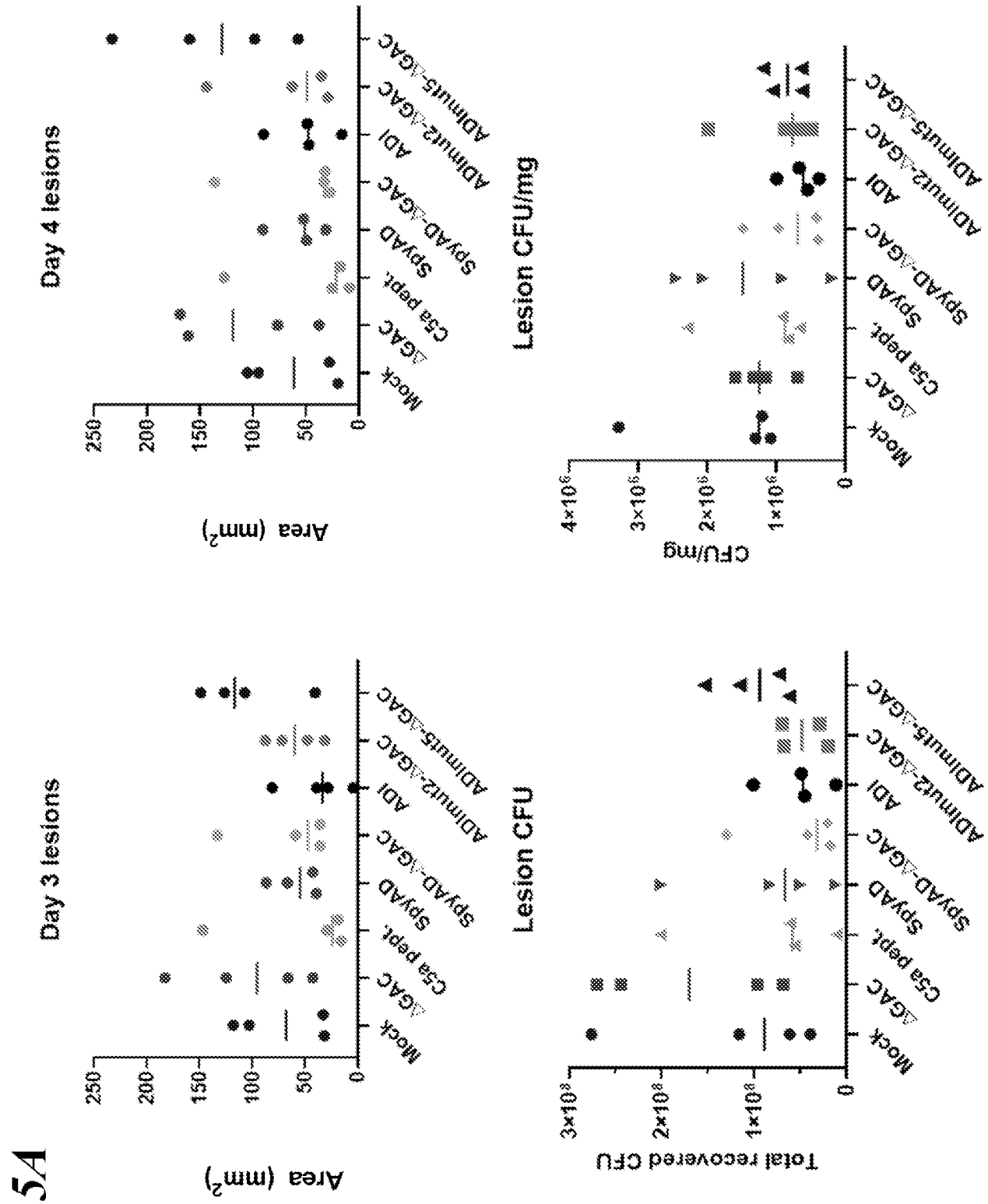

Day 3 lesions
Area (mm²)
Day 4 lesions
Area (mm²)
Lesion CFU
Total recovered CFU
Lesion CFU/mg
CFU/mg
Mock
ΔGAC
Csa pept.
SpyAD
SpyAD-ΔGAC
ADI
ADImut2-ΔGAC
ADImut5-ΔGAC

FIG. 5A

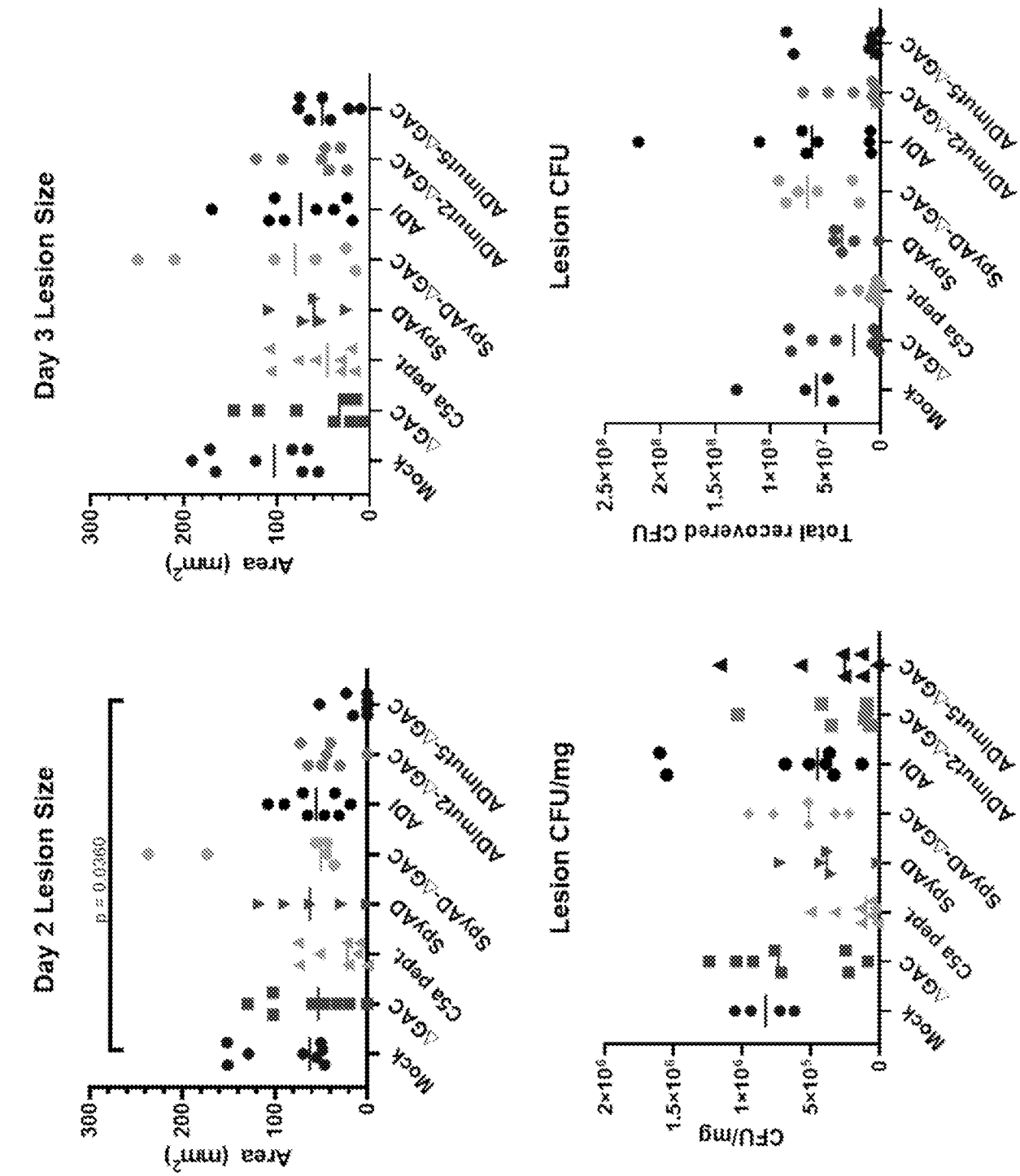

Day 3 Lesion Size
Area (mm²)
300
200
100
0
Mock
ΔGAC
Csa pept.
SpyAD
SpyAD-ΔGAC
ADI
ADImut2-ΔGAC
ADImut5-ΔGAC
Lesion CFU
Total recovered CFU
2.5×10⁸
2×10⁸
1.5×10⁸
1×10⁸
5×10⁷
0
Day 2 Lesion Size
p = 0.0360
Lesion CFU/mg
CFU/mg
2×10⁶
1.5×10⁶
1×10⁶
5×10⁵
0

FIG. 5B

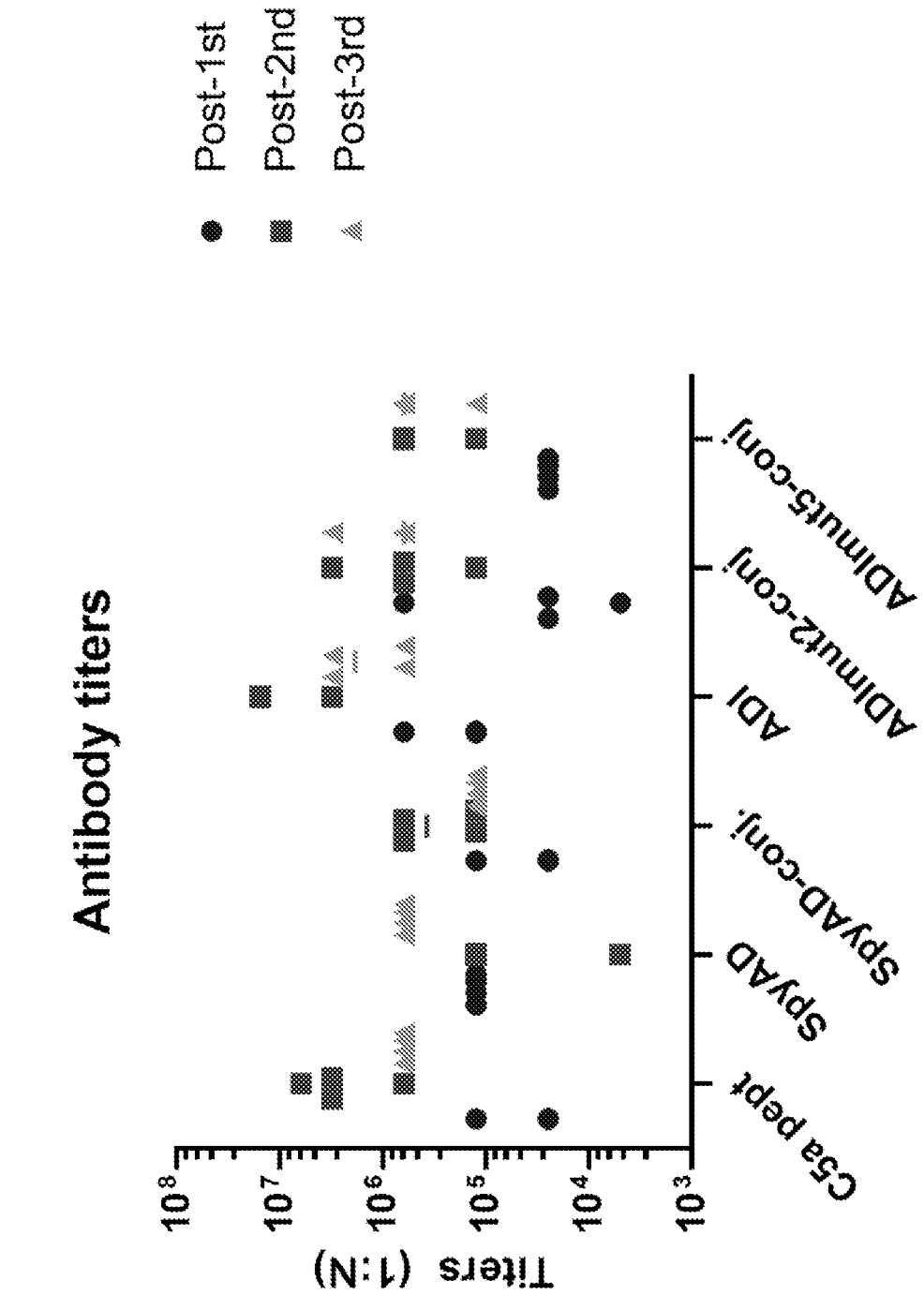

Post-1st
Post-2nd
Post-3rd
Antibody titers
Titers (1:N)
10^8
10^7
10^6
10^5
10^4
10^3
C5a pept
SpyAD
SpyAD-conj.
ADI
ADImut2-conj.
ADImut5-conj.

FIG. 5C

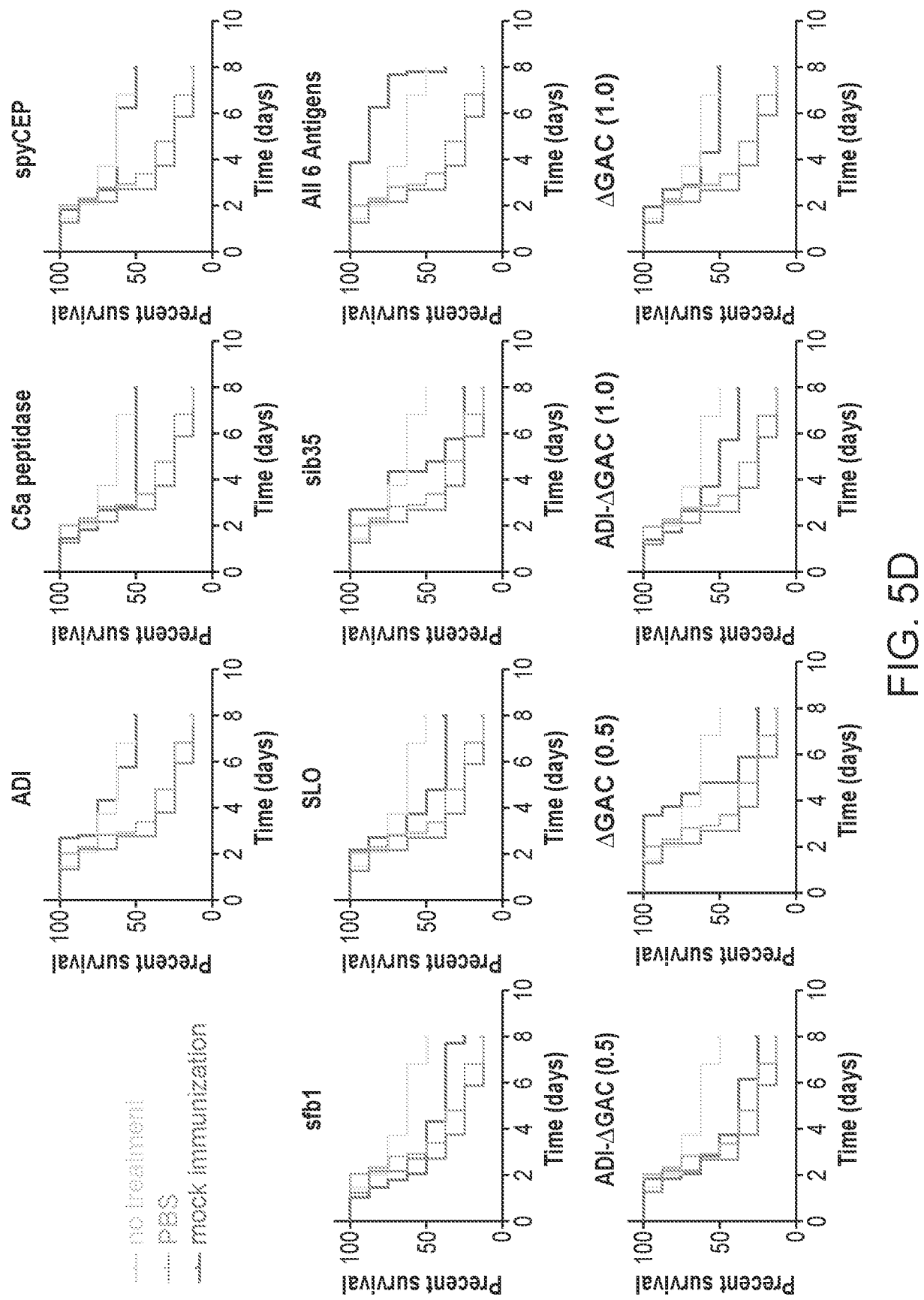

no treatment
PBS
mock immunization
ADI
C5a peptidase
spyCEP
sfb1
sib35
All 6 Antigens
SLO
ΔGAC (0.5)
ΔGAC (1.0)
ADI-ΔGAC (0.5)
ADI-ΔGAC (1.0)
Percent survival
Time (days)
0
50
100
0
2
4
6
8
10

FIG. 5D

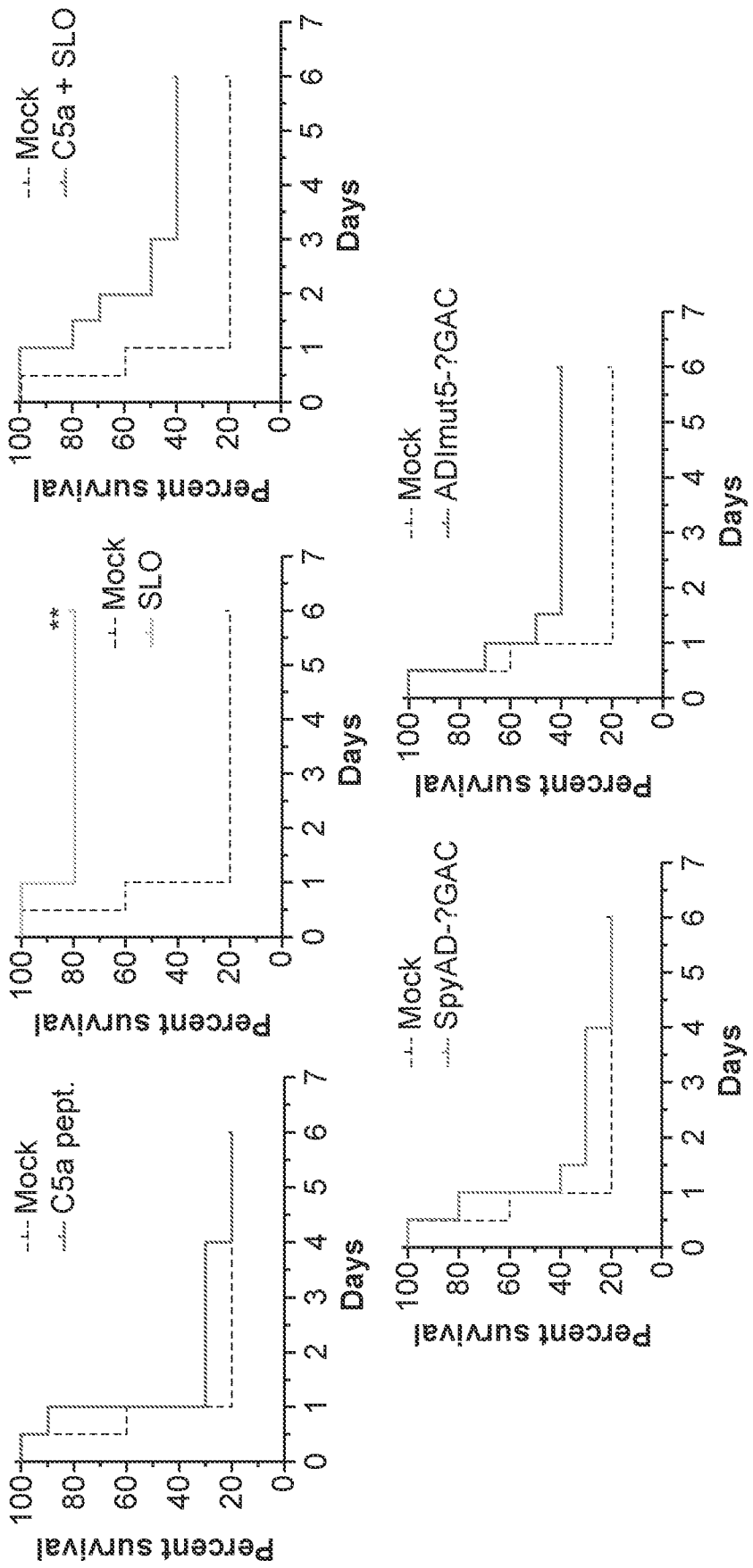

Mock
C5a pept.
Percent survival
Days
Mock
SLO
**
Mock
C5a + SLO
Mock
SpyAD-?GAC
Mock
ADImut5-?GAC

FIG. 5E

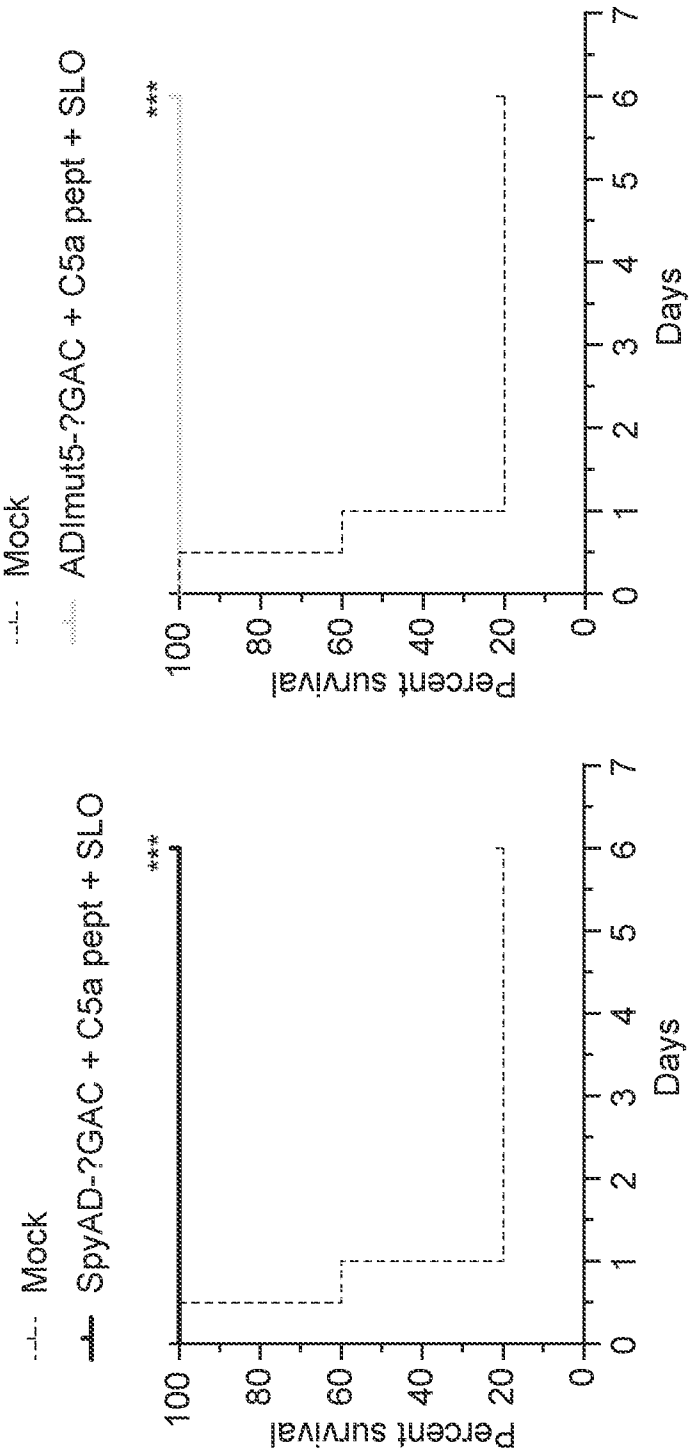

Mock
SpyAD-?GAC + C5a pept + SLO
***
Percent survival
100
80
60
40
20
0
0 1 2 3 4 5 6 7
Days
Mock
ADImut5-?GAC + C5a pept + SLO
***
Percent survival
100
80
60
40
20
0
0 1 2 3 4 5 6 7
Days

FIG. 5F

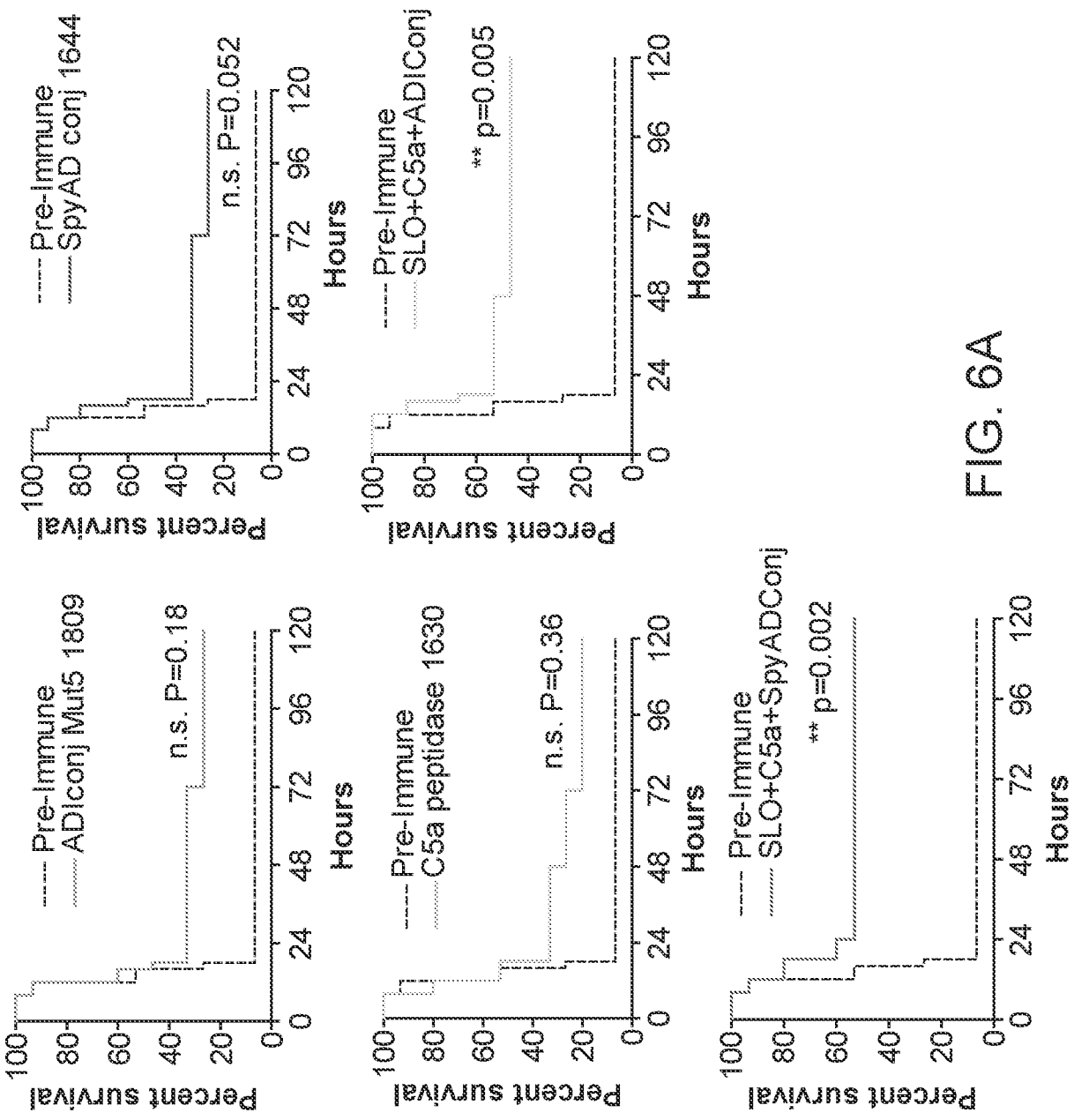

Pre-Immune
SpyAD conj 1644
n.s. P=0.052
Pre-Immune
SLO+C5a+ADIConj
** p=0.005
Pre-Immune
ADIconj Mut5 1809
n.s. P=0.18
Pre-Immune
C5a peptidase 1630
n.s. P=0.36
Pre-Immune
SLO+C5a+SpyADConj
** p=0.002
Pre-Immune
SLO 1627
* p=0.02
Pre-Immune
SLO+C5aPeptidase
* p=0.044
Percent survival
Hours
0
20
40
60
80
100
0
24
48
72
96
120

FIG. 6A

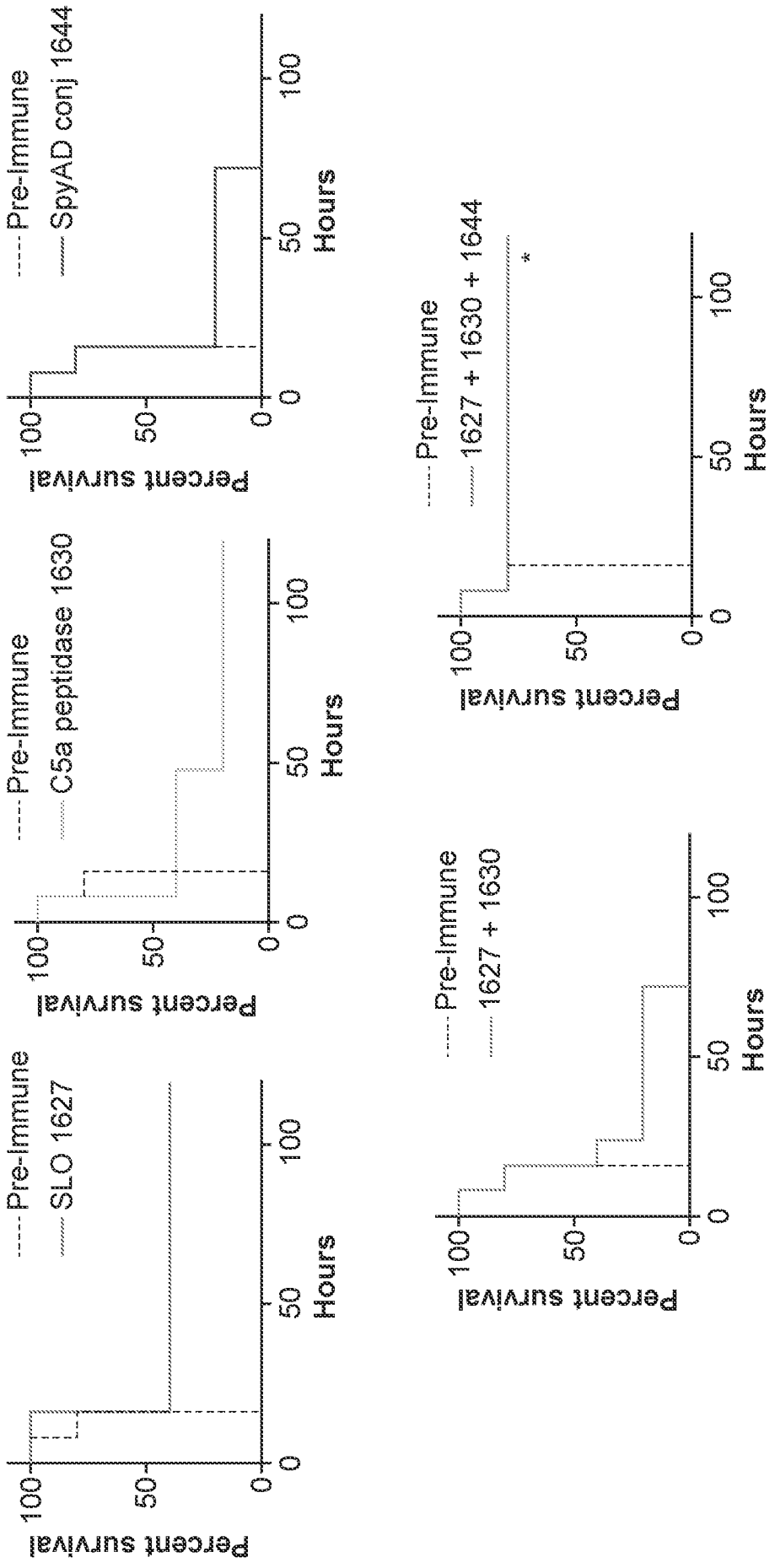

Pre-Immune
SLO 1627
Percent survival
Hours
Pre-Immune
C5a peptidase 1630
Percent survival
Hours
Pre-Immune
SpyAD conj 1644
Percent survival
Hours
Pre-Immune
1627 + 1630
Percent survival
Hours
Pre-Immune
1627 + 1630 + 1644
*
Percent survival
Hours

FIG. 6B

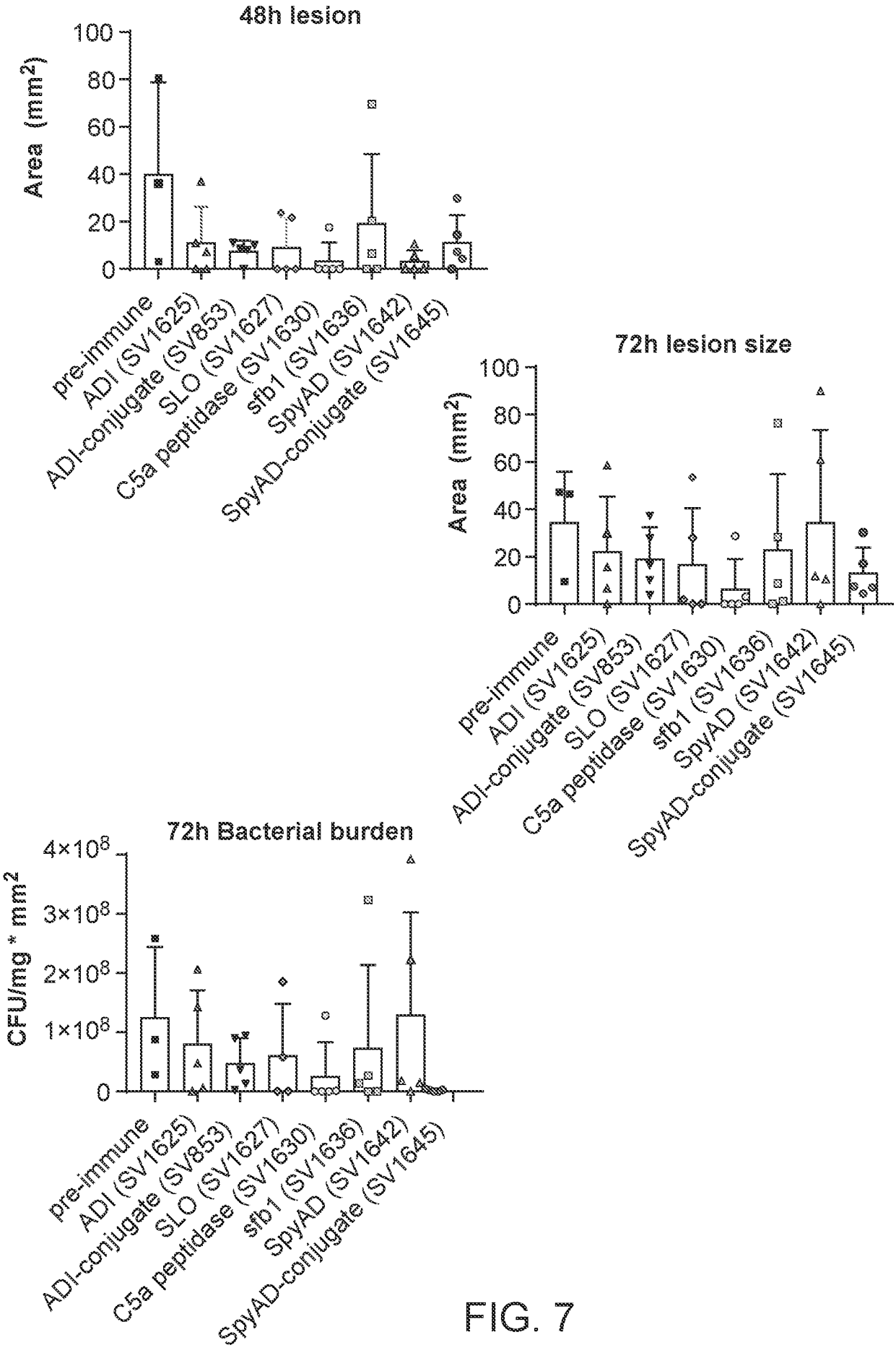
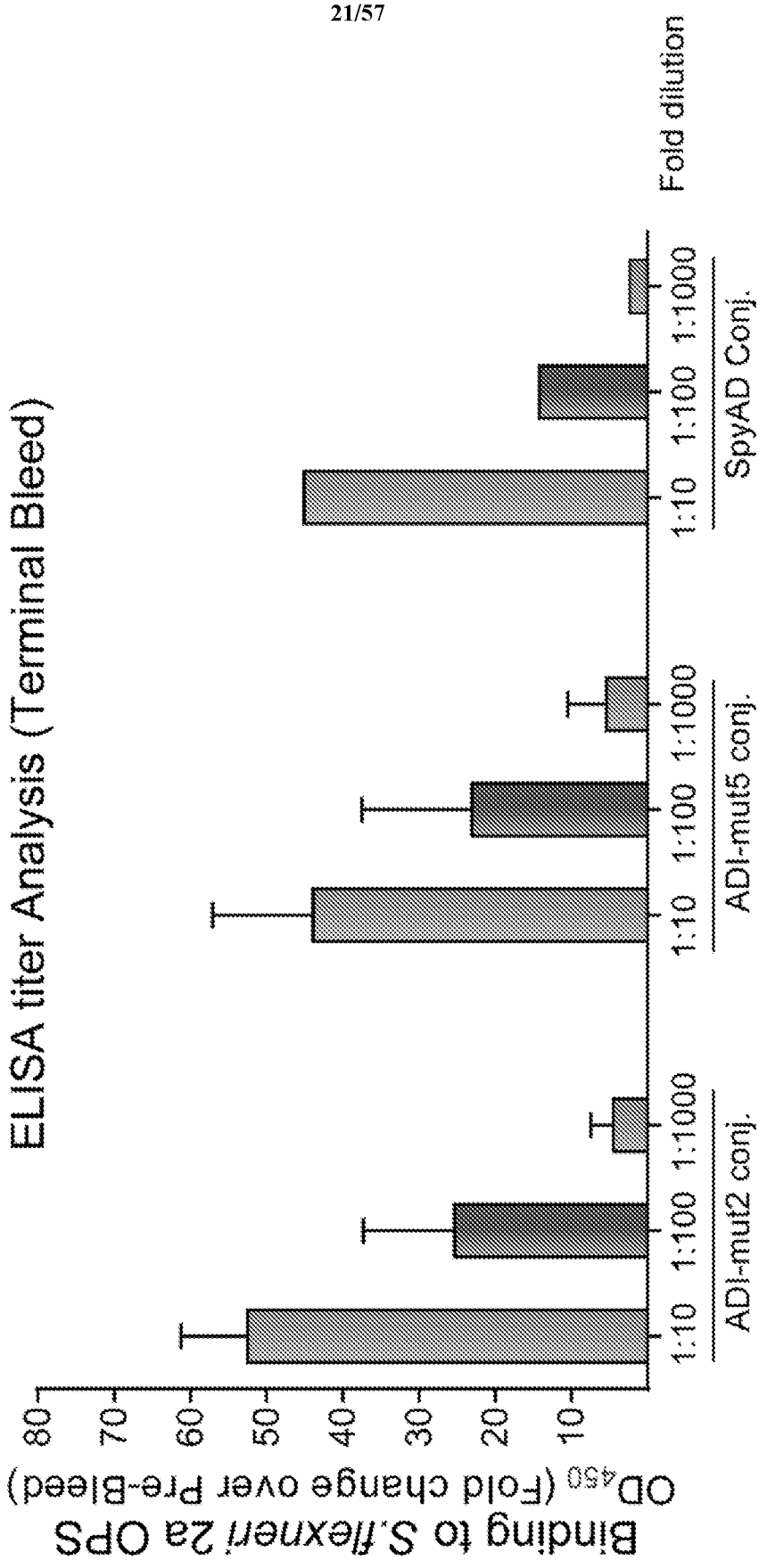

48h lesion
Area (mm2)
100
80
60
40
20
0
pre-immune
ADI (SV1625)
ADI-conjugate (SV853)
SLO (SV1627)
C5a peptidase (SV1630)
sfb1 (SV1636)
SpyAD (SV1642)
SpyAD-conjugate (SV1645)
72h lesion size
Area (mm2)
100
80
60
40
20
0
pre-immune
ADI (SV1625)
ADI-conjugate (SV853)
SLO (SV1627)
C5a peptidase (SV1630)
sfb1 (SV1636)
SpyAD (SV1642)
SpyAD-conjugate (SV1645)
72h Bacterial burden
CFU/mg * mm2
4×10^8
3×10^8
2×10^8
1×10^8
0
pre-immune
ADI (SV1625)
ADI-conjugate (SV853)
SLO (SV1627)
C5a peptidase (SV1630)
sfb1 (SV1636)
SpyAD (SV1642)
SpyAD-conjugate (SV1645)

FIG. 7

FIG. 8


ELISA titer Analysis (Terminal Bleed)
Binding to S.flexneri 2a OPS
OD450 (Fold change over Pre-Bleed)
80
70
60
50
40
30
20
10
1:10
1:100
1:1000
ADI-mut2 conj.
1:10
1:100
1:1000
ADI-mut5 conj.
1:10
1:100
1:1000
SpyAD Conj.
Fold dilution

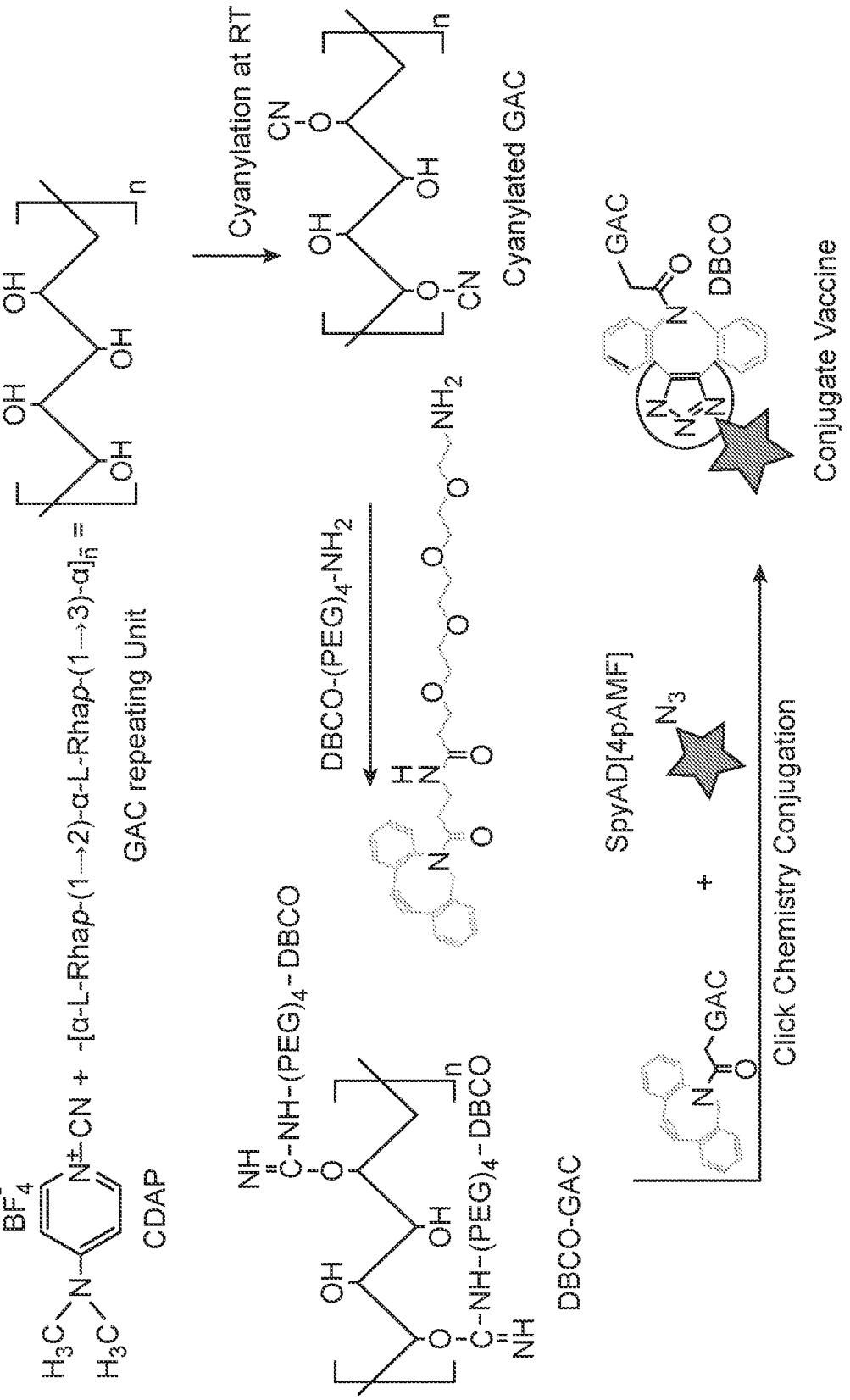

CDAP
GAC repeating Unit
Cyanylation at RT
Cyanylated GAC
DBCO-(PEG)4-NH2
DBCO-GAC
SpyAD[4pAMF]
Click Chemistry Conjugation
Conjugate Vaccine

FIG. 9

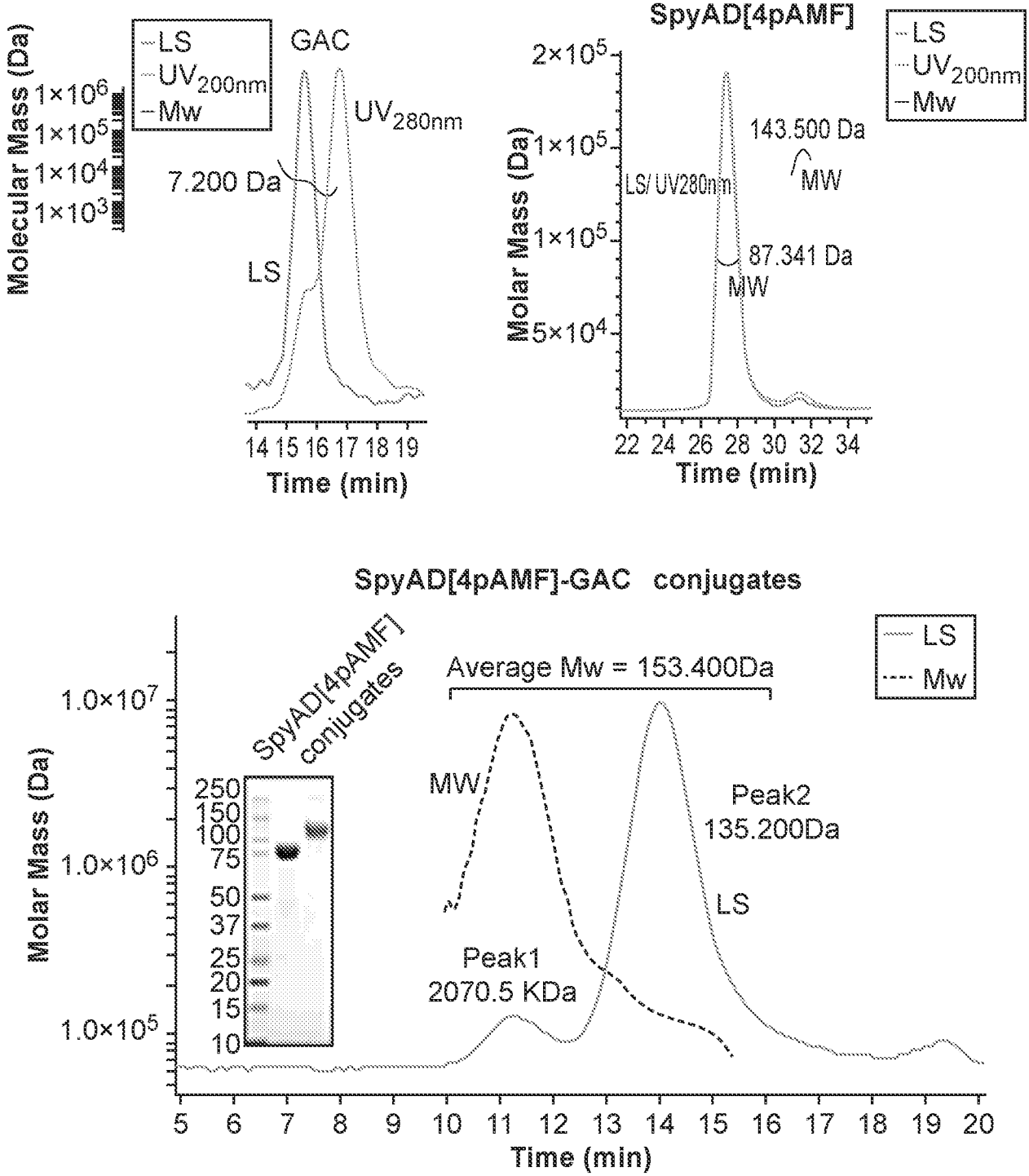

GAC
LS
UV200nm
Mw
UV280nm
7.200 Da
LS
Molecular Mass (Da)
1×10^6
1×10^5
1×10^4
1×10^3
14 15 16 17 18 19
Time (min)
SpyAD[4pAMF]
LS
UV200nm
Mw
2×10^5
1×10^5
1×10^5
5×10^4
Molar Mass (Da)
143.500 Da
MW
LS/ UV280nm
87.341 Da
MW
22 24 26 28 30 32 34
Time (min)
SpyAD[4pAMF]-GAC conjugates
SpyAD[4pAMF] conjugates
250
150
100
75
50
37
25
20
15
10
Average Mw = 153.400Da
LS
Mw
MW
Peak2
135.200Da
LS
Peak1
2070.5 KDa
1.0×10^7
1.0×10^6
1.0×10^5
Molar Mass (Da)
5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Time (min)

FIG. 10

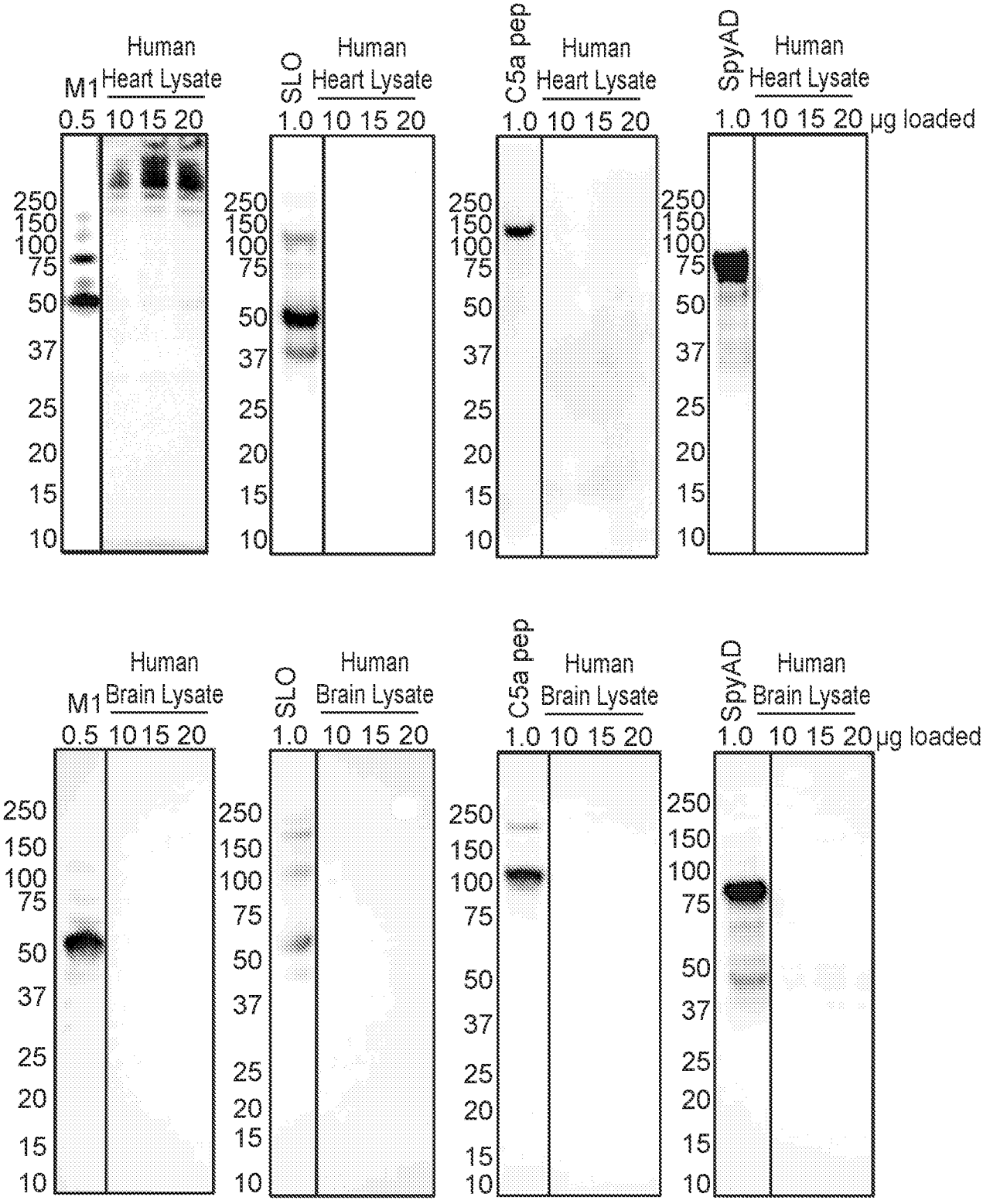
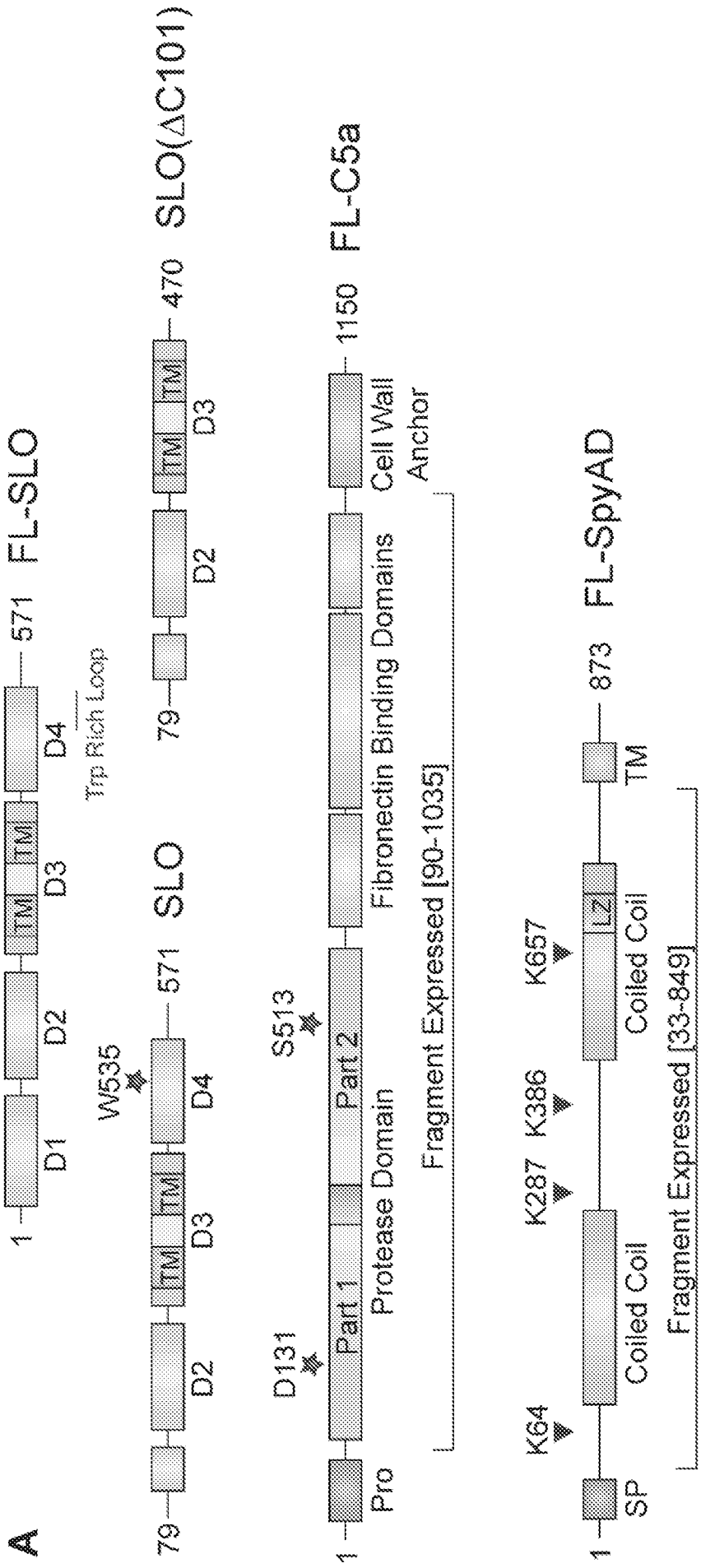

M1
Human Heart Lysate
0.5 10 15 20
SLO
Human Heart Lysate
1.0 10 15 20
C5a pep
Human Heart Lysate
1.0 10 15 20
SpyAD
Human Heart Lysate
1.0 10 15 20 µg loaded
M1
Human Brain Lysate
0.5 10 15 20
SLO
Human Brain Lysate
1.0 10 15 20
C5a pep
Human Brain Lysate
1.0 10 15 20
SpyAD
Human Brain Lysate
1.0 10 15 20 µg loaded
250
150
100
75
50
37
25
20
15
10

FIG. 11

FIG. 12


A
FL-SLO
1
571
D1
D2
TM
TM
D3
D4
Trp Rich Loop
W535
SLO(ΔC101)
79
470
D2
TM
TM
D3
SLO
79
571
D2
TM
TM
D3
D4
S513
D131
FL-C5a
1
1150
Pro
Part 1
Part 2
Protease Domain
Fibronectin Binding Domains
Cell Wall Anchor
Fragment Expressed [90-1035]
K64
K287
K386
K657
FL-SpyAD
1
873
SP
Coiled Coil
Coiled Coil
LZ
TM
Fragment Expressed [33-849]

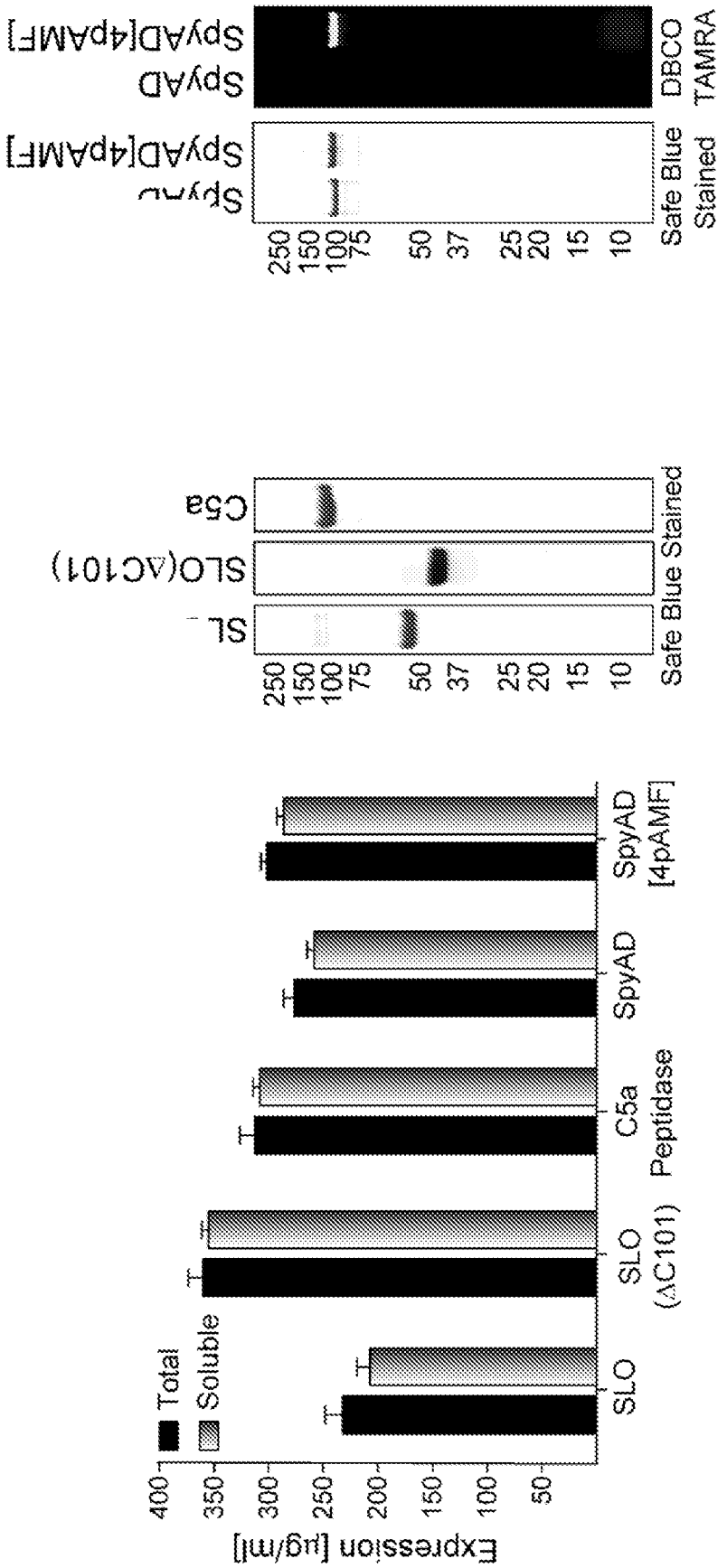
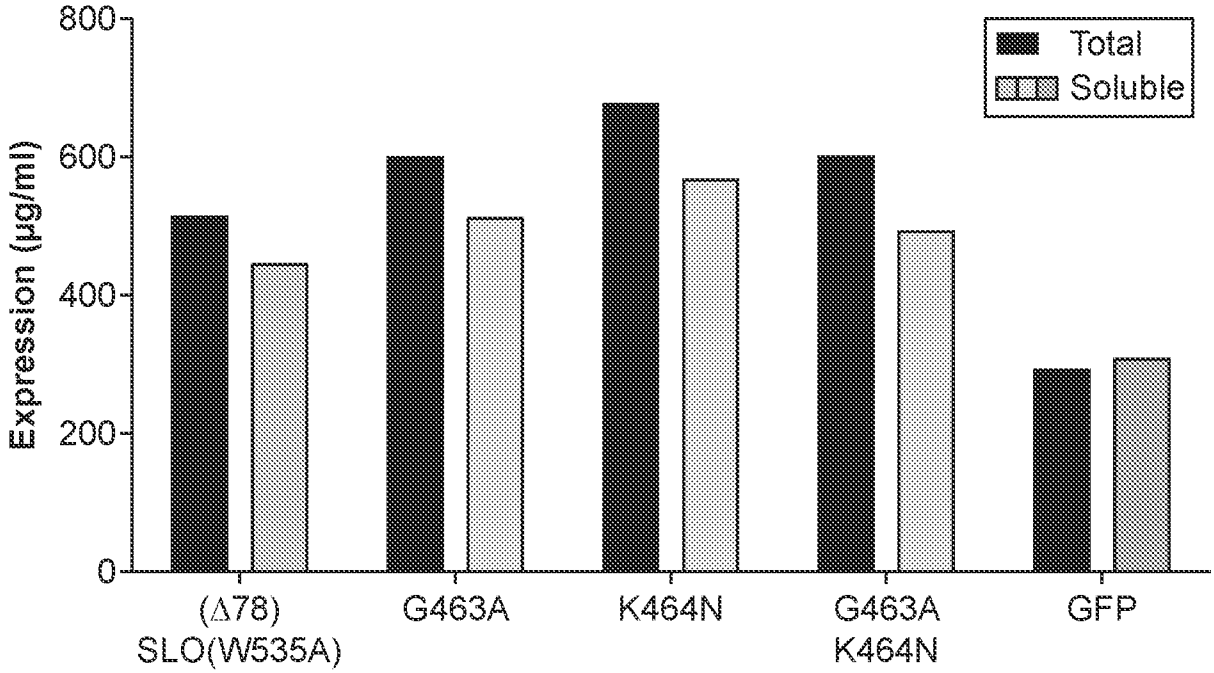

Expression [µg/ml]
400
350
300
250
200
150
100
50
Total
Soluble
SLO
SLO (ΔC101)
C5a Peptidase
SpyAD
SpyAD [4pAMF]
250
150
100
75
50
37
25
20
15
10
Safe Blue Stained
SL_
SLO(ΔC101)
C5a
Safe Blue Stained
SpyAD
SpyAD[4pAMF]
DBCO TAMRA

FIG. 13


800
600
400
200
0
Expression (µg/ml)
Total
Soluble
(Δ78) SLO(W535A)
G463A
K464N
G463A K464N
GFP

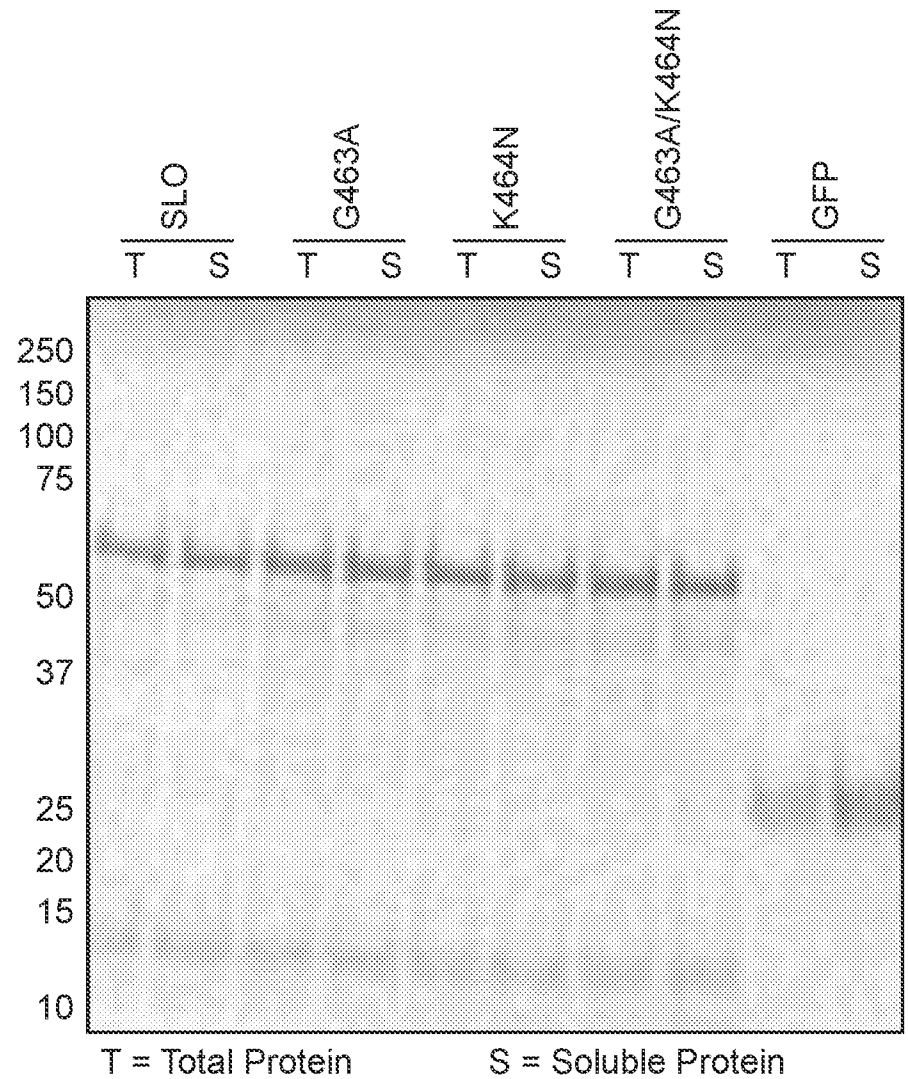

SLO
G463A
K464N
G463A/K464N
GFP
T S T S T S T S T S
250
150
100
75
50
37
25
20
15
10
T = Total Protein
S = Soluble Protein

FIG. 14

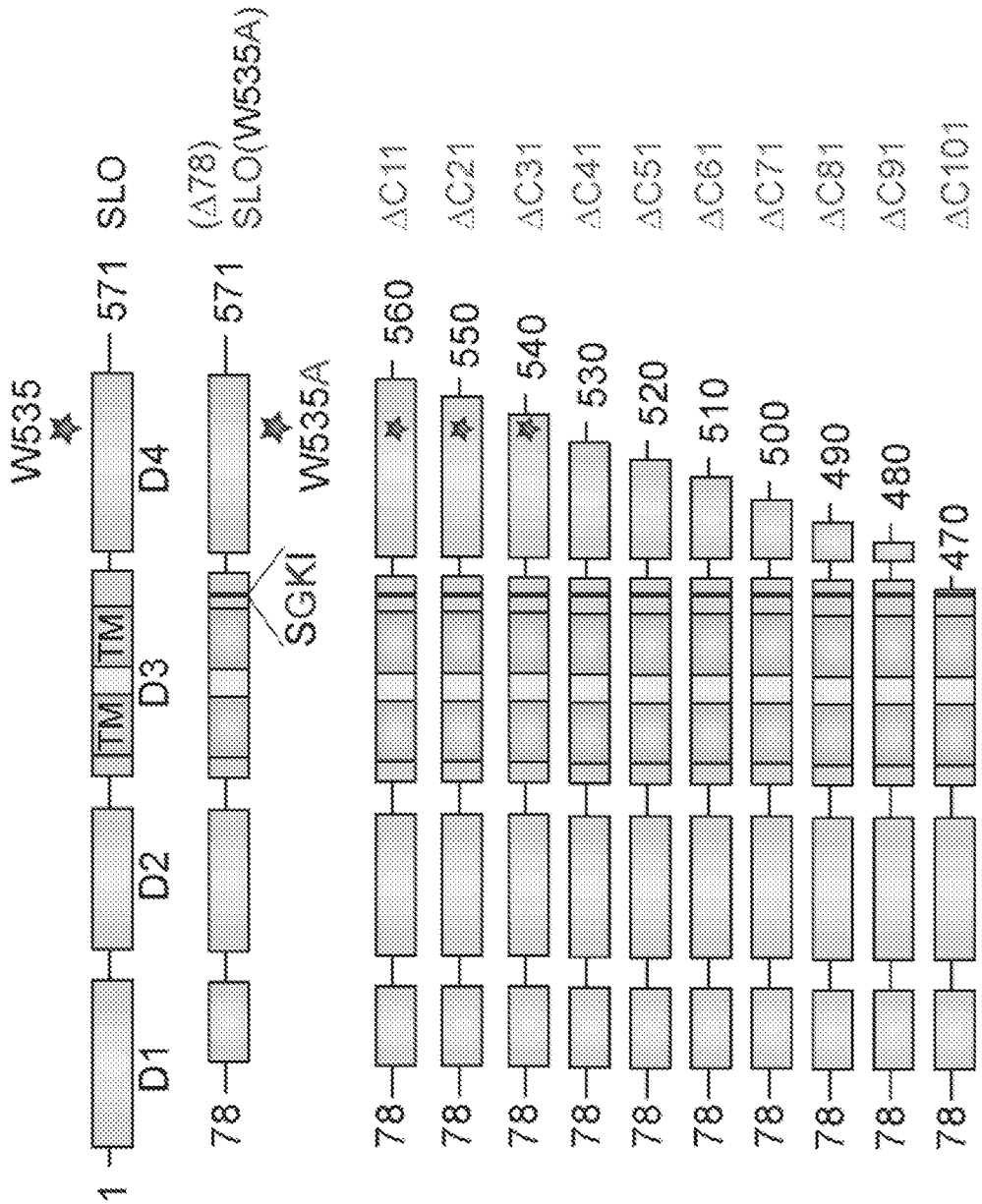

W535
1
D1
D2
TM
TM
D3
D4
571
SLO
78
(Δ78)
SLO(W535A)
571
W535A
SGKI
78
560
ΔC11
78
550
ΔC21
78
540
ΔC31
78
530
ΔC41
78
520
ΔC51
78
510
ΔC61
78
500
ΔC71
78
490
ΔC81
78
480
ΔC91
78
470
ΔC101

FIG. 15

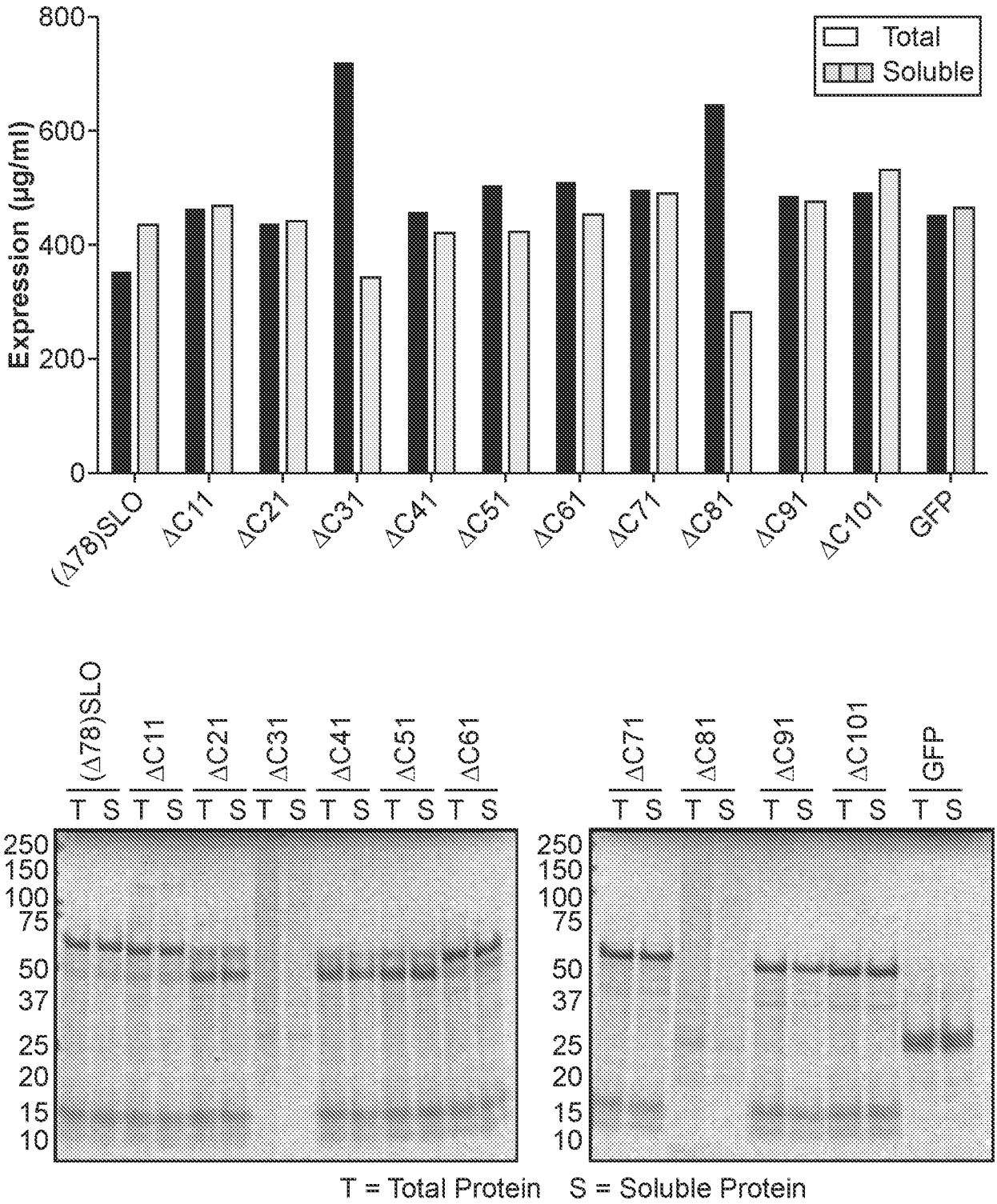
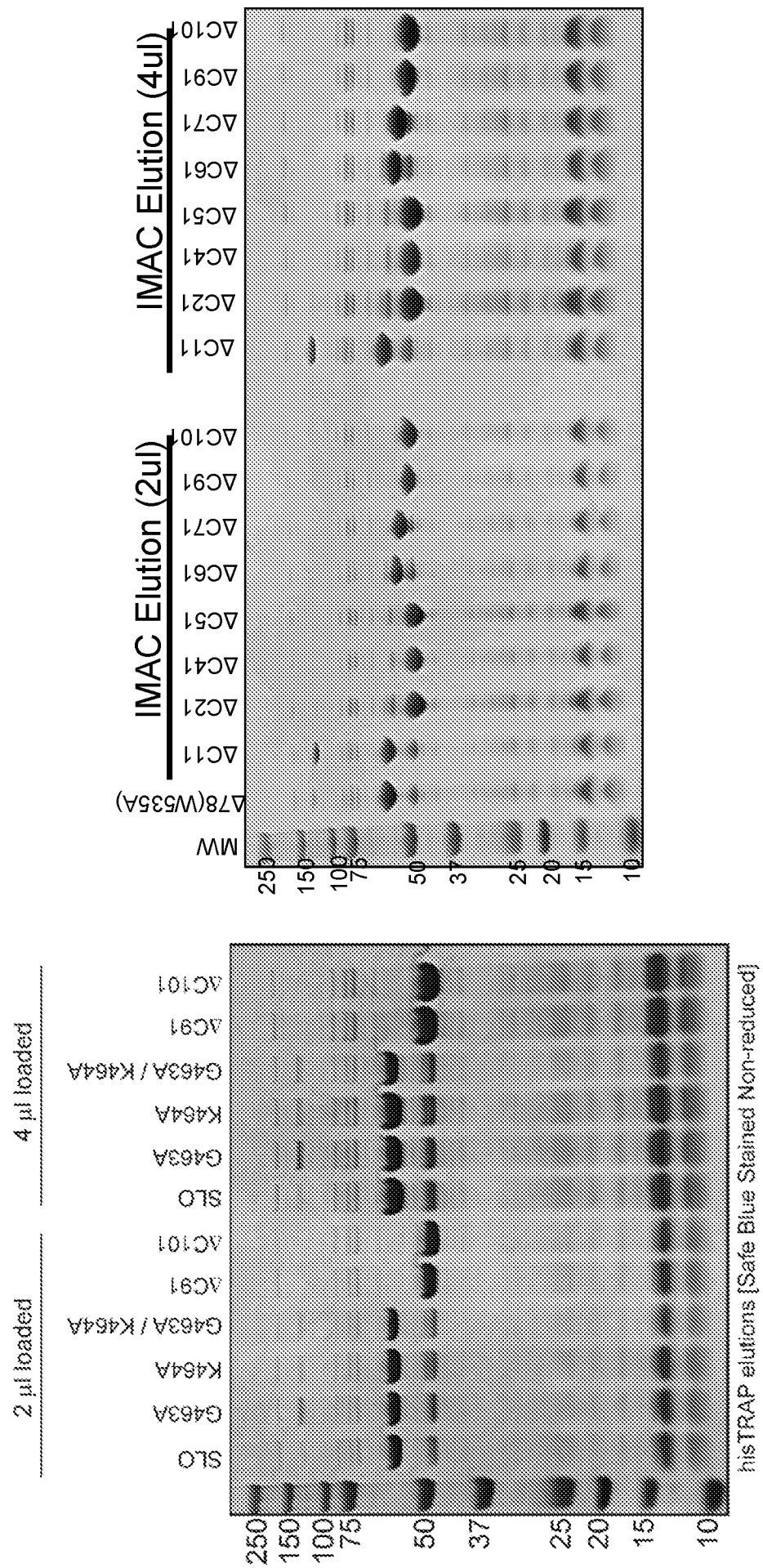

800
600
400
200
0
Expression (µg/ml)
Total
Soluble
(Δ78)SLO
ΔC11
ΔC21
ΔC31
ΔC41
ΔC51
ΔC61
ΔC71
ΔC81
ΔC91
ΔC101
GFP
T S
250
150
100
75
50
37
25
20
15
10
T = Total Protein S = Soluble Protein

FIG. 16

FIG. 17


IMAC Elution (4ul)
IMAC Elution (2ul)
MW
Δ78(W535A)
ΔC11
ΔC21
ΔC41
ΔC51
ΔC61
ΔC71
ΔC91
ΔC101
250
150
100
75
50
37
25
20
15
10
2 µl loaded
4 µl loaded
SLO
G463A
K464A
G463A / K464A
ΔC91
ΔC101
hisTRAP elutions [Safe Blue Stained Non-reduced]

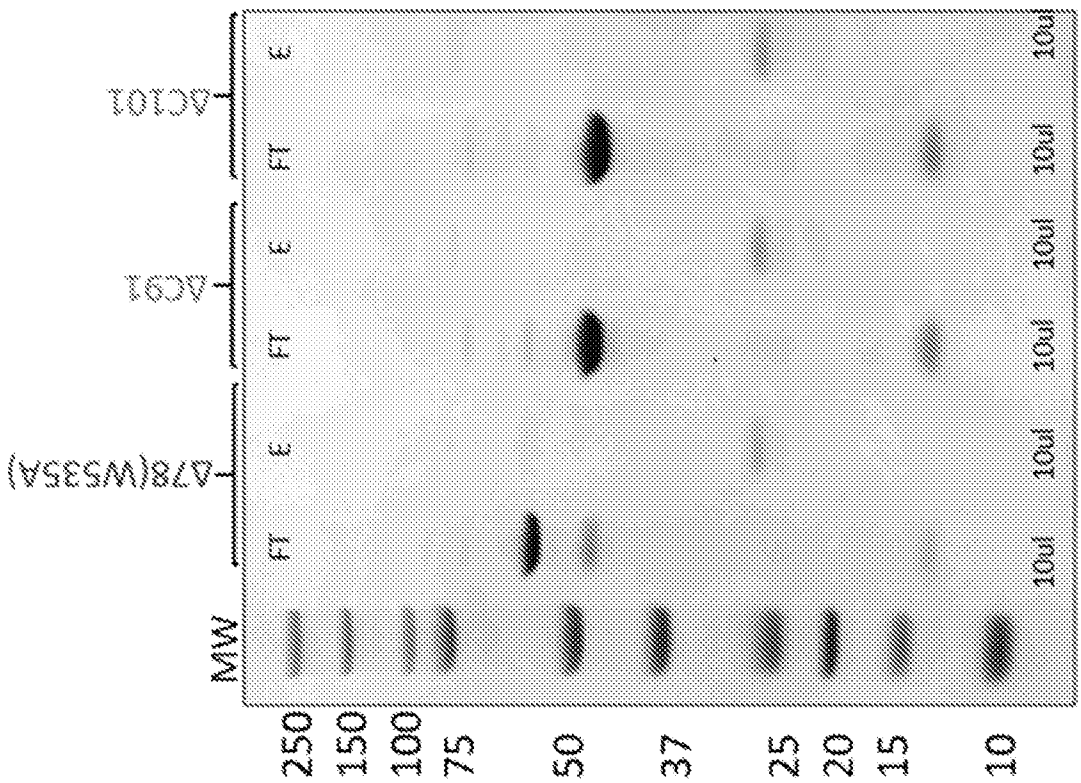


FIG. 18

FIG. 19

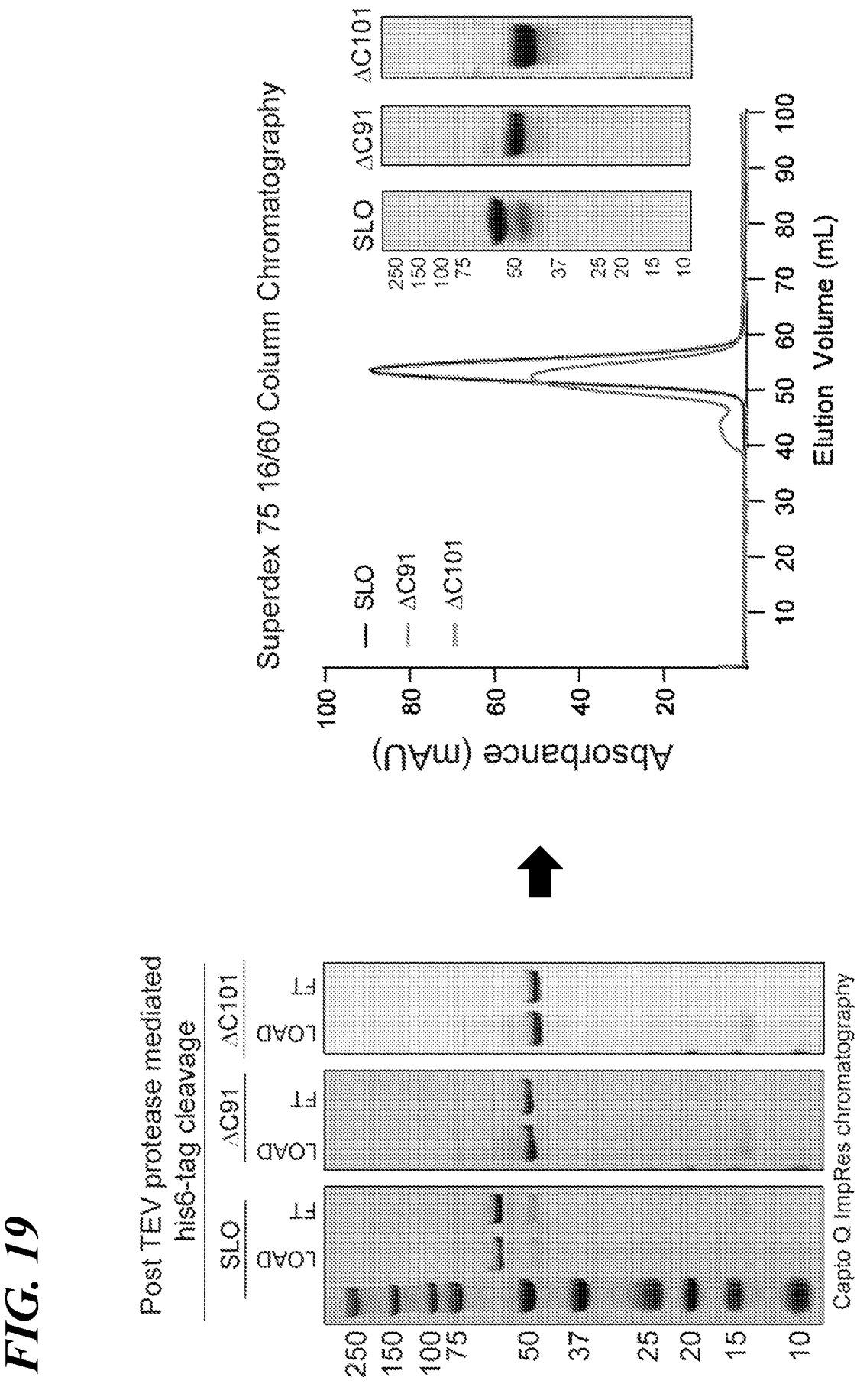
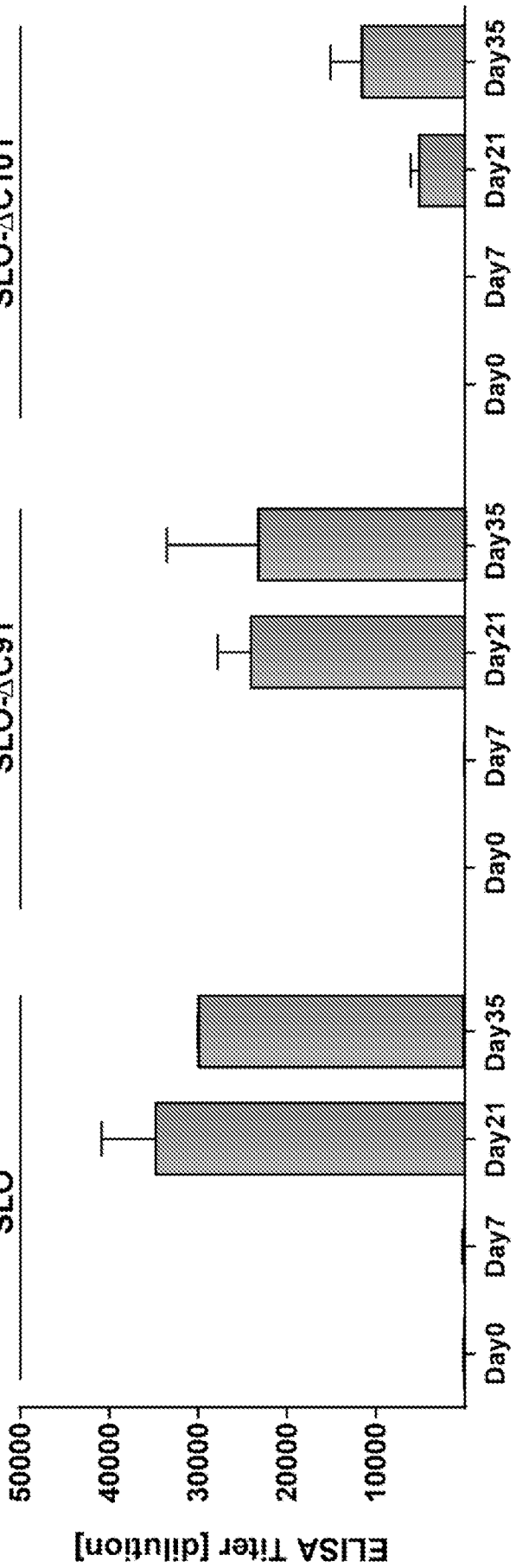

Post TEV protease mediated his6-tag cleavage
SLO
ΔC91
ΔC101
LOAD
FT
250
150
100
75
50
37
25
20
15
10
Capto Q ImpRes chromatography
Superdex 75 16/60 Column Chromatography
SLO
ΔC91
ΔC101
Absorbance (mAU)
Elution Volume (mL)

FIG. 20


ELISA Titer [dilution]
50000
40000
30000
20000
10000
SLO
SLO-ΔC91
SLO-ΔC101
Day0
Day7
Day21
Day35
Immunization Dose : 50 μg + Alum / I.M. injection
ELISA performed against in respective antigen

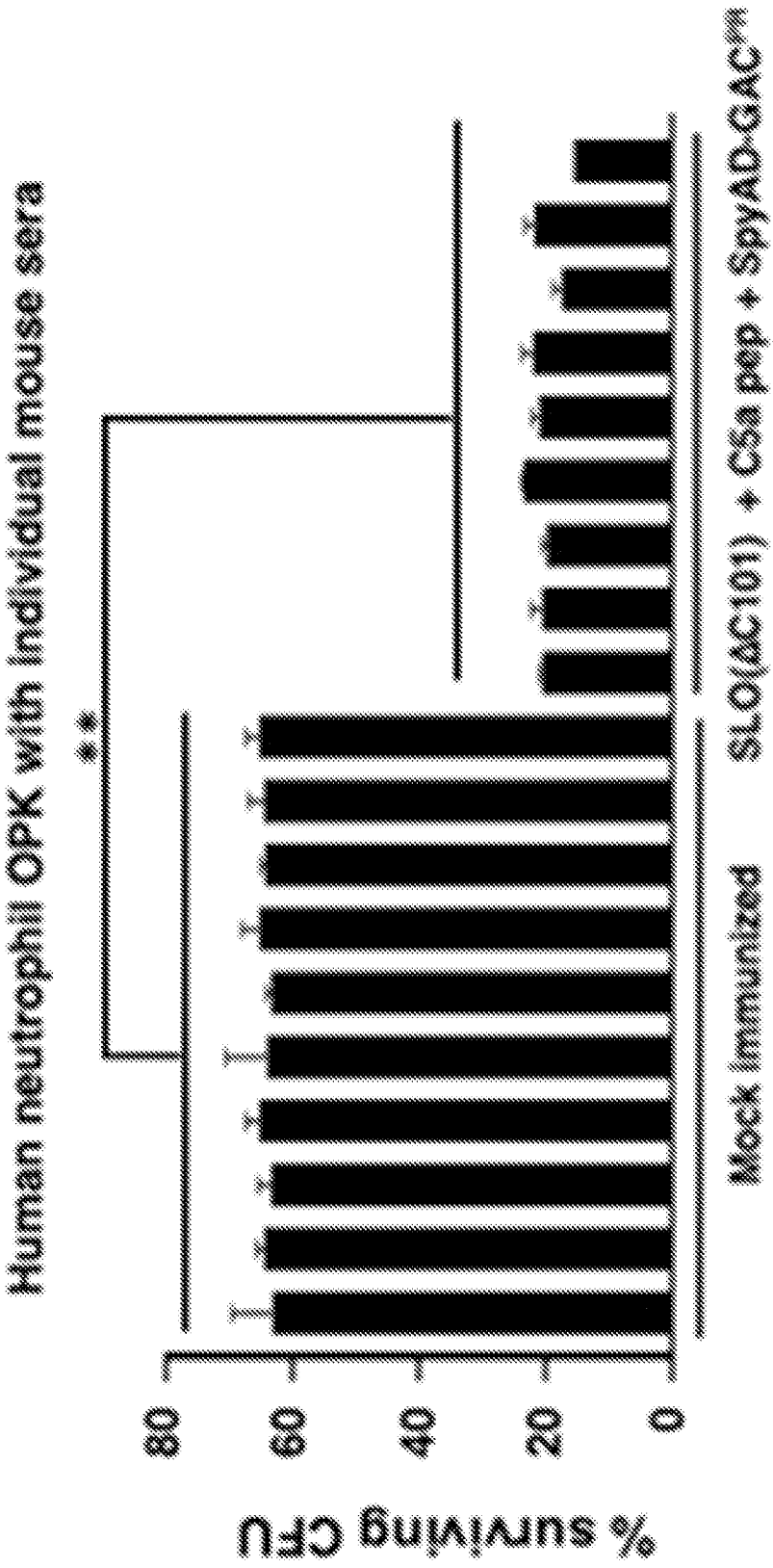

Human neutrophil OPK with individual mouse sera
**
% surviving CFU
80
60
40
20
0
Mock immunized
SLO(ΔC101) + CSa pep + SpyAD-GAC^{m}

FIG. 21

FIG. 22A

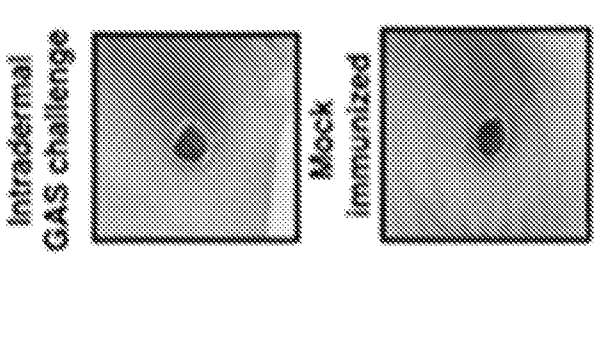


FIG. 22B

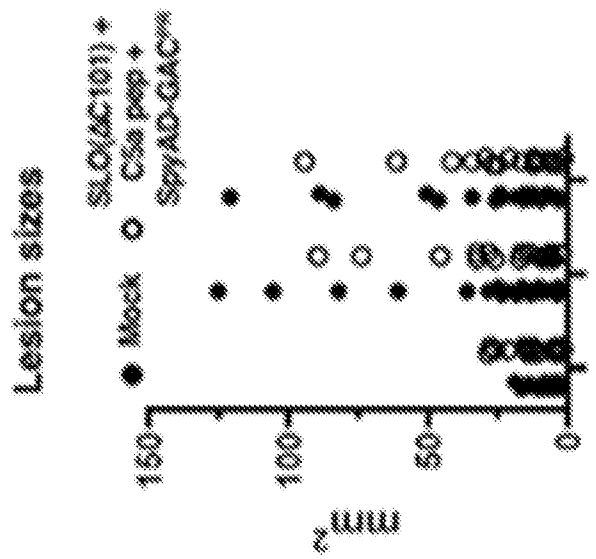


FIG. 22C

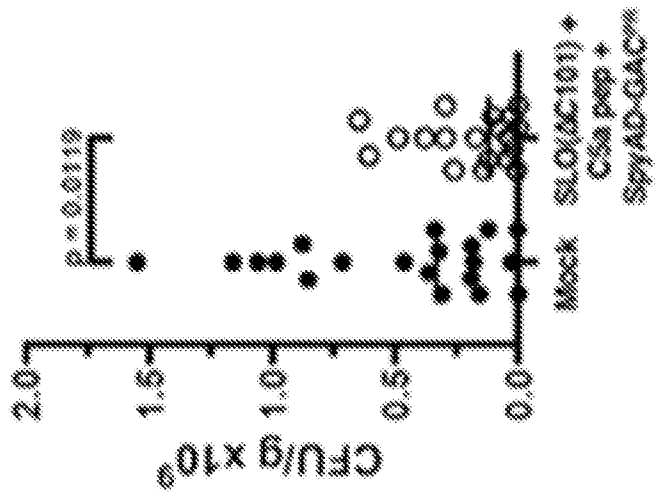
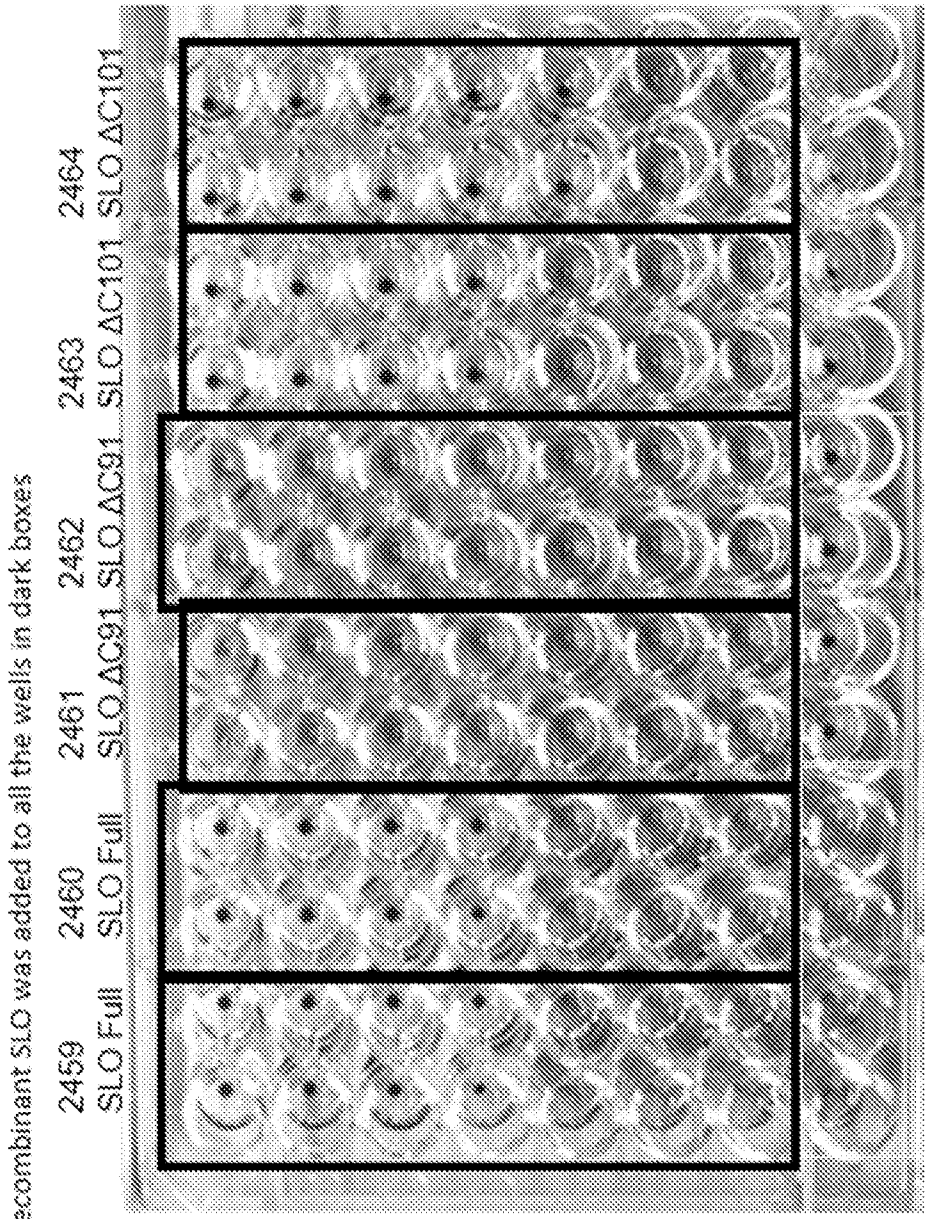


FIG. 23


0.1ug/ml recombinant SLO was added to all the wells in dark boxes
2459 SLO Full
2460 SLO Full
2461 SLO ΔC91
2462 SLO ΔC91
2463 SLO ΔC101
2464 SLO ΔC101
Serum dilution
1:16
1:32
1:64
1:128
1:256
1:512
1:1024
Full-length SLO and SLO-ΔC101 anti-sera block lysis while SLO-ΔC91 anti-sera **does not** block lysis.
Water - lysis
PBS – no lysis

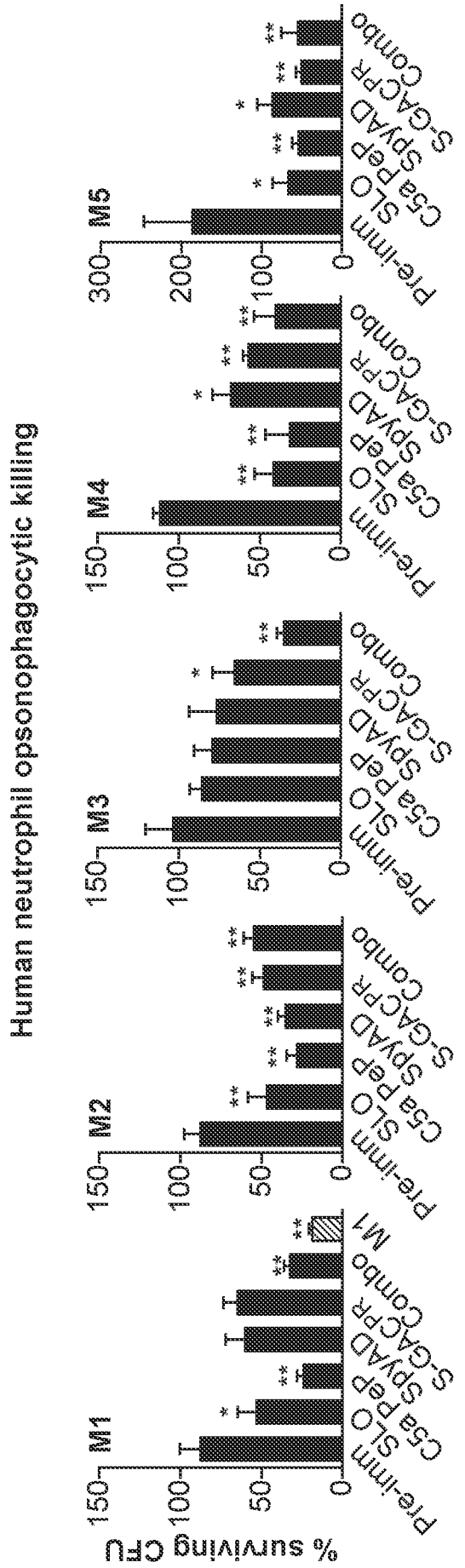


FIG. 24A

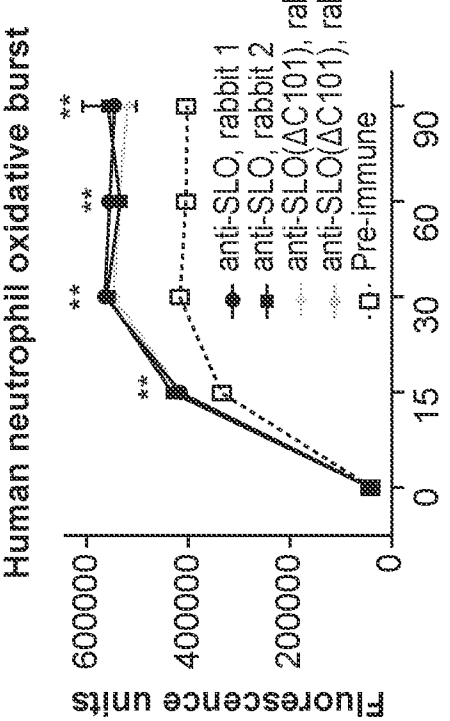


FIG. 24C

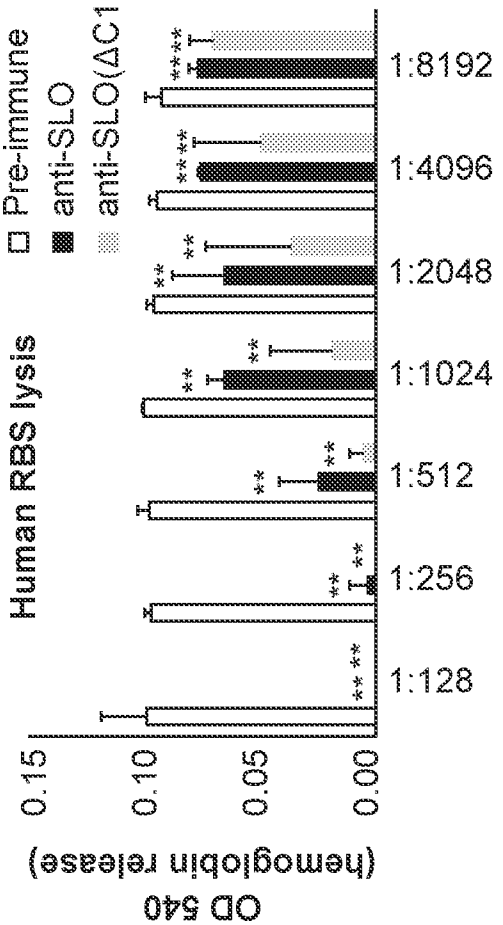


FIG. 24B

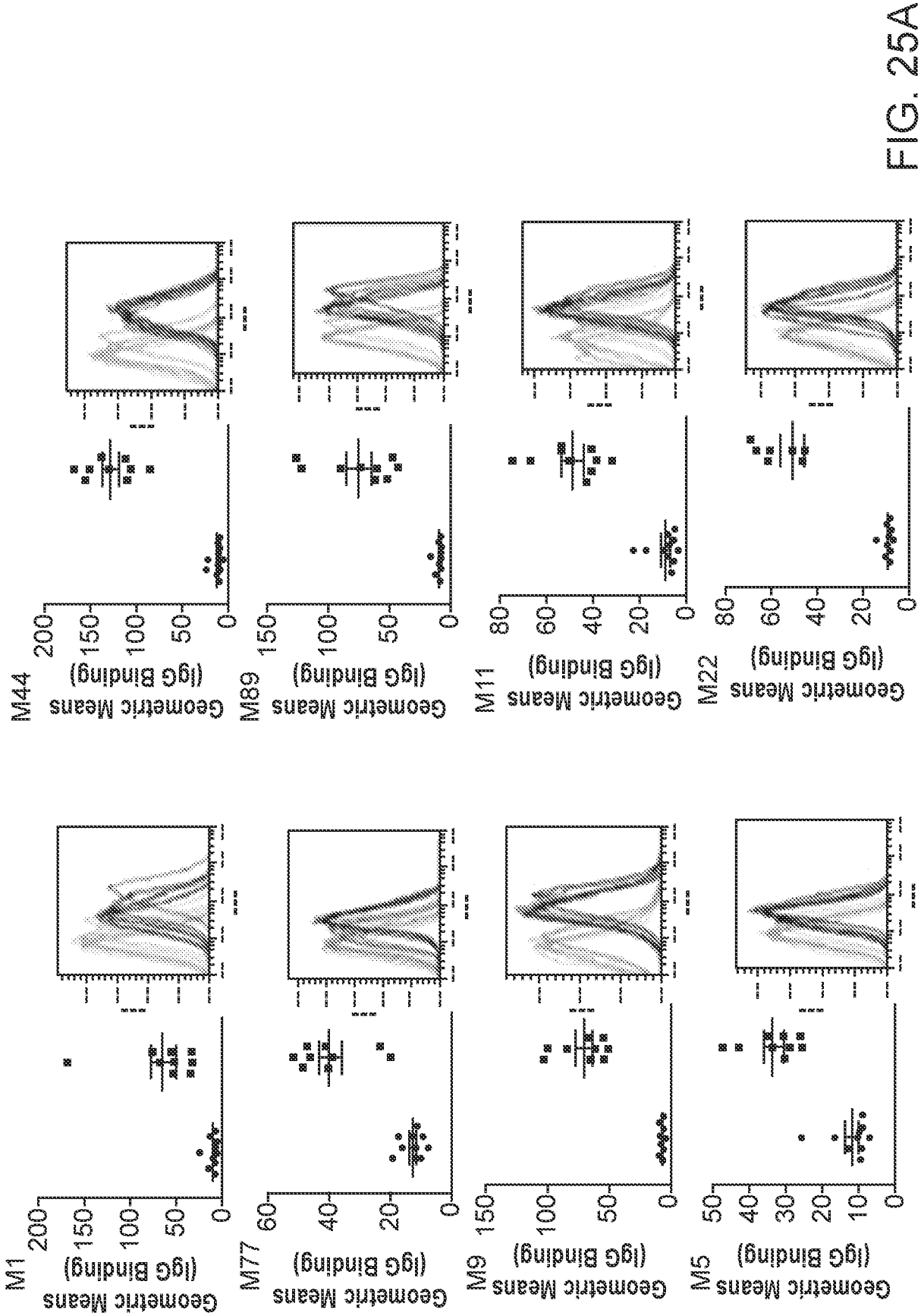


FIG. 25A

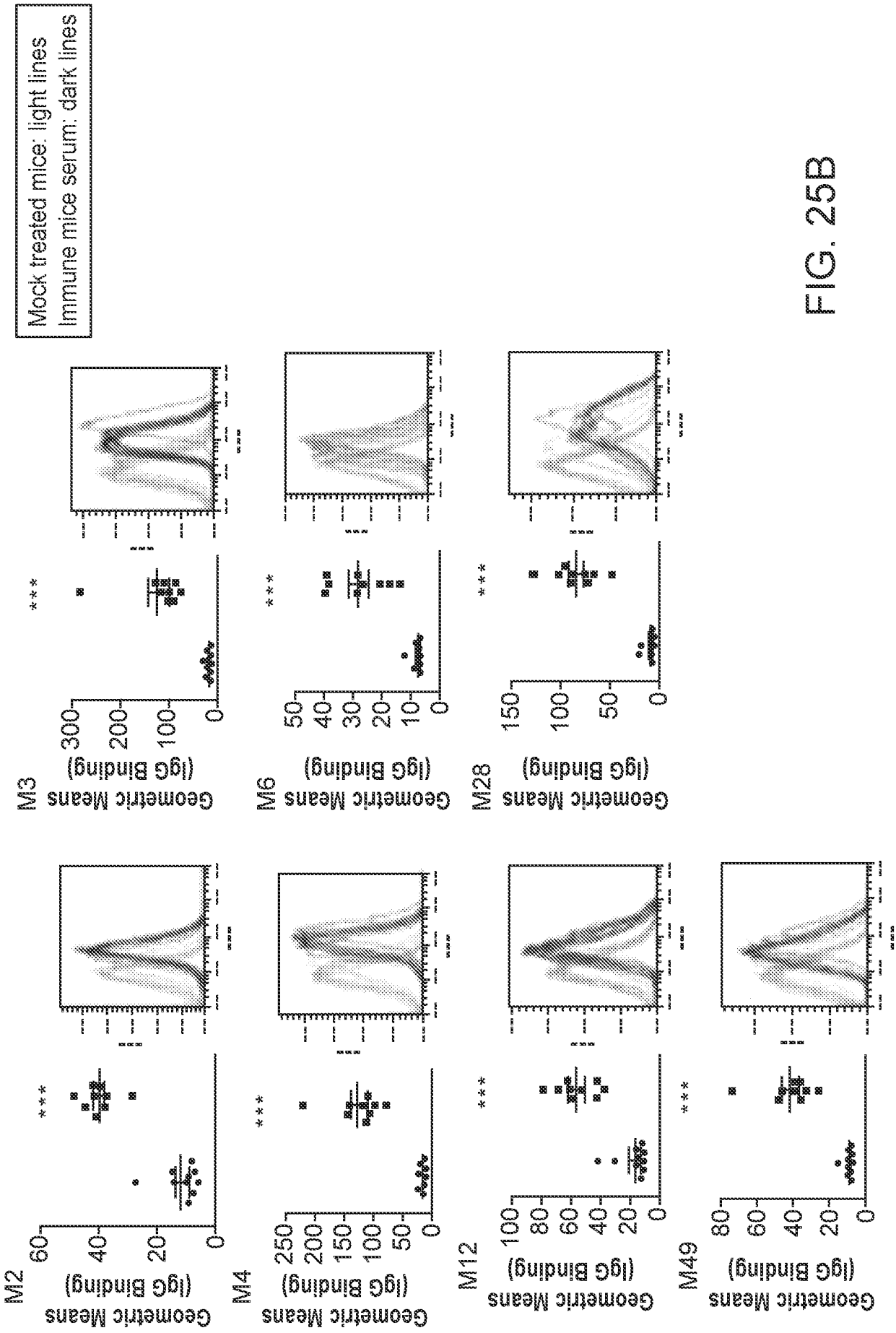

Mock treated mice: light lines
Immune mice serum: dark lines
M2
M3
M4
M6
M12
M28
M49
Geometric Means (IgG Binding)
***

FIG. 25B

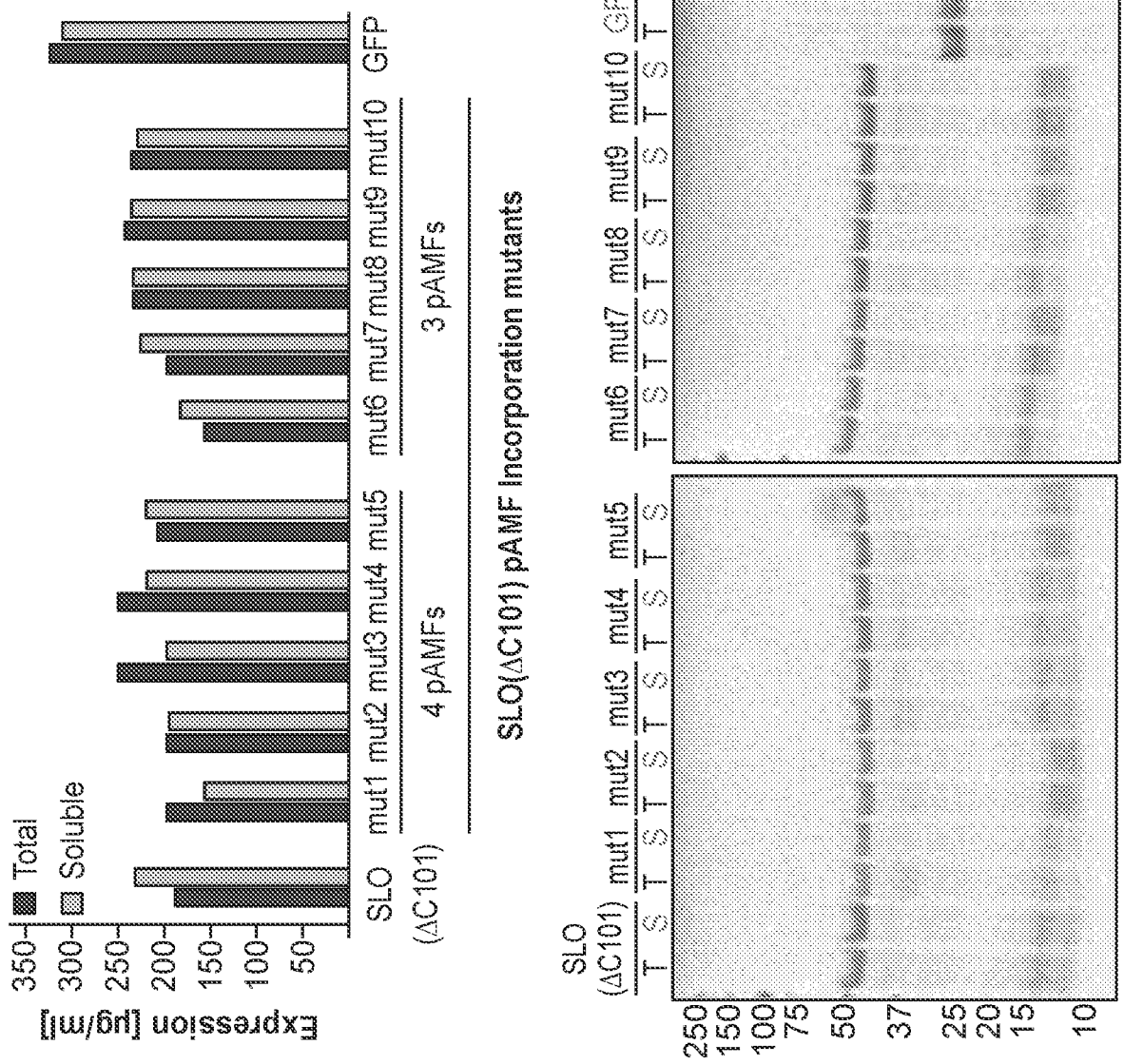
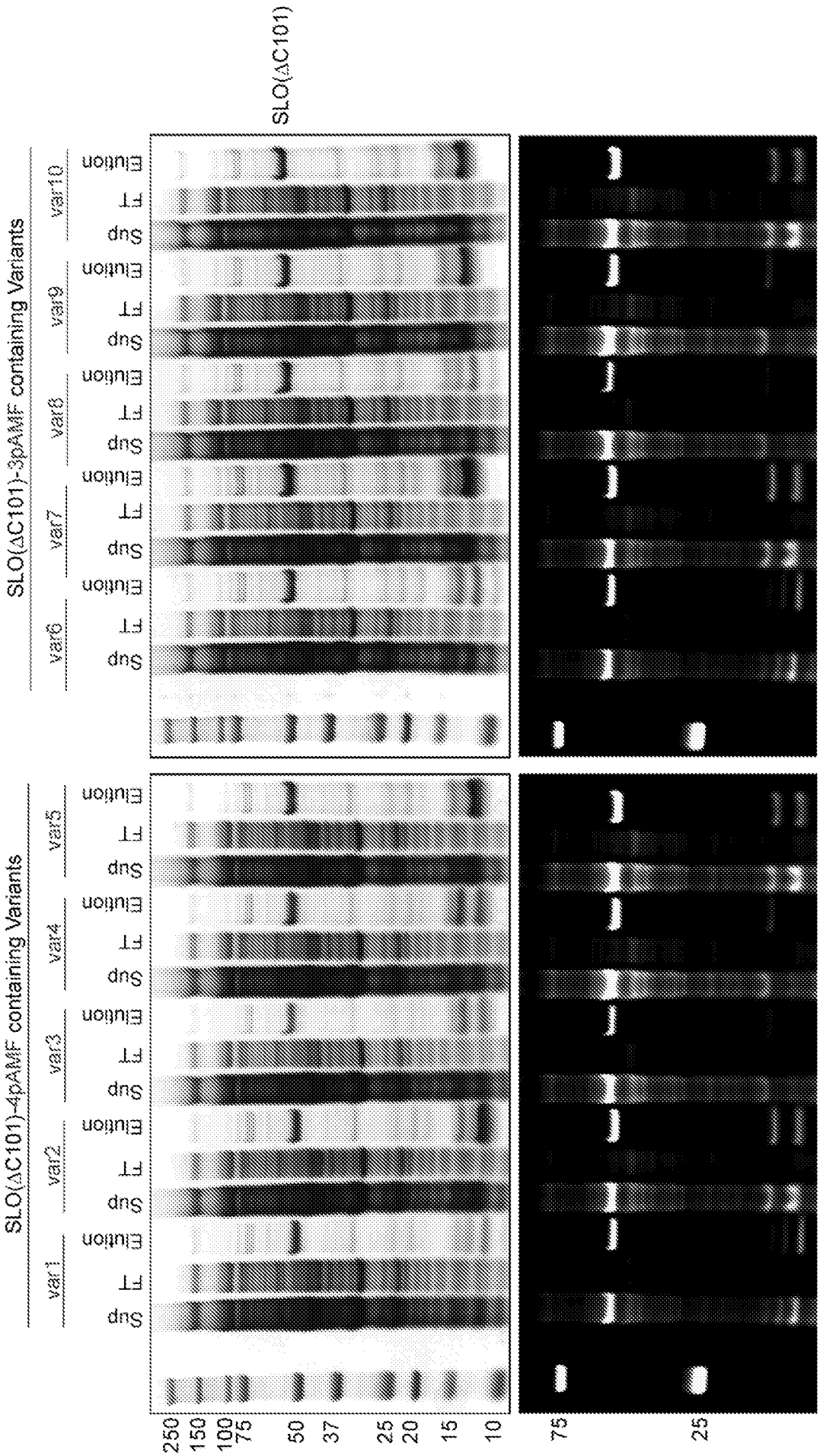

Total
Soluble
Expression [µg/ml]
350
300
250
200
150
100
50
SLO (ΔC101)
mut1 mut2 mut3 mut4 mut5
4 pAMFs
mut6 mut7 mut8 mut9 mut10
3 pAMFs
GFP
SLO(ΔC101) pAMF Incorporation mutants
SLO (ΔC101)
mut1 mut2 mut3 mut4 mut5
mut6 mut7 mut8 mut9 mut10 GFP
T S T S T S T S T S
T S T S T S T S T S T S
SLO (ΔC101)
250
150
100
75
50
37
25
20
15
10
14C-Leucine Incorporation Autoradiogram

FIG. 26

FIG. 27A


SLO(ΔC101)
SLO(ΔC101)-4pAMF containing Variants
var1
var2
var3
var4
var5
Sup
FT
Elution
SLO(ΔC101)-3pAMF containing Variants
var6
var7
var8
var9
var10
250
150
100
75
50
37
25
20
15
10
75
25

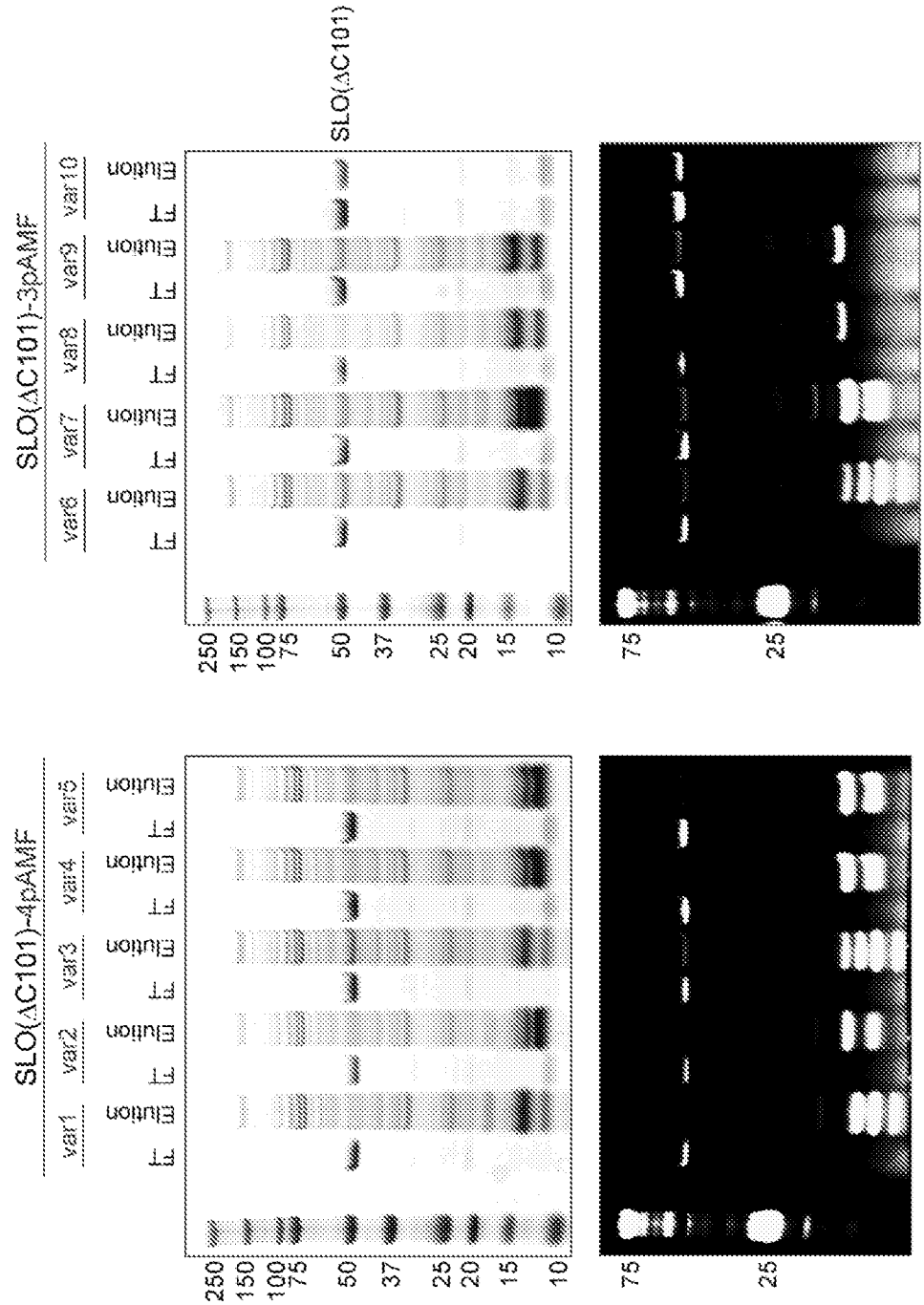

SLO(ΔC101)-4pAMF
var1
var2
var3
var4
var5
FT
Elution
SLO(ΔC101)-3pAMF
var6
var7
var8
var9
var10
SLO(ΔC101)
250
150
100
75
50
37
25
20
15
10
75
25

FIG. 27B

FIG. 28

Incubate DBCO derivatized GAC with SLO variants for conjugated at 25C overnight

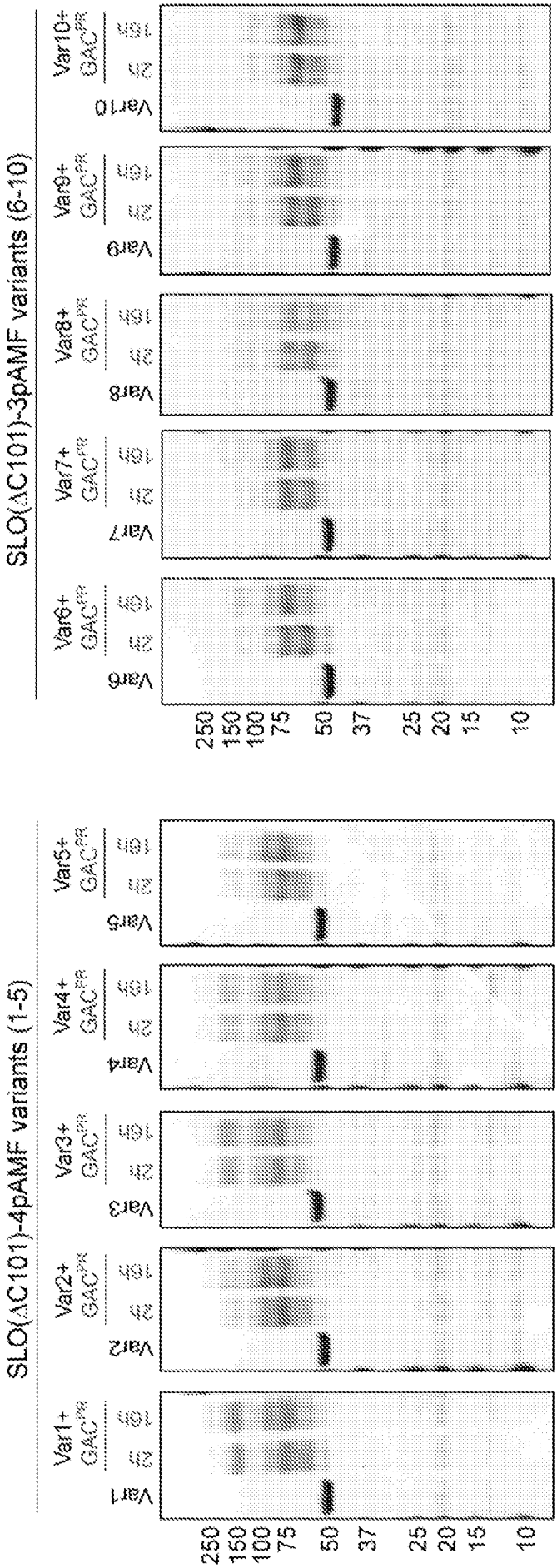
Reaction	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	
Protein	60.0	48.0	80.0	48.0	40.0	17.8	20.9	48.0	21.8	30.0	
PS-DBCO	42.5	42.5	42.5	42.5	42.5	42.5	42.5	42.5	42.5	42.5	424.8
Buffer (10x PBS)	6.0	12.0	6.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	
Water	11.5	17.5	-8.5	17.5	25.5	47.7	44.7	17.5	43.7	35.5	
Total	120.0	120.0	120.0	120.0	120.0	120.0	120.0	120.0	120.0	120.0	

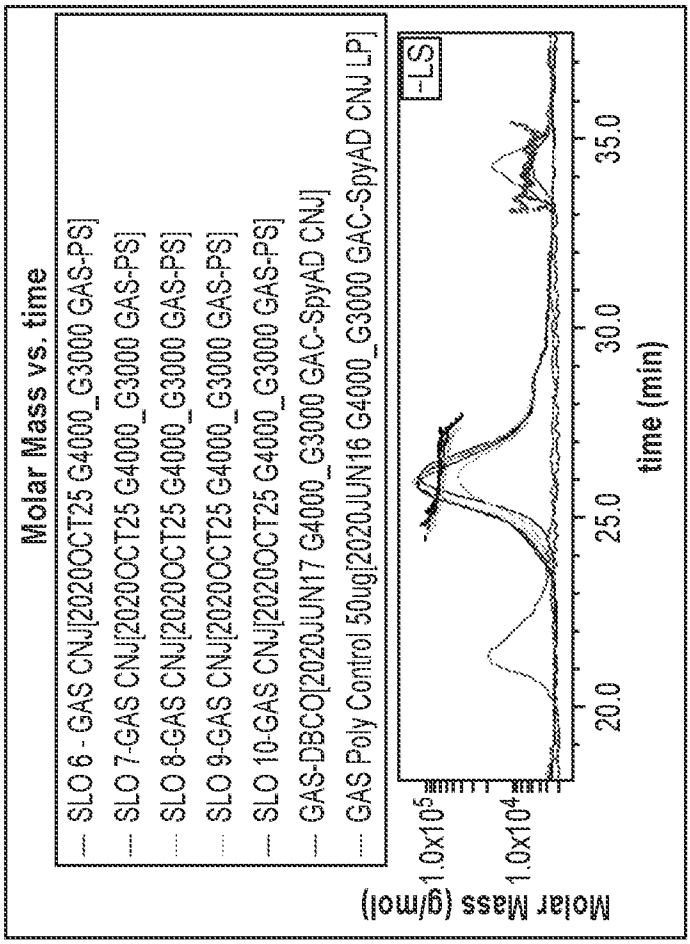
Protein	var1	var2	var3	var4	var5	var6	var7	var8	var9	var10	mg/ml
Stock	0.8	1.0	0.6	1.0	1.2	2.7	2.3	1.0	2.2	1.6	

PS-DBCO stock	1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.13	mg/ml

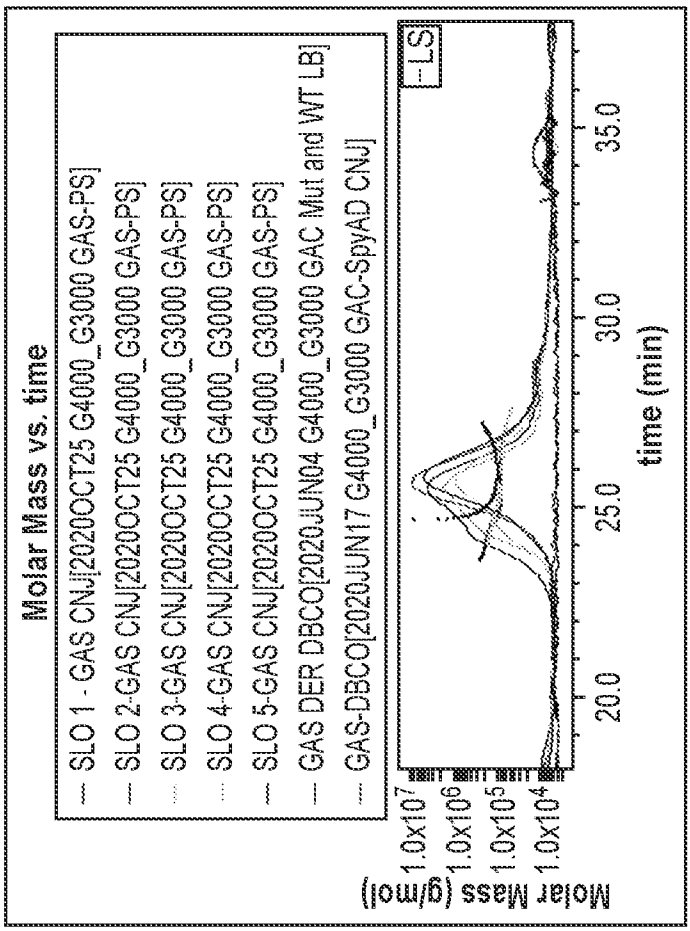
SUBSTITUTE SHEET (RULE 26)

FIG. 29


SLO(ΔC101)-4pAMF variants (1-5)
SLO(ΔC101)-3pAMF variants (6-10)
Var1+ GAC^PR
Var2+ GAC^PR
Var3+ GAC^PR
Var4+ GAC^PR
Var5+ GAC^PR
Var6+ GAC^PR
Var7+ GAC^PR
Var8+ GAC^PR
Var9+ GAC^PR
Var10+ GAC^PR
Var1
Var2
Var3
Var4
Var5
Var6
Var7
Var8
Var9
Var10
2h
16h
250
150
100
75
50
37
25
20
15
10



Sample ID	Mw (kDa)
SLO 6 - GAC CNJ	67.4
SLO 7 - GAC CNJ	72.6
SLO 8 - GAC CNJ	62.6
SLO 9 - GAC CNJ	69.6
SLO 10 - GAC CNJ	73.3
GAC DER DBCO	6.6
GAC-Mut no Der	6.9



Sample ID	Mw (kDa)
SLO 1 - GAC CNJ	97.1
SLO 2 - GAC CNJ	78.5
SLO 3 - GAC CNJ	90.4
SLO 4 - GAC CNJ	79.1
SLO 5 - GAC CNJ	116
GAC DER DBCO	6.2
GAC-DBCO	6.6

FIG. 30

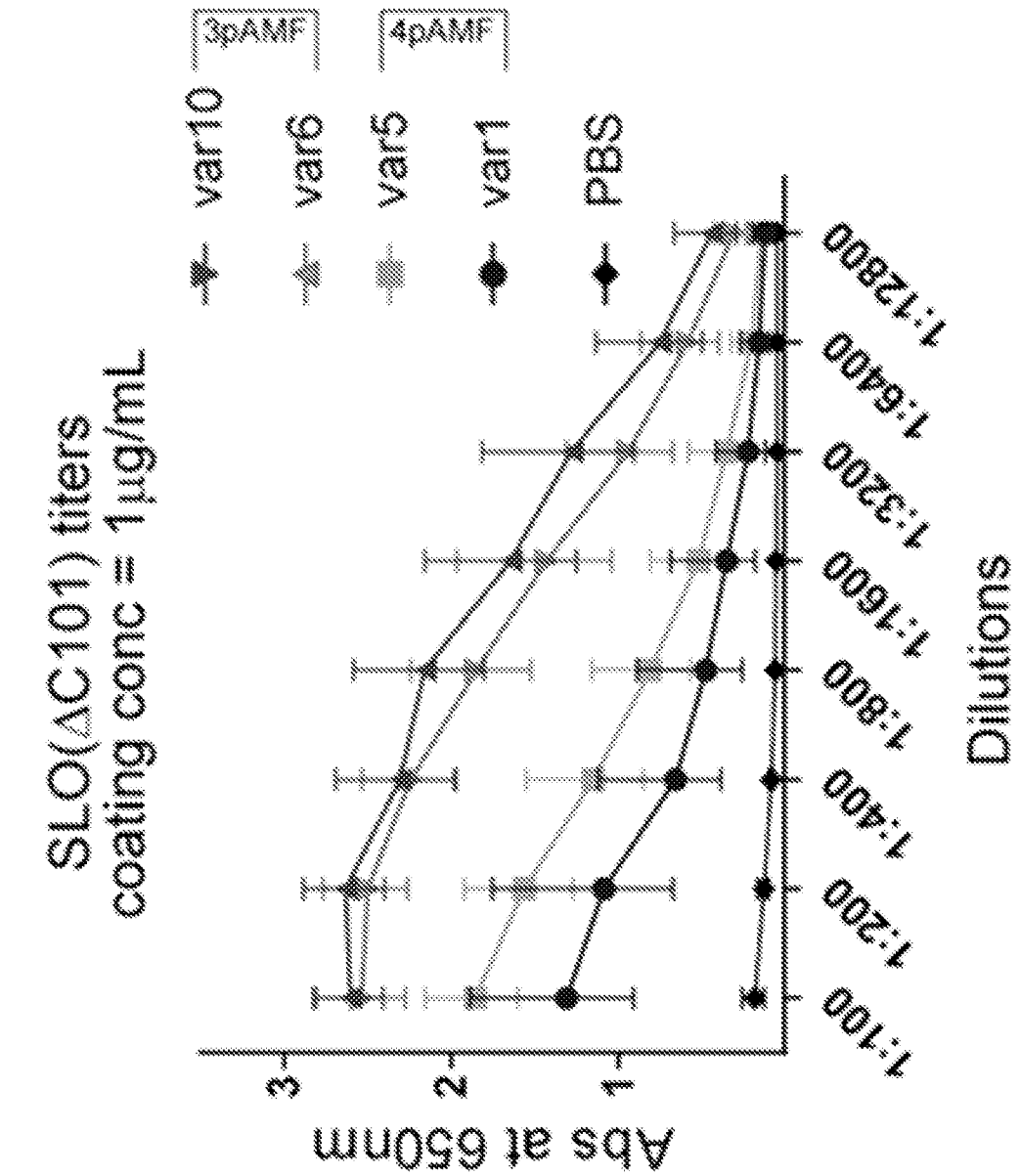
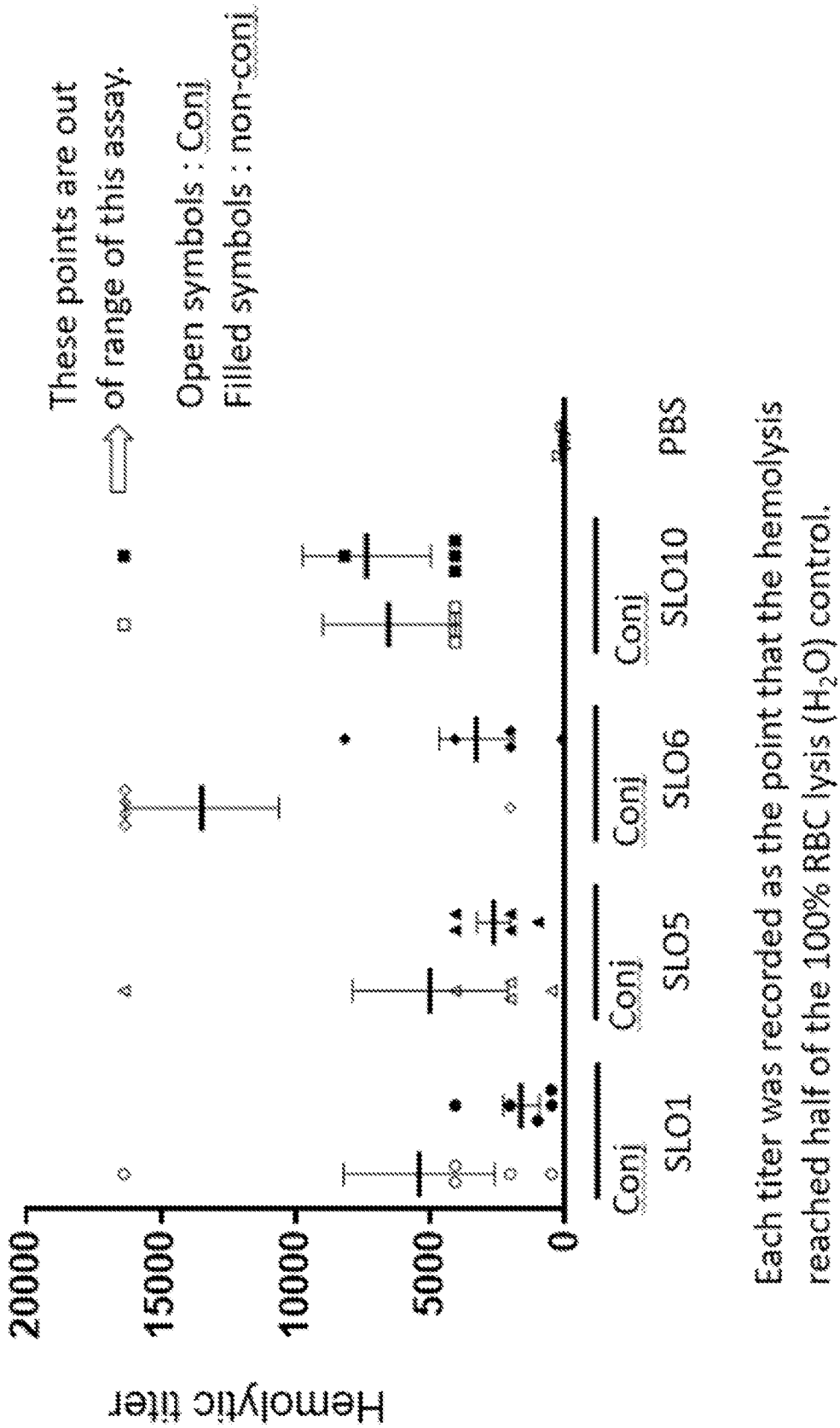

SLO(ΔC101) titers
coating conc = 1μg/mL
3pAMF
4pAMF
var10
var6
var5
var1
PBS
Abs at 650nm
3
2
1
1:100
1:200
1:400
1:800
1:1600
1:3200
1:6400
1:12800
Dilutions

FIG. 31A

FIG. 31B


These points are out
of range of this assay.
Open symbols : Conj
Filled symbols : non-conj
Hemolytic titer
20000
15000
10000
5000
0
Conj
SLO1
Conj
SLO5
Conj
SLO6
Conj
SLO10
PBS
Each titer was recorded as the point that the hemolysis
reached half of the 100% RBC lysis (H_2O) control.

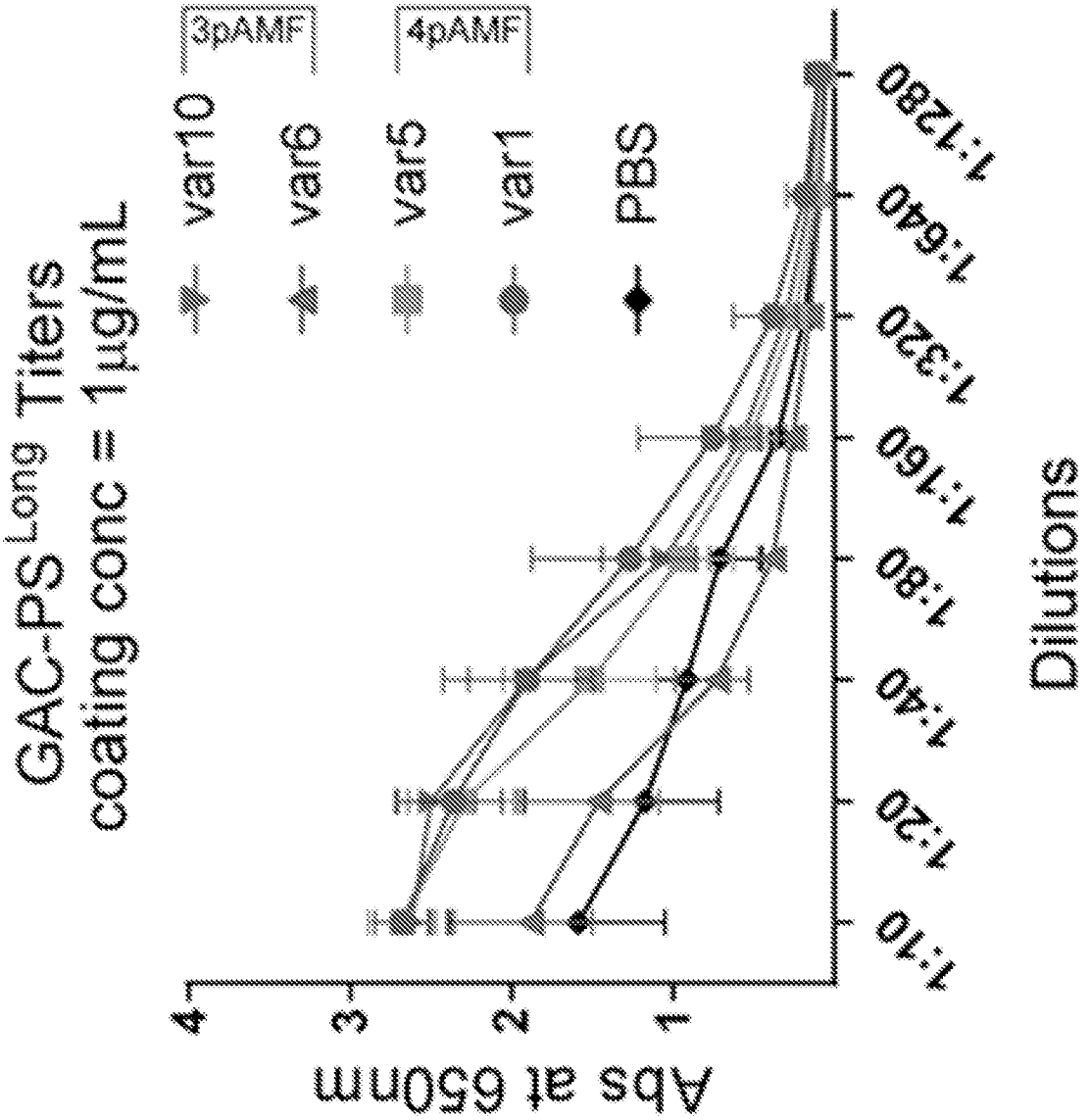

GAC-PS Long Titers
coating conc = 1µg/mL
3pAMF
4pAMF
var10
var6
var5
var1
PBS
Abs at 650nm
4
3
2
1
1:10
1:20
1:40
1:80
1:160
1:320
1:640
1:1280
Dilutions

FIG. 31C

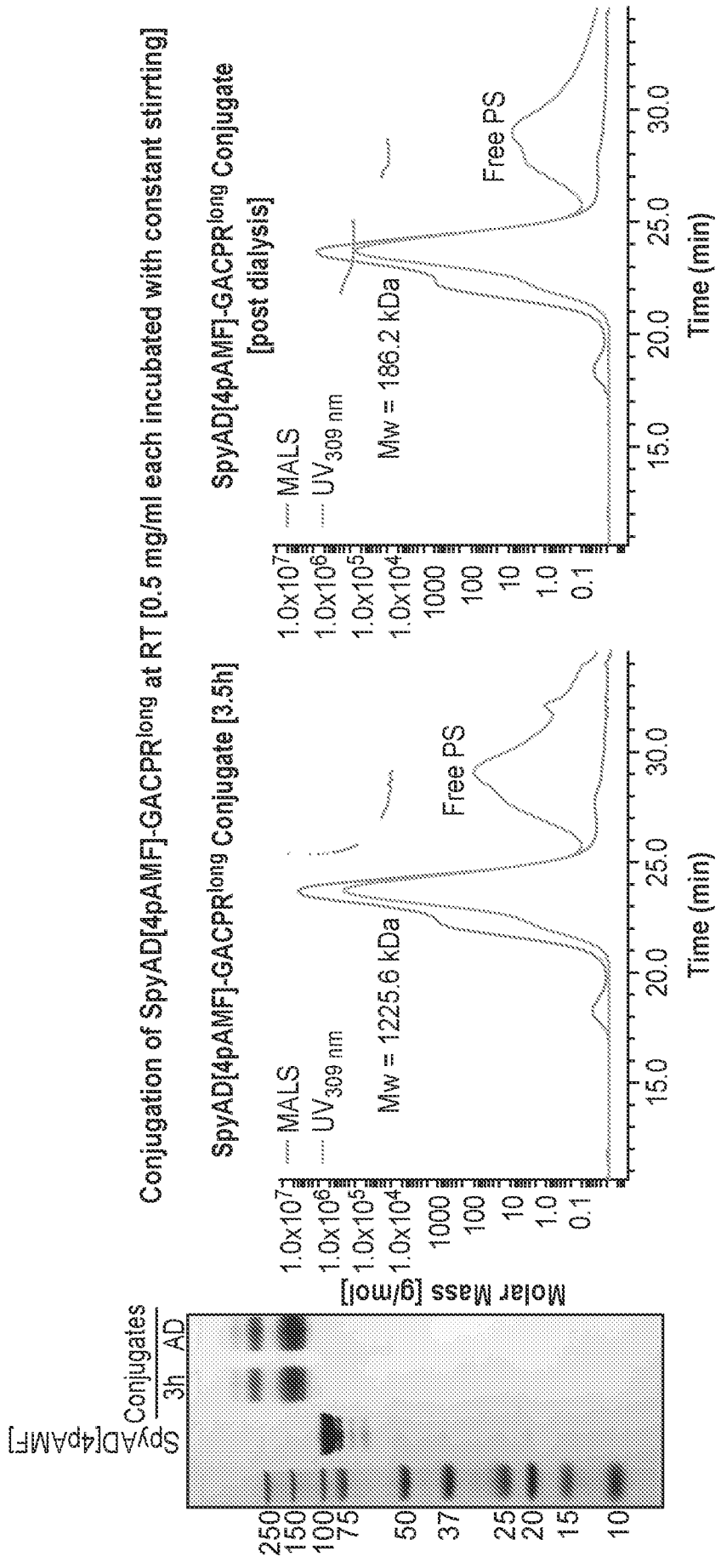

Conjugation of SpyAD[4pAMF]-GACPR^long at RT [0.5 mg/ml each incubated with constant stirring]
SpyAD[4pAMF]-GACPR^long Conjugate [3.5h]
SpyAD[4pAMF]-GACPR^long Conjugate [post dialysis]
MALS
UV309 nm
Mw = 1225.6 kDa
Mw = 186.2 kDa
Free PS
Molar Mass [g/mol]
Time (min)
SpyAD[4pAMF]
Conjugates
3h
AD

FIG. 32

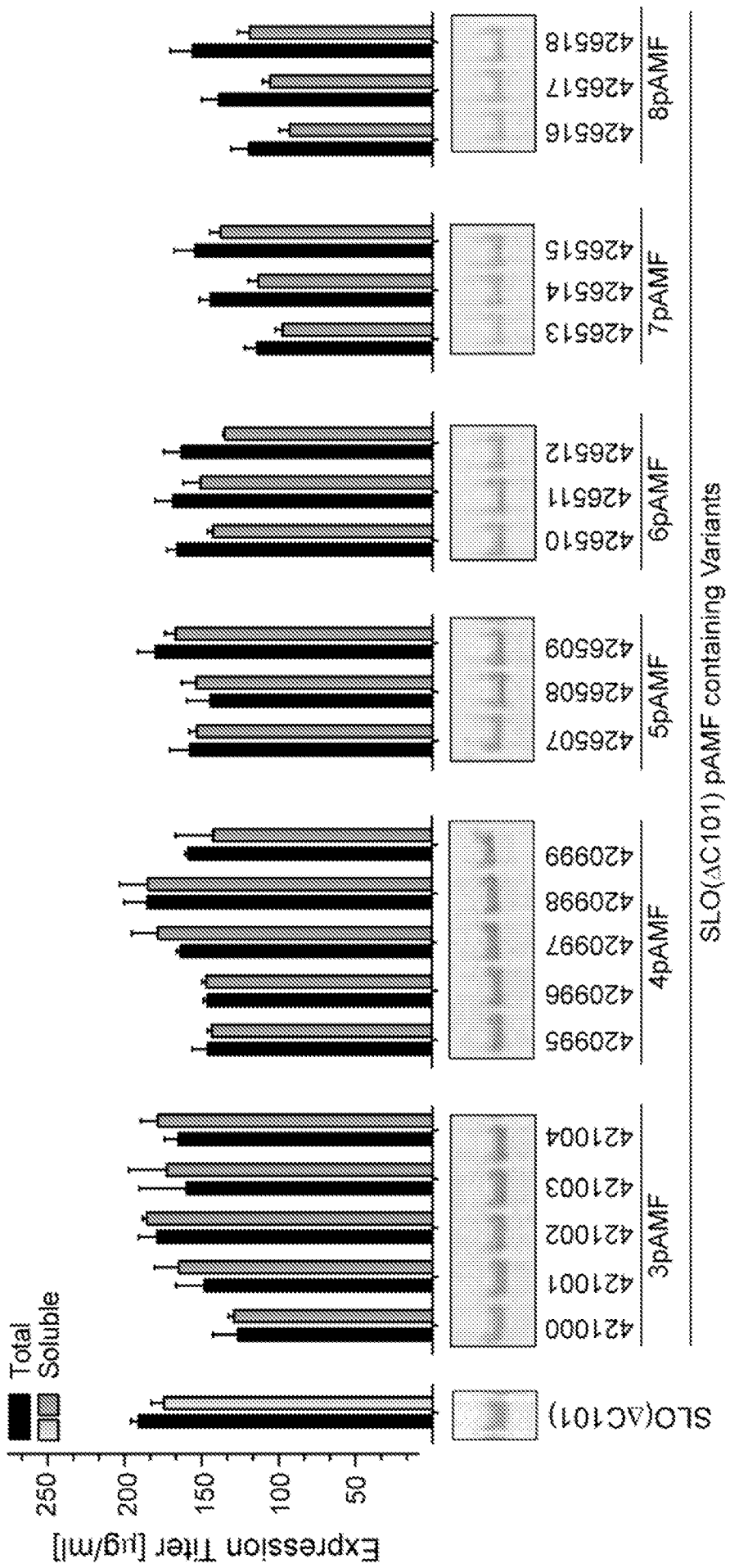

Expression Titer [μg/ml]
250
200
150
100
50
Total
Soluble
SLO(ΔC101)
421000
421001
421002
421003
421004
3pAMF
420995
420996
420997
420998
420999
4pAMF
426507
426508
426509
5pAMF
426510
426511
426512
6pAMF
426513
426514
426515
7pAMF
426516
426517
426518
8pAMF
SLO(ΔC101) pAMF containing Variants

FIG. 33A

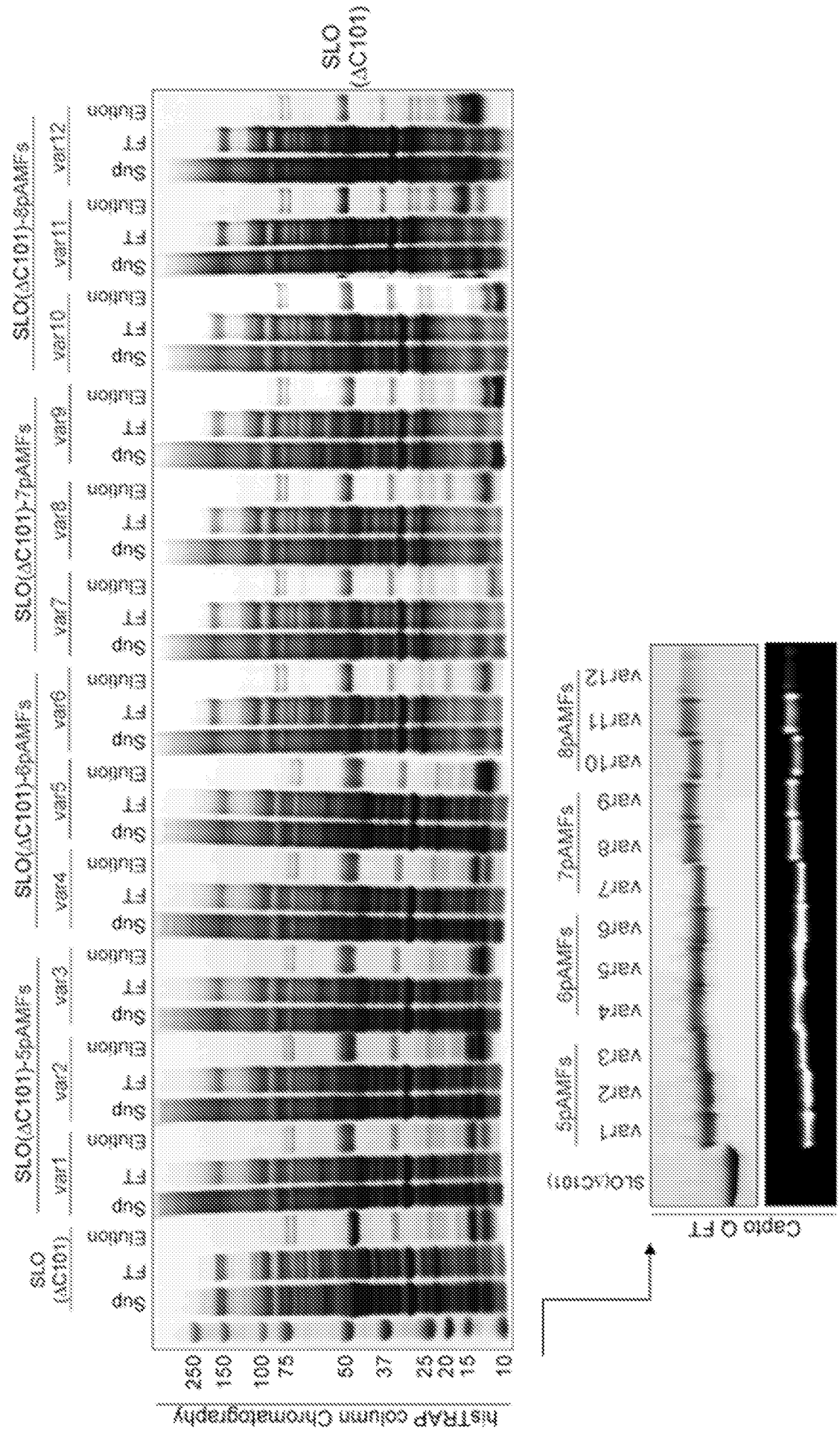
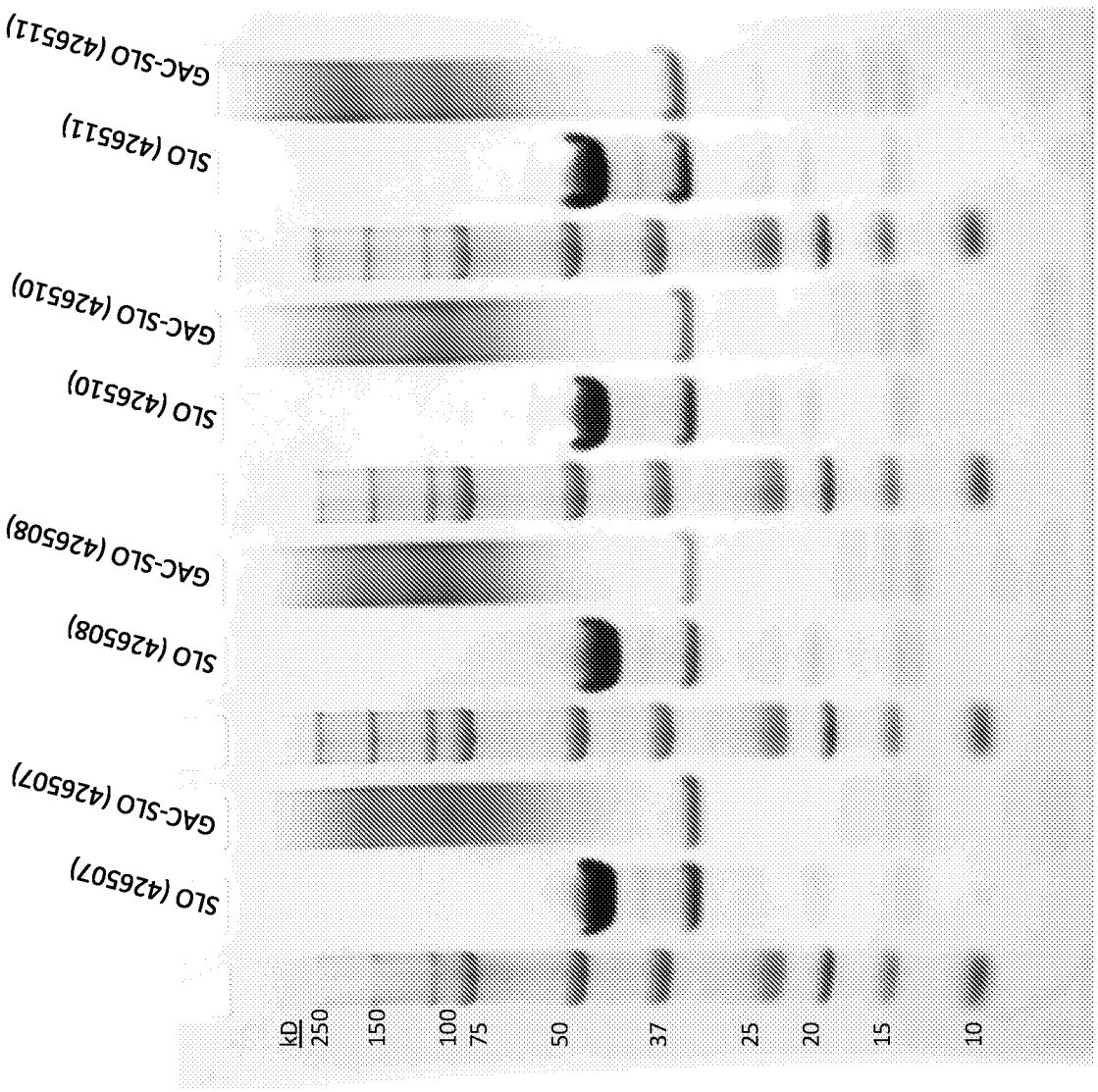
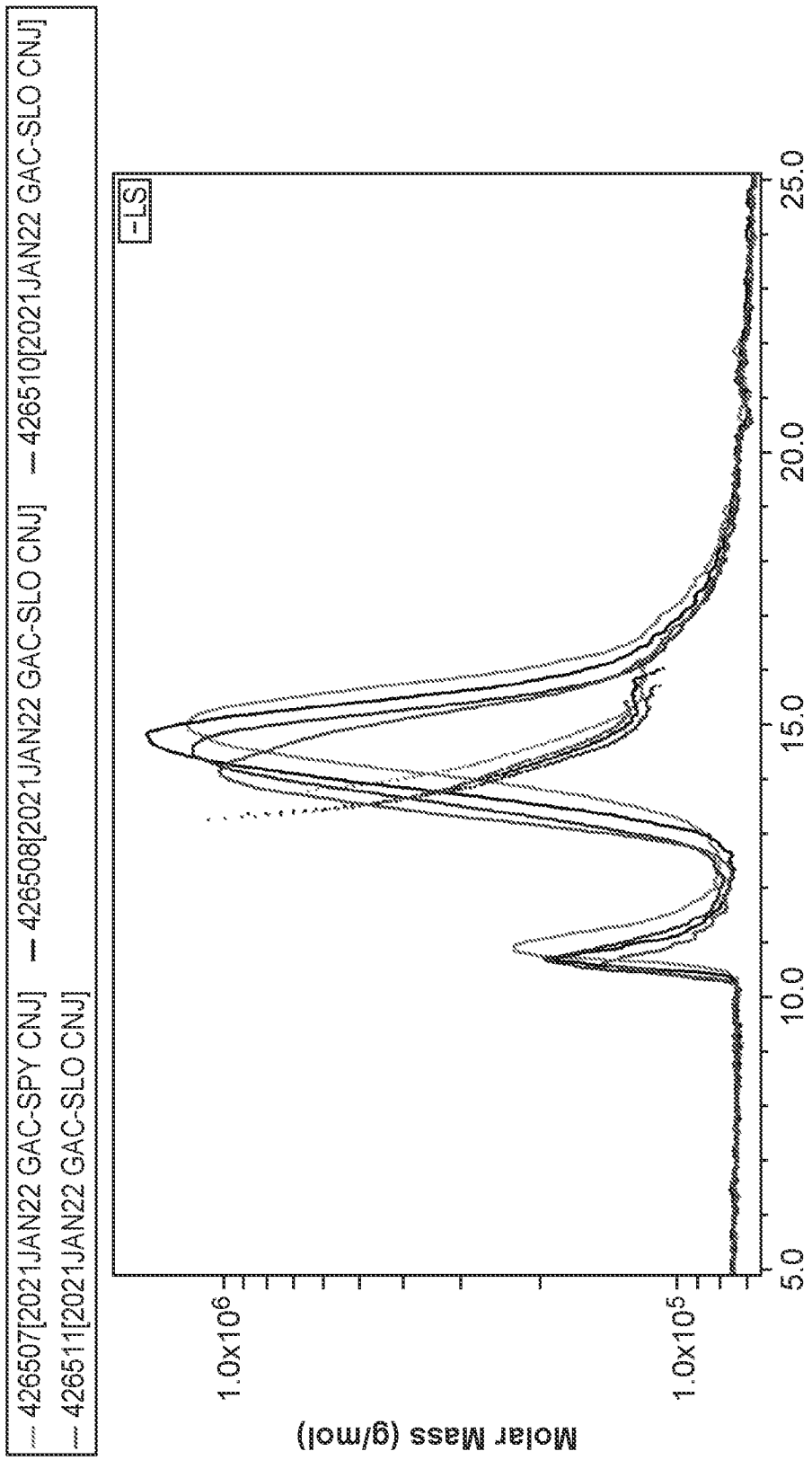

SLO (ΔC101)
SLO(ΔC101)-5pAMFs
SLO(ΔC101)-6pAMFs
SLO(ΔC101)-7pAMFs
SLO(ΔC101)-8pAMFs
var1
var2
var3
var4
var5
var6
var7
var8
var9
var10
var11
var12
Sup
FT
Elution
250
150
100
75
50
37
25
20
15
10
HisTRAP column Chromatography
SLO(ΔC101)
5pAMFs
6pAMFs
7pAMFs
8pAMFs
Capto Q FT

FIG. 33B

FIG. 34


GAC Long:SLO – 0.5:0.5
Reaction went overnight, 650 RPM 22C
Sample Load: 10uL
Protein Load: 5ug
kD
250
150
100
75
50
37
25
20
15
10
SLO (426507)
GAC-SLO (426507)
SLO (426508)
GAC-SLO (426508)
SLO (426510)
GAC-SLO (426510)
SLO (426511)
GAC-SLO (426511)



Sample ID	Mw (kDa)
426507SLO(ΔC101)-GAC conjugate	161.1
426508 SLO(ΔC101)-GAC conjugate	162.2
426510 SLO(ΔC101)-GAC conjugate	159.1
4265011 SLO(ΔC101)-GAC conjugate	208.9

FIG. 35

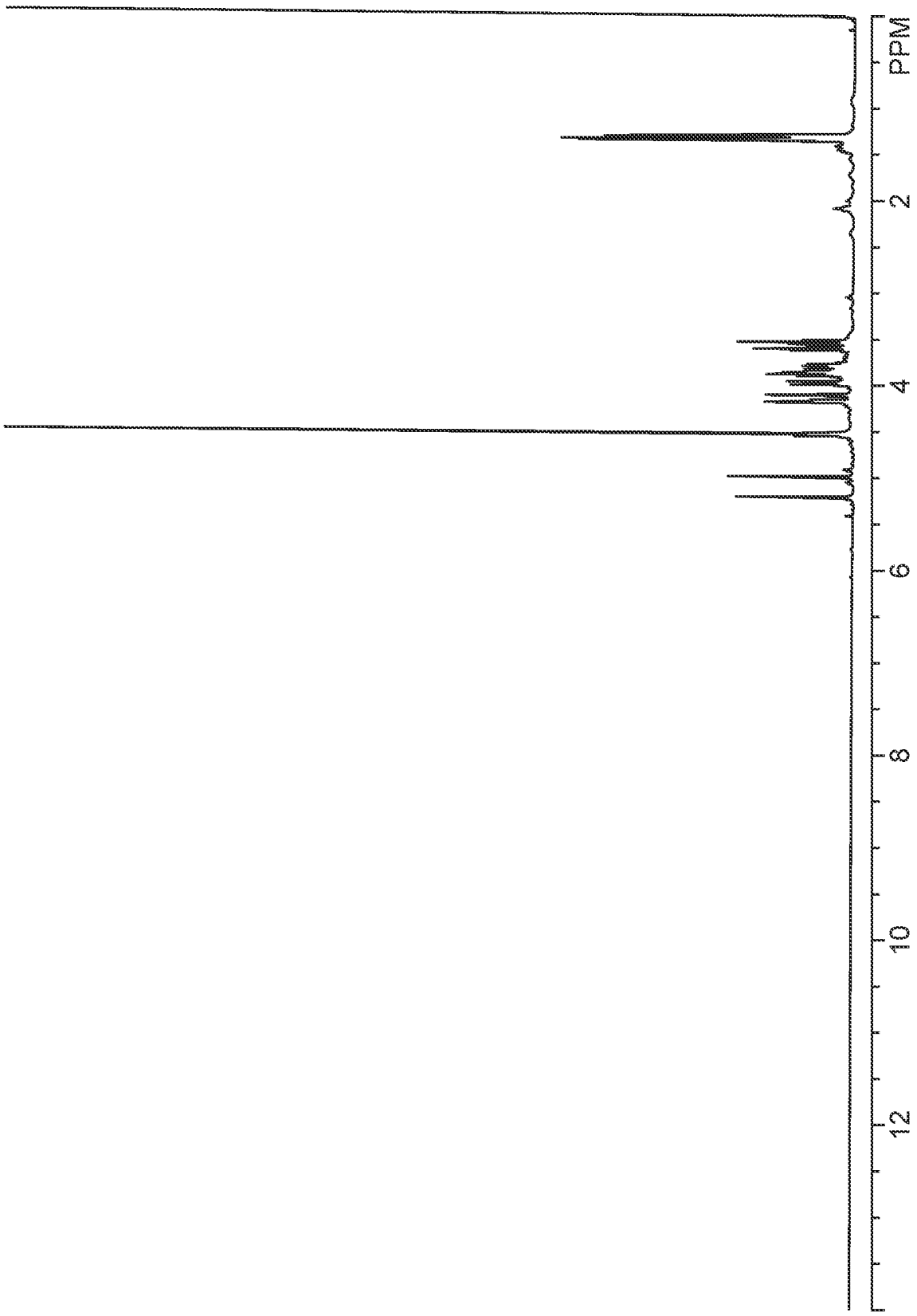


FIG. 36A

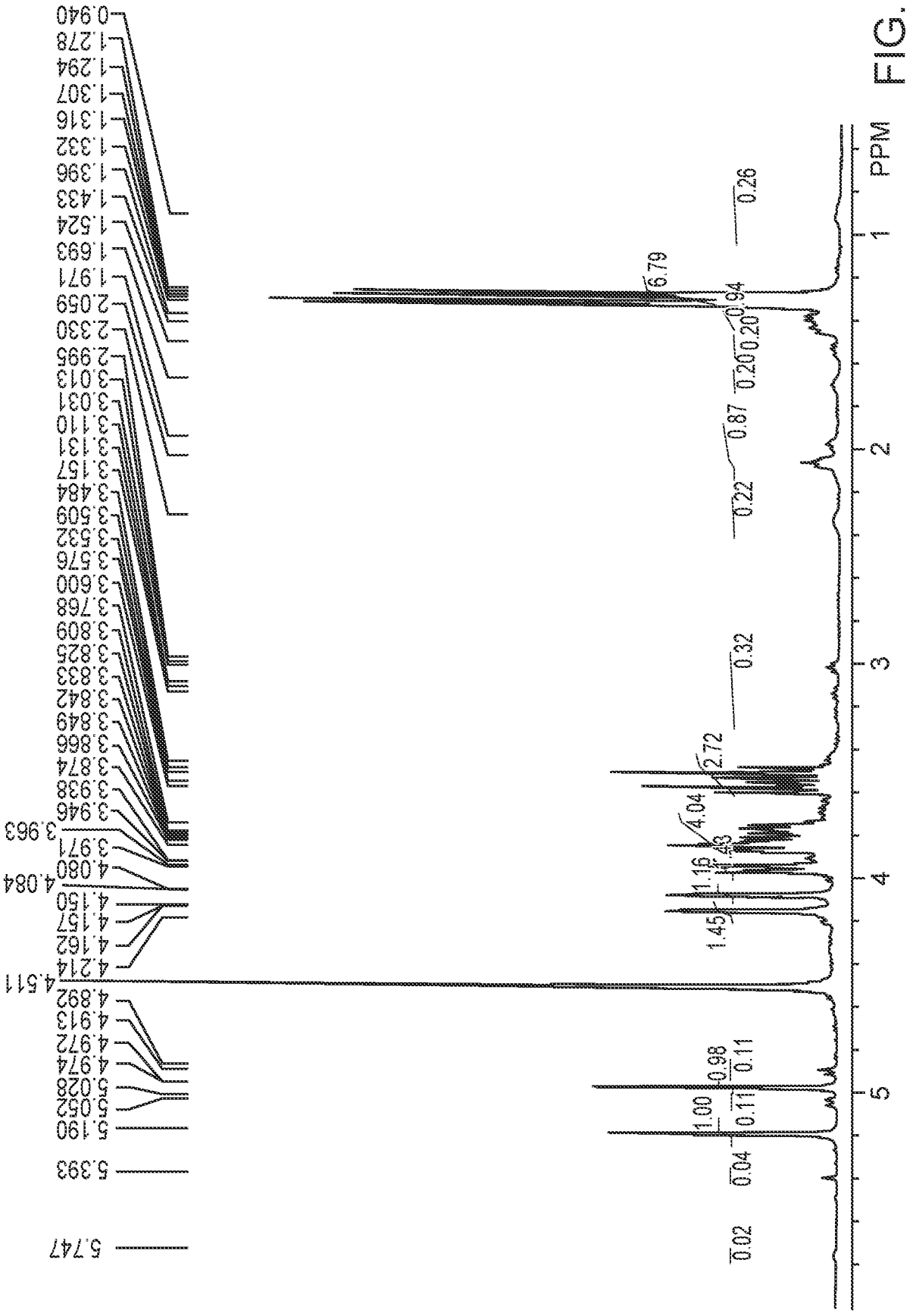

0.940
1.278
1.294
1.307
1.316
1.332
1.396
1.433
1.524
1.693
1.971
2.059
2.330
2.995
3.013
3.031
3.110
3.131
3.157
3.484
3.509
3.532
3.576
3.600
3.768
3.809
3.825
3.833
3.842
3.849
3.866
3.874
3.938
3.946
3.963
3.971
4.080
4.084
4.150
4.157
4.162
4.214
4.511
4.892
4.913
4.972
4.974
5.028
5.052
5.190
5.393
5.747
0.02
0.04
1.00
0.11
0.98
0.11
1.45
1.16
1.43
4.04
2.72
0.32
0.22
0.87
0.20
0.20
0.94
6.79
0.26
PPM
1
2
3
4
5

FIG. 36B

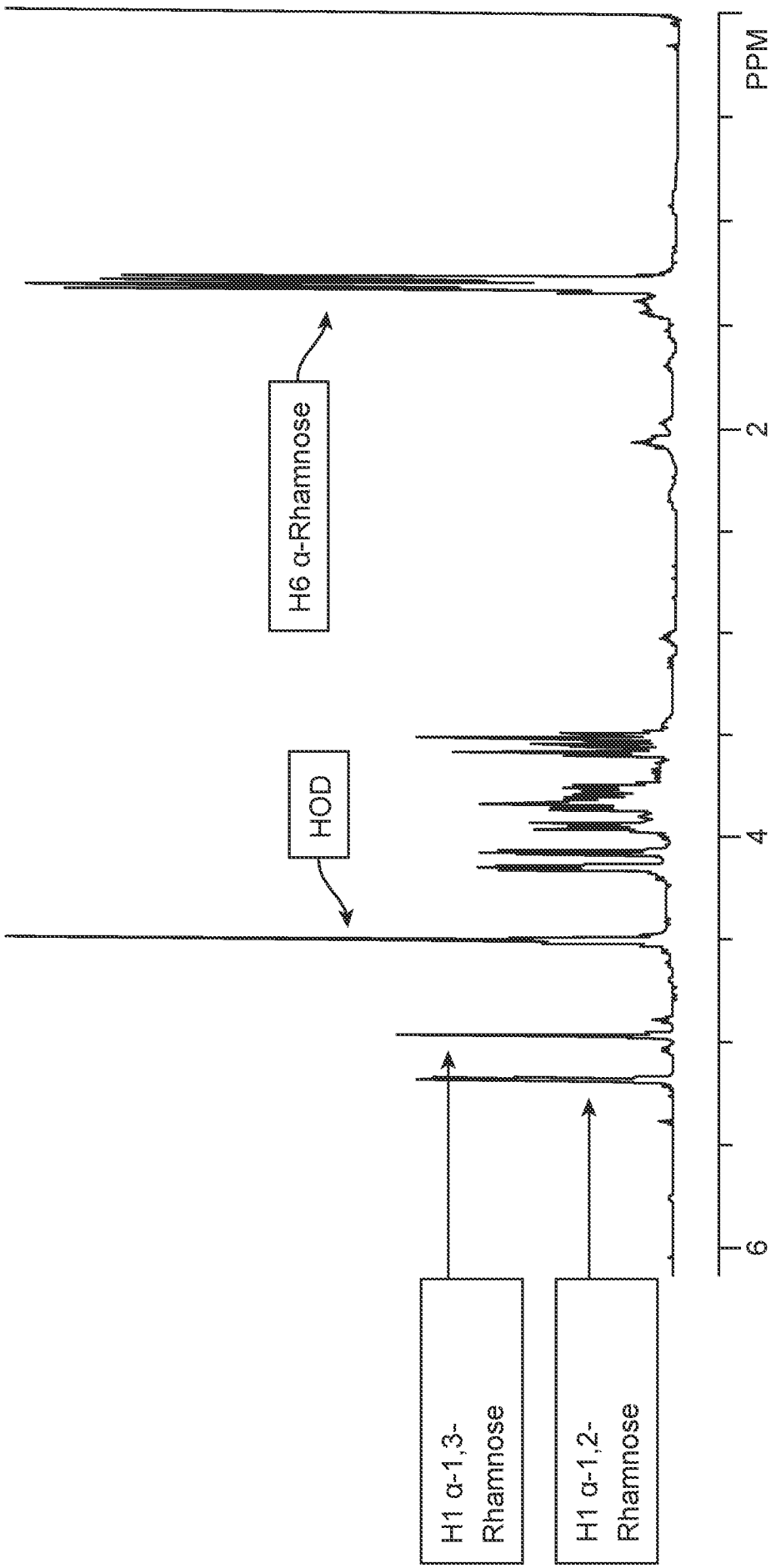


FIG. 36C

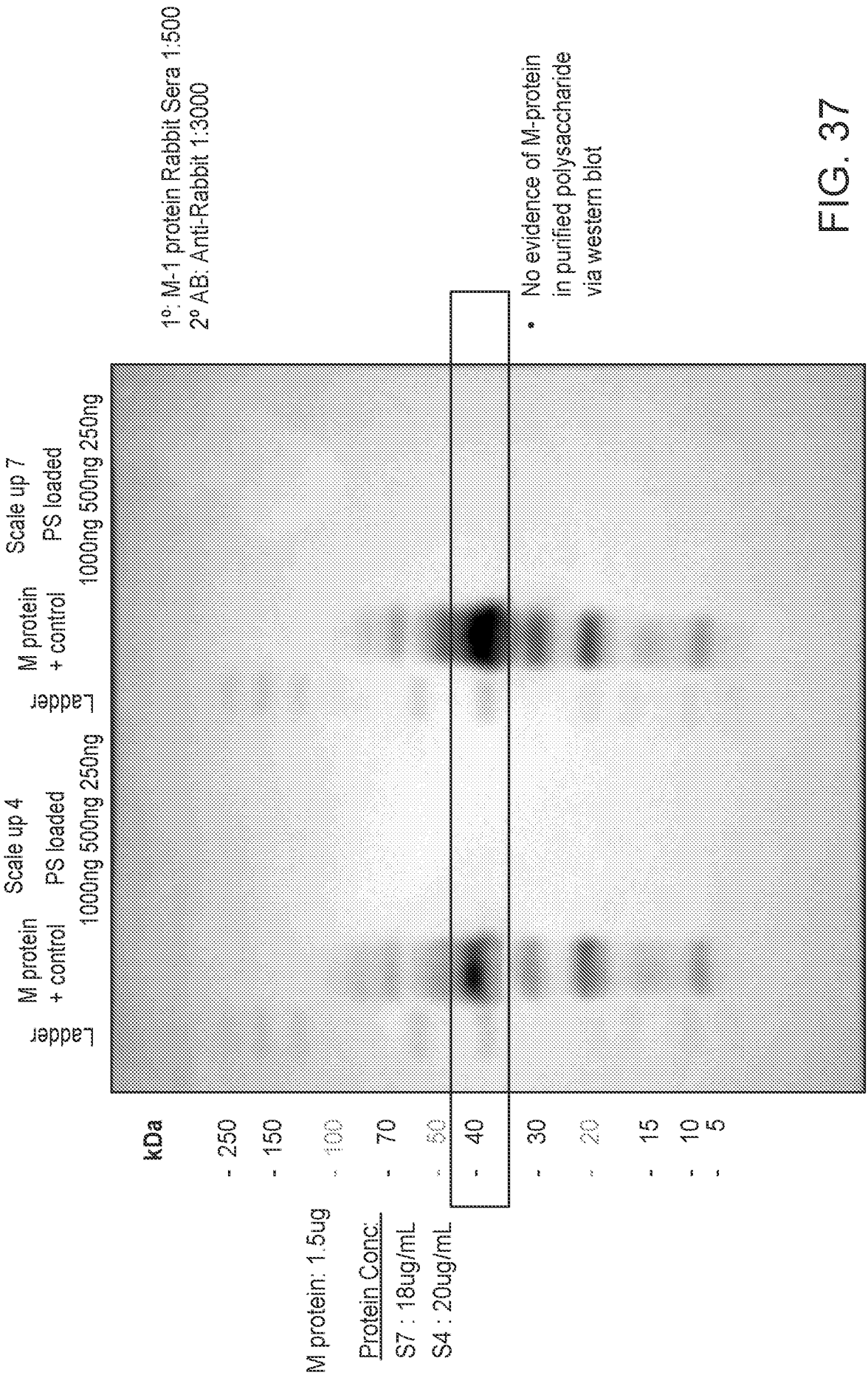

1°: M-1 protein Rabbit Sera 1:500
2° AB: Anti-Rabbit 1:3000
No evidence of M-protein in purified polysaccharide via western blot
kDa
- 250
- 150
- 100
- 70
- 50
- 40
- 30
- 20
- 15
- 10
- 5
M protein: 1.5ug
Protein Conc.
S7 : 18ug/mL
S4 : 20ug/mL
Ladder
M protein + control
Scale up 4 PS loaded 1000ng 500ng 250ng
Ladder
M protein + control
Scale up 7 PS loaded 1000ng 500ng 250ng

FIG. 37

SEQUENCE LISTING

<110> Vaxcyte, INC.

<120> GROUP A STREP IMMUNOGENIC COMPOSITIONS WITH POLYSACCHARIDE-PROTEIN CONJUGATES

<130> STRO-011/04WO 331540-2094

<150> US 62/977,886
<151> 2020-02-18

<150> US 63/013,924
<151> 2020-04-22

<150> US 63/090,069
<151> 2020-10-09

<150> US 63/123,293
<151> 2020-12-09

<160> 76

<170> PatentIn version 3.5

<210> 1
<211> 962
<212> PRT
<213> Artificial Sequence

<220>
<223> C5a [90-1035 fragment] - WT w/ leader

<400> 1

Met His His His His His His Gly Ser Gly Glu Asn Leu Tyr Phe Gln
1 5 10 15

Gly Lys Thr Ala Asp Thr Pro Val Thr Ser Lys Ala Thr Ile Arg Asp
20 25 30

Leu Asn Asp Pro Ser Gln Val Lys Thr Leu Gln Glu Lys Ala Gly Lys
35 40 45

Gly Ala Gly Thr Val Val Ala Val Ile Asp Ala Gly Phe Asp Lys Asn
50 55 60

His Glu Ala Trp Arg Leu Thr Asp Lys Thr Lys Ala Arg Tyr Gln Ser
65 70 75 80

Lys Glu Asp Leu Glu Lys Ala Lys Lys Glu His Gly Ile Thr Tyr Gly
85 90 95

Glu Trp Val Asn Asp Lys Val Ala Tyr Tyr His Asp Tyr Ser Lys Asp
100 105 110

Gly Lys Thr Ala Val Asp Gln Glu His Gly Thr His Val Ser Gly Ile
115 120 125

Leu Ser Gly Asn Ala Pro Ser Glu Thr Lys Glu Pro Tyr Arg Leu Glu

```
    130                 135                 140

Gly Ala Met Pro Glu Ala Gln Leu Leu Leu Met Arg Val Glu Ile Val
145                 150                 155                 160

Asn Gly Leu Ala Asp Tyr Ala Arg Asn Tyr Ala Gln Ala Ile Arg Asp
                165                 170                 175

Ala Val Asn Leu Gly Ala Lys Val Ile Asn Met Ser Phe Gly Asn Ala
            180                 185                 190

Ala Leu Ala Tyr Ala Asn Leu Pro Asp Glu Thr Lys Lys Ala Phe Asp
        195                 200                 205

Tyr Ala Lys Ser Lys Gly Val Ser Ile Val Thr Ser Ala Gly Asn Asp
    210                 215                 220

Ser Ser Phe Gly Gly Lys Thr Arg Leu Pro Leu Ala Asp His Pro Asp
225                 230                 235                 240

Tyr Gly Val Val Gly Thr Pro Ala Ala Ala Asp Ser Thr Leu Thr Val
                245                 250                 255

Ala Ser Tyr Ser Pro Asp Lys Gln Leu Thr Glu Thr Ala Thr Val Lys
            260                 265                 270

Thr Ala Asp Lys Gln Asp Lys Glu Met Pro Val Leu Ser Thr Asn Arg
        275                 280                 285

Phe Glu Pro Asn Lys Ala Tyr Asp Tyr Ala Tyr Ala Asn Arg Gly Met
    290                 295                 300

Lys Glu Asp Asp Phe Lys Asp Val Lys Gly Lys Ile Ala Leu Ile Glu
305                 310                 315                 320

Arg Gly Asp Ile Asp Phe Lys Asp Lys Ile Ala Asn Ala Lys Lys Ala
                325                 330                 335

Gly Ala Val Gly Val Leu Ile Tyr Asp Asn Gln Asp Lys Gly Phe Pro
            340                 345                 350

Ile Glu Leu Pro Asn Val Asp Gln Met Pro Ala Ala Phe Ile Ser Arg
        355                 360                 365

Lys Asp Gly Leu Leu Leu Lys Asp Asn Pro Lys Lys Thr Ile Thr Phe
    370                 375                 380

Asn Ala Thr Pro Lys Val Leu Pro Thr Ala Ser Gly Thr Lys Leu Ser
385                 390                 395                 400

Arg Phe Ser Ser Trp Gly Leu Thr Ala Asp Gly Asn Ile Lys Pro Asp
                405                 410                 415
```

```
Ile Ala Ala Pro Gly Gln Asp Ile Leu Ser Ser Val Ala Asn Asn Lys
            420                 425                 430

Tyr Ala Lys Leu Ser Gly Thr Ser Met Ser Ala Pro Leu Val Ala Gly
        435                 440                 445

Ile Met Gly Leu Leu Gln Lys Gln Tyr Glu Thr Gln Tyr Pro Asp Met
    450                 455                 460

Thr Pro Ser Glu Arg Leu Asp Leu Ala Lys Lys Val Leu Met Ser Ser
465                 470                 475                 480

Ala Thr Ala Leu Tyr Asp Glu Asp Glu Lys Ala Tyr Phe Ser Pro Arg
                485                 490                 495

Gln Gln Gly Ala Gly Ala Val Asp Ala Lys Lys Ala Ser Ala Ala Thr
            500                 505                 510

Met Tyr Val Thr Asp Lys Asp Asn Thr Ser Ser Lys Val His Leu Asn
        515                 520                 525

Asn Val Ser Asp Thr Phe Glu Val Thr Val Thr Val His Asn Lys Ser
    530                 535                 540

Asp Lys Pro Gln Glu Leu Tyr Tyr Gln Ala Thr Val Gln Thr Asp Lys
545                 550                 555                 560

Val Asp Gly Lys His Phe Ala Leu Ala Pro Lys Ala Leu Tyr Glu Thr
                565                 570                 575

Ser Trp Gln Lys Ile Thr Ile Pro Ala Asn Ser Ser Lys Gln Val Thr
            580                 585                 590

Val Pro Ile Asp Ala Ser Arg Phe Ser Lys Asp Leu Leu Ala Gln Met
        595                 600                 605

Lys Asn Gly Tyr Phe Leu Glu Gly Phe Val Arg Phe Lys Gln Asp Pro
    610                 615                 620

Thr Lys Glu Glu Leu Met Ser Ile Pro Tyr Ile Gly Phe Arg Gly Asp
625                 630                 635                 640

Phe Gly Asn Leu Ser Ala Leu Glu Lys Pro Ile Tyr Asp Ser Lys Asp
                645                 650                 655

Gly Ser Ser Tyr Tyr His Glu Ala Asn Ser Asp Ala Lys Asp Gln Leu
            660                 665                 670

Asp Gly Asp Gly Leu Gln Phe Tyr Ala Leu Lys Asn Asn Phe Thr Ala
        675                 680                 685
```

```
Leu Thr Thr Glu Ser Asn Pro Trp Thr Ile Ile Lys Ala Val Lys Glu
    690                 695                 700

Gly Val Glu Asn Ile Glu Asp Ile Glu Ser Ser Glu Ile Thr Glu Thr
705                 710                 715                 720

Ile Phe Ala Gly Thr Phe Ala Lys Gln Asp Asp Asp Ser His Tyr Tyr
                725                 730                 735

Ile His Arg His Ala Asn Gly Lys Pro Tyr Ala Ala Ile Ser Pro Asn
            740                 745                 750

Gly Asp Gly Asn Arg Asp Tyr Val Gln Phe Gln Gly Thr Phe Leu Arg
        755                 760                 765

Asn Ala Lys Asn Leu Val Ala Glu Val Leu Asp Lys Glu Gly Asn Val
    770                 775                 780

Val Trp Thr Ser Glu Val Thr Glu Gln Val Val Lys Asn Tyr Asn Asn
785                 790                 795                 800

Asp Leu Ala Ser Thr Leu Gly Ser Thr Arg Phe Glu Lys Thr Arg Trp
                805                 810                 815

Asp Gly Lys Asp Lys Asp Gly Lys Val Val Ala Asn Gly Thr Tyr Thr
            820                 825                 830

Tyr Arg Val Arg Tyr Thr Pro Ile Ser Ser Gly Ala Lys Glu Gln His
        835                 840                 845

Thr Asp Phe Asp Val Ile Val Asp Asn Thr Thr Pro Glu Val Ala Thr
    850                 855                 860

Ser Ala Thr Phe Ser Thr Glu Asp Arg Arg Leu Thr Leu Ala Ser Lys
865                 870                 875                 880

Pro Lys Thr Ser Gln Pro Val Tyr Arg Glu Arg Ile Ala Tyr Thr Tyr
                885                 890                 895

Met Asp Glu Asp Leu Pro Thr Thr Glu Tyr Ile Ser Pro Asn Glu Asp
            900                 905                 910

Gly Thr Phe Thr Leu Pro Glu Glu Ala Glu Thr Met Glu Gly Ala Thr
        915                 920                 925

Val Pro Leu Lys Met Ser Asp Phe Thr Tyr Val Val Glu Asp Met Ala
    930                 935                 940

Gly Asn Ile Thr Tyr Thr Pro Val Thr Lys Leu Leu Glu Gly His Ser
945                 950                 955                 960
```

Asn Lys

<210> 2
<211> 945
<212> PRT
<213> Streptococcus pyogenes

<400> 2

Lys Thr Ala Asp Thr Pro Val Thr Ser Lys Ala Thr Ile Arg Asp Leu
1 5 10 15

Asn Asp Pro Ser Gln Val Lys Thr Leu Gln Glu Lys Ala Gly Lys Gly
20 25 30

Ala Gly Thr Val Val Ala Val Ile Asp Ala Gly Phe Asp Lys Asn His
35 40 45

Glu Ala Trp Arg Leu Thr Asp Lys Thr Lys Ala Arg Tyr Gln Ser Lys
50 55 60

Glu Asp Leu Glu Lys Ala Lys Lys Glu His Gly Ile Thr Tyr Gly Glu
65 70 75 80

Trp Val Asn Asp Lys Val Ala Tyr Tyr His Asp Tyr Ser Lys Asp Gly
85 90 95

Lys Thr Ala Val Asp Gln Glu His Gly Thr His Val Ser Gly Ile Leu
100 105 110

Ser Gly Asn Ala Pro Ser Glu Thr Lys Glu Pro Tyr Arg Leu Glu Gly
115 120 125

Ala Met Pro Glu Ala Gln Leu Leu Leu Met Arg Val Glu Ile Val Asn
130 135 140

Gly Leu Ala Asp Tyr Ala Arg Asn Tyr Ala Gln Ala Ile Arg Asp Ala
145 150 155 160

Val Asn Leu Gly Ala Lys Val Ile Asn Met Ser Phe Gly Asn Ala Ala
165 170 175

Leu Ala Tyr Ala Asn Leu Pro Asp Glu Thr Lys Lys Ala Phe Asp Tyr
180 185 190

Ala Lys Ser Lys Gly Val Ser Ile Val Thr Ser Ala Gly Asn Asp Ser
195 200 205

Ser Phe Gly Gly Lys Thr Arg Leu Pro Leu Ala Asp His Pro Asp Tyr
210 215 220

Gly Val Val Gly Thr Pro Ala Ala Ala Asp Ser Thr Leu Thr Val Ala
225 230 235 240

```
Ser Tyr Ser Pro Asp Lys Gln Leu Thr Glu Thr Ala Thr Val Lys Thr
                245                 250                 255

Ala Asp Lys Gln Asp Lys Glu Met Pro Val Leu Ser Thr Asn Arg Phe
            260                 265                 270

Glu Pro Asn Lys Ala Tyr Asp Tyr Ala Tyr Ala Asn Arg Gly Met Lys
        275                 280                 285

Glu Asp Asp Phe Lys Asp Val Lys Gly Lys Ile Ala Leu Ile Glu Arg
    290                 295                 300

Gly Asp Ile Asp Phe Lys Asp Lys Ile Ala Asn Ala Lys Lys Ala Gly
305                 310                 315                 320

Ala Val Gly Val Leu Ile Tyr Asp Asn Gln Asp Lys Gly Phe Pro Ile
                325                 330                 335

Glu Leu Pro Asn Val Asp Gln Met Pro Ala Ala Phe Ile Ser Arg Lys
            340                 345                 350

Asp Gly Leu Leu Leu Lys Asp Asn Pro Lys Lys Thr Ile Thr Phe Asn
        355                 360                 365

Ala Thr Pro Lys Val Leu Pro Thr Ala Ser Gly Thr Lys Leu Ser Arg
    370                 375                 380

Phe Ser Ser Trp Gly Leu Thr Ala Asp Gly Asn Ile Lys Pro Asp Ile
385                 390                 395                 400

Ala Ala Pro Gly Gln Asp Ile Leu Ser Ser Val Ala Asn Asn Lys Tyr
                405                 410                 415

Ala Lys Leu Ser Gly Thr Ser Met Ser Ala Pro Leu Val Ala Gly Ile
            420                 425                 430

Met Gly Leu Leu Gln Lys Gln Tyr Glu Thr Gln Tyr Pro Asp Met Thr
        435                 440                 445

Pro Ser Glu Arg Leu Asp Leu Ala Lys Lys Val Leu Met Ser Ser Ala
    450                 455                 460

Thr Ala Leu Tyr Asp Glu Asp Glu Lys Ala Tyr Phe Ser Pro Arg Gln
465                 470                 475                 480

Gln Gly Ala Gly Ala Val Asp Ala Lys Lys Ala Ser Ala Ala Thr Met
                485                 490                 495

Tyr Val Thr Asp Lys Asp Asn Thr Ser Ser Lys Val His Leu Asn Asn
            500                 505                 510
```

```
Val Ser Asp Thr Phe Glu Val Thr Val Thr Val His Asn Lys Ser Asp
        515                 520                 525

Lys Pro Gln Glu Leu Tyr Tyr Gln Ala Thr Val Gln Thr Asp Lys Val
    530                 535                 540

Asp Gly Lys His Phe Ala Leu Ala Pro Lys Ala Leu Tyr Glu Thr Ser
545                 550                 555                 560

Trp Gln Lys Ile Thr Ile Pro Ala Asn Ser Ser Lys Gln Val Thr Val
                565                 570                 575

Pro Ile Asp Ala Ser Arg Phe Ser Lys Asp Leu Leu Ala Gln Met Lys
            580                 585                 590

Asn Gly Tyr Phe Leu Glu Gly Phe Val Arg Phe Lys Gln Asp Pro Thr
        595                 600                 605

Lys Glu Glu Leu Met Ser Ile Pro Tyr Ile Gly Phe Arg Gly Asp Phe
    610                 615                 620

Gly Asn Leu Ser Ala Leu Glu Lys Pro Ile Tyr Asp Ser Lys Asp Gly
625                 630                 635                 640

Ser Ser Tyr Tyr His Glu Ala Asn Ser Asp Ala Lys Asp Gln Leu Asp
                645                 650                 655

Gly Asp Gly Leu Gln Phe Tyr Ala Leu Lys Asn Asn Phe Thr Ala Leu
            660                 665                 670

Thr Thr Glu Ser Asn Pro Trp Thr Ile Ile Lys Ala Val Lys Glu Gly
        675                 680                 685

Val Glu Asn Ile Glu Asp Ile Glu Ser Ser Glu Ile Thr Glu Thr Ile
    690                 695                 700

Phe Ala Gly Thr Phe Ala Lys Gln Asp Asp Asp Ser His Tyr Tyr Ile
705                 710                 715                 720

His Arg His Ala Asn Gly Lys Pro Tyr Ala Ala Ile Ser Pro Asn Gly
                725                 730                 735

Asp Gly Asn Arg Asp Tyr Val Gln Phe Gln Gly Thr Phe Leu Arg Asn
            740                 745                 750

Ala Lys Asn Leu Val Ala Glu Val Leu Asp Lys Glu Gly Asn Val Val
        755                 760                 765

Trp Thr Ser Glu Val Thr Glu Gln Val Val Lys Asn Tyr Asn Asn Asp
    770                 775                 780
```

```
Leu Ala Ser Thr Leu Gly Ser Thr Arg Phe Glu Lys Thr Arg Trp Asp
785                 790                 795                 800


Gly Lys Asp Lys Asp Gly Lys Val Val Ala Asn Gly Thr Tyr Thr Tyr
                805                 810                 815


Arg Val Arg Tyr Thr Pro Ile Ser Ser Gly Ala Lys Glu Gln His Thr
            820                 825                 830


Asp Phe Asp Val Ile Val Asp Asn Thr Thr Pro Glu Val Ala Thr Ser
        835                 840                 845


Ala Thr Phe Ser Thr Glu Asp Arg Arg Leu Thr Leu Ala Ser Lys Pro
    850                 855                 860


Lys Thr Ser Gln Pro Val Tyr Arg Glu Arg Ile Ala Tyr Thr Tyr Met
865                 870                 875                 880


Asp Glu Asp Leu Pro Thr Thr Glu Tyr Ile Ser Pro Asn Glu Asp Gly
                885                 890                 895


Thr Phe Thr Leu Pro Glu Glu Ala Glu Thr Met Glu Gly Ala Thr Val
            900                 905                 910


Pro Leu Lys Met Ser Asp Phe Thr Tyr Val Val Glu Asp Met Ala Gly
        915                 920                 925


Asn Ile Thr Tyr Thr Pro Val Thr Lys Leu Leu Glu Gly His Ser Asn
    930                 935                 940


Lys
945


<210>  3
<211>  962
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  C5a [90-1035 fragment] D131A/S513A mutant w/ leader

<400>  3

Met His His His His His His Gly Ser Gly Glu Asn Leu Tyr Phe Gln
1               5                   10                  15


Gly Lys Thr Ala Asp Thr Pro Val Thr Ser Lys Ala Thr Ile Arg Asp
            20                  25                  30


Leu Asn Asp Pro Ser Gln Val Lys Thr Leu Gln Glu Lys Ala Gly Lys
        35                  40                  45


Gly Ala Gly Thr Val Val Ala Val Ile Ala Ala Gly Phe Asp Lys Asn
    50                  55                  60
```

```
His Glu Ala Trp Arg Leu Thr Asp Lys Thr Lys Ala Arg Tyr Gln Ser
65                  70                  75                  80

Lys Glu Asp Leu Glu Lys Ala Lys Lys Glu His Gly Ile Thr Tyr Gly
                85                  90                  95

Glu Trp Val Asn Asp Lys Val Ala Tyr Tyr His Asp Tyr Ser Lys Asp
            100                 105                 110

Gly Lys Thr Ala Val Asp Gln Glu His Gly Thr His Val Ser Gly Ile
        115                 120                 125

Leu Ser Gly Asn Ala Pro Ser Glu Thr Lys Glu Pro Tyr Arg Leu Glu
    130                 135                 140

Gly Ala Met Pro Glu Ala Gln Leu Leu Leu Met Arg Val Glu Ile Val
145                 150                 155                 160

Asn Gly Leu Ala Asp Tyr Ala Arg Asn Tyr Ala Gln Ala Ile Arg Asp
                165                 170                 175

Ala Val Asn Leu Gly Ala Lys Val Ile Asn Met Ser Phe Gly Asn Ala
            180                 185                 190

Ala Leu Ala Tyr Ala Asn Leu Pro Asp Glu Thr Lys Lys Ala Phe Asp
        195                 200                 205

Tyr Ala Lys Ser Lys Gly Val Ser Ile Val Thr Ser Ala Gly Asn Asp
    210                 215                 220

Ser Ser Phe Gly Gly Lys Thr Arg Leu Pro Leu Ala Asp His Pro Asp
225                 230                 235                 240

Tyr Gly Val Val Gly Thr Pro Ala Ala Ala Asp Ser Thr Leu Thr Val
                245                 250                 255

Ala Ser Tyr Ser Pro Asp Lys Gln Leu Thr Glu Thr Ala Thr Val Lys
            260                 265                 270

Thr Ala Asp Lys Gln Asp Lys Glu Met Pro Val Leu Ser Thr Asn Arg
        275                 280                 285

Phe Glu Pro Asn Lys Ala Tyr Asp Tyr Ala Tyr Ala Asn Arg Gly Met
    290                 295                 300

Lys Glu Asp Asp Phe Lys Asp Val Lys Gly Lys Ile Ala Leu Ile Glu
305                 310                 315                 320

Arg Gly Asp Ile Asp Phe Lys Asp Lys Ile Ala Asn Ala Lys Lys Ala
                325                 330                 335
```

```
Gly Ala Val Gly Val Leu Ile Tyr Asp Asn Gln Asp Lys Gly Phe Pro
            340                 345                 350

Ile Glu Leu Pro Asn Val Asp Gln Met Pro Ala Ala Phe Ile Ser Arg
        355                 360                 365

Lys Asp Gly Leu Leu Leu Lys Asp Asn Pro Lys Lys Thr Ile Thr Phe
    370                 375                 380

Asn Ala Thr Pro Lys Val Leu Pro Thr Ala Ser Gly Thr Lys Leu Ser
385                 390                 395                 400

Arg Phe Ser Ser Trp Gly Leu Thr Ala Asp Gly Asn Ile Lys Pro Asp
                405                 410                 415

Ile Ala Ala Pro Gly Gln Asp Ile Leu Ser Ser Val Ala Asn Asn Lys
            420                 425                 430

Tyr Ala Lys Leu Ser Gly Thr Ala Met Ser Ala Pro Leu Val Ala Gly
        435                 440                 445

Ile Met Gly Leu Leu Gln Lys Gln Tyr Glu Thr Gln Tyr Pro Asp Met
    450                 455                 460

Thr Pro Ser Glu Arg Leu Asp Leu Ala Lys Lys Val Leu Met Ser Ser
465                 470                 475                 480

Ala Thr Ala Leu Tyr Asp Glu Asp Glu Lys Ala Tyr Phe Ser Pro Arg
                485                 490                 495

Gln Gln Gly Ala Gly Ala Val Asp Ala Lys Lys Ala Ser Ala Ala Thr
            500                 505                 510

Met Tyr Val Thr Asp Lys Asp Asn Thr Ser Ser Lys Val His Leu Asn
        515                 520                 525

Asn Val Ser Asp Thr Phe Glu Val Thr Val Thr Val His Asn Lys Ser
    530                 535                 540

Asp Lys Pro Gln Glu Leu Tyr Tyr Gln Ala Thr Val Gln Thr Asp Lys
545                 550                 555                 560

Val Asp Gly Lys His Phe Ala Leu Ala Pro Lys Ala Leu Tyr Glu Thr
                565                 570                 575

Ser Trp Gln Lys Ile Thr Ile Pro Ala Asn Ser Ser Lys Gln Val Thr
            580                 585                 590

Val Pro Ile Asp Ala Ser Arg Phe Ser Lys Asp Leu Leu Ala Gln Met
        595                 600                 605

Lys Asn Gly Tyr Phe Leu Glu Gly Phe Val Arg Phe Lys Gln Asp Pro
```

```
    610                 615                 620

Thr Lys Glu Glu Leu Met Ser Ile Pro Tyr Ile Gly Phe Arg Gly Asp
625                 630                 635                 640

Phe Gly Asn Leu Ser Ala Leu Glu Lys Pro Ile Tyr Asp Ser Lys Asp
                645                 650                 655

Gly Ser Ser Tyr Tyr His Glu Ala Asn Ser Asp Ala Lys Asp Gln Leu
            660                 665                 670

Asp Gly Asp Gly Leu Gln Phe Tyr Ala Leu Lys Asn Asn Phe Thr Ala
        675                 680                 685

Leu Thr Thr Glu Ser Asn Pro Trp Thr Ile Ile Lys Ala Val Lys Glu
    690                 695                 700

Gly Val Glu Asn Ile Glu Asp Ile Glu Ser Ser Glu Ile Thr Glu Thr
705                 710                 715                 720

Ile Phe Ala Gly Thr Phe Ala Lys Gln Asp Asp Asp Ser His Tyr Tyr
                725                 730                 735

Ile His Arg His Ala Asn Gly Lys Pro Tyr Ala Ala Ile Ser Pro Asn
            740                 745                 750

Gly Asp Gly Asn Arg Asp Tyr Val Gln Phe Gln Gly Thr Phe Leu Arg
        755                 760                 765

Asn Ala Lys Asn Leu Val Ala Glu Val Leu Asp Lys Glu Gly Asn Val
    770                 775                 780

Val Trp Thr Ser Glu Val Thr Glu Gln Val Val Lys Asn Tyr Asn Asn
785                 790                 795                 800

Asp Leu Ala Ser Thr Leu Gly Ser Thr Arg Phe Glu Lys Thr Arg Trp
                805                 810                 815

Asp Gly Lys Asp Lys Asp Gly Lys Val Val Ala Asn Gly Thr Tyr Thr
            820                 825                 830

Tyr Arg Val Arg Tyr Thr Pro Ile Ser Ser Gly Ala Lys Glu Gln His
        835                 840                 845

Thr Asp Phe Asp Val Ile Val Asp Asn Thr Thr Pro Glu Val Ala Thr
    850                 855                 860

Ser Ala Thr Phe Ser Thr Glu Asp Arg Arg Leu Thr Leu Ala Ser Lys
865                 870                 875                 880

Pro Lys Thr Ser Gln Pro Val Tyr Arg Glu Arg Ile Ala Tyr Thr Tyr
                885                 890                 895
```

```
Met Asp Glu Asp Leu Pro Thr Thr Glu Tyr Ile Ser Pro Asn Glu Asp
            900                 905                 910

Gly Thr Phe Thr Leu Pro Glu Glu Ala Glu Thr Met Glu Gly Ala Thr
        915                 920                 925

Val Pro Leu Lys Met Ser Asp Phe Thr Tyr Val Val Glu Asp Met Ala
    930                 935                 940

Gly Asn Ile Thr Tyr Thr Pro Val Thr Lys Leu Leu Glu Gly His Ser
945                 950                 955                 960

Asn Lys
```

<210> 4
<211> 945
<212> PRT
<213> Artificial Sequence

<220>
<223> C5a [90-1035 fragment] D131A/S513A mutant w/o leader

<400> 4

```
Lys Thr Ala Asp Thr Pro Val Thr Ser Lys Ala Thr Ile Arg Asp Leu
1               5                   10                  15

Asn Asp Pro Ser Gln Val Lys Thr Leu Gln Glu Lys Ala Gly Lys Gly
            20                  25                  30

Ala Gly Thr Val Val Ala Val Ile Ala Ala Gly Phe Asp Lys Asn His
        35                  40                  45

Glu Ala Trp Arg Leu Thr Asp Lys Thr Lys Ala Arg Tyr Gln Ser Lys
    50                  55                  60

Glu Asp Leu Glu Lys Ala Lys Lys Glu His Gly Ile Thr Tyr Gly Glu
65                  70                  75                  80

Trp Val Asn Asp Lys Val Ala Tyr Tyr His Asp Tyr Ser Lys Asp Gly
                85                  90                  95

Lys Thr Ala Val Asp Gln Glu His Gly Thr His Val Ser Gly Ile Leu
            100                 105                 110

Ser Gly Asn Ala Pro Ser Glu Thr Lys Glu Pro Tyr Arg Leu Glu Gly
        115                 120                 125

Ala Met Pro Glu Ala Gln Leu Leu Leu Met Arg Val Glu Ile Val Asn
    130                 135                 140

Gly Leu Ala Asp Tyr Ala Arg Asn Tyr Ala Gln Ala Ile Arg Asp Ala
```

```
145                 150                 155                 160

Val Asn Leu Gly Ala Lys Val Ile Asn Met Ser Phe Gly Asn Ala Ala
                165                 170                 175

Leu Ala Tyr Ala Asn Leu Pro Asp Glu Thr Lys Lys Ala Phe Asp Tyr
            180                 185                 190

Ala Lys Ser Lys Gly Val Ser Ile Val Thr Ser Ala Gly Asn Asp Ser
        195                 200                 205

Ser Phe Gly Gly Lys Thr Arg Leu Pro Leu Ala Asp His Pro Asp Tyr
    210                 215                 220

Gly Val Val Gly Thr Pro Ala Ala Ala Asp Ser Thr Leu Thr Val Ala
225                 230                 235                 240

Ser Tyr Ser Pro Asp Lys Gln Leu Thr Glu Thr Ala Thr Val Lys Thr
                245                 250                 255

Ala Asp Lys Gln Asp Lys Glu Met Pro Val Leu Ser Thr Asn Arg Phe
            260                 265                 270

Glu Pro Asn Lys Ala Tyr Asp Tyr Ala Tyr Ala Asn Arg Gly Met Lys
        275                 280                 285

Glu Asp Asp Phe Lys Asp Val Lys Gly Lys Ile Ala Leu Ile Glu Arg
    290                 295                 300

Gly Asp Ile Asp Phe Lys Asp Lys Ile Ala Asn Ala Lys Lys Ala Gly
305                 310                 315                 320

Ala Val Gly Val Leu Ile Tyr Asp Asn Gln Asp Lys Gly Phe Pro Ile
                325                 330                 335

Glu Leu Pro Asn Val Asp Gln Met Pro Ala Ala Phe Ile Ser Arg Lys
            340                 345                 350

Asp Gly Leu Leu Leu Lys Asp Asn Pro Lys Lys Thr Ile Thr Phe Asn
        355                 360                 365

Ala Thr Pro Lys Val Leu Pro Thr Ala Ser Gly Thr Lys Leu Ser Arg
    370                 375                 380

Phe Ser Ser Trp Gly Leu Thr Ala Asp Gly Asn Ile Lys Pro Asp Ile
385                 390                 395                 400

Ala Ala Pro Gly Gln Asp Ile Leu Ser Ser Val Ala Asn Asn Lys Tyr
                405                 410                 415

Ala Lys Leu Ser Gly Thr Ala Met Ser Ala Pro Leu Val Ala Gly Ile
            420                 425                 430
```

```
Met Gly Leu Leu Gln Lys Gln Tyr Glu Thr Gln Tyr Pro Asp Met Thr
        435                 440                 445

Pro Ser Glu Arg Leu Asp Leu Ala Lys Lys Val Leu Met Ser Ser Ala
    450                 455                 460

Thr Ala Leu Tyr Asp Glu Asp Glu Lys Ala Tyr Phe Ser Pro Arg Gln
465                 470                 475                 480

Gln Gly Ala Gly Ala Val Asp Ala Lys Lys Ala Ser Ala Ala Thr Met
                485                 490                 495

Tyr Val Thr Asp Lys Asp Asn Thr Ser Ser Lys Val His Leu Asn Asn
            500                 505                 510

Val Ser Asp Thr Phe Glu Val Thr Val Thr Val His Asn Lys Ser Asp
        515                 520                 525

Lys Pro Gln Glu Leu Tyr Tyr Gln Ala Thr Val Gln Thr Asp Lys Val
    530                 535                 540

Asp Gly Lys His Phe Ala Leu Ala Pro Lys Ala Leu Tyr Glu Thr Ser
545                 550                 555                 560

Trp Gln Lys Ile Thr Ile Pro Ala Asn Ser Ser Lys Gln Val Thr Val
                565                 570                 575

Pro Ile Asp Ala Ser Arg Phe Ser Lys Asp Leu Leu Ala Gln Met Lys
            580                 585                 590

Asn Gly Tyr Phe Leu Glu Gly Phe Val Arg Phe Lys Gln Asp Pro Thr
        595                 600                 605

Lys Glu Glu Leu Met Ser Ile Pro Tyr Ile Gly Phe Arg Gly Asp Phe
    610                 615                 620

Gly Asn Leu Ser Ala Leu Glu Lys Pro Ile Tyr Asp Ser Lys Asp Gly
625                 630                 635                 640

Ser Ser Tyr Tyr His Glu Ala Asn Ser Asp Ala Lys Asp Gln Leu Asp
                645                 650                 655

Gly Asp Gly Leu Gln Phe Tyr Ala Leu Lys Asn Asn Phe Thr Ala Leu
            660                 665                 670

Thr Thr Glu Ser Asn Pro Trp Thr Ile Ile Lys Ala Val Lys Glu Gly
        675                 680                 685

Val Glu Asn Ile Glu Asp Ile Glu Ser Ser Glu Ile Thr Glu Thr Ile
    690                 695                 700
```

```
Phe Ala Gly Thr Phe Ala Lys Gln Asp Asp Asp Ser His Tyr Tyr Ile
705                 710                 715                 720

His Arg His Ala Asn Gly Lys Pro Tyr Ala Ala Ile Ser Pro Asn Gly
                725                 730                 735

Asp Gly Asn Arg Asp Tyr Val Gln Phe Gln Gly Thr Phe Leu Arg Asn
            740                 745                 750

Ala Lys Asn Leu Val Ala Glu Val Leu Asp Lys Glu Gly Asn Val Val
        755                 760                 765

Trp Thr Ser Glu Val Thr Glu Gln Val Val Lys Asn Tyr Asn Asn Asp
    770                 775                 780

Leu Ala Ser Thr Leu Gly Ser Thr Arg Phe Glu Lys Thr Arg Trp Asp
785                 790                 795                 800

Gly Lys Asp Lys Asp Gly Lys Val Val Ala Asn Gly Thr Tyr Thr Tyr
                805                 810                 815

Arg Val Arg Tyr Thr Pro Ile Ser Ser Gly Ala Lys Glu Gln His Thr
            820                 825                 830

Asp Phe Asp Val Ile Val Asp Asn Thr Thr Pro Glu Val Ala Thr Ser
        835                 840                 845

Ala Thr Phe Ser Thr Glu Asp Arg Arg Leu Thr Leu Ala Ser Lys Pro
    850                 855                 860

Lys Thr Ser Gln Pro Val Tyr Arg Glu Arg Ile Ala Tyr Thr Tyr Met
865                 870                 875                 880

Asp Glu Asp Leu Pro Thr Thr Glu Tyr Ile Ser Pro Asn Glu Asp Gly
                885                 890                 895

Thr Phe Thr Leu Pro Glu Glu Ala Glu Thr Met Glu Gly Ala Thr Val
            900                 905                 910

Pro Leu Lys Met Ser Asp Phe Thr Tyr Val Val Glu Asp Met Ala Gly
        915                 920                 925

Asn Ile Thr Tyr Thr Pro Val Thr Lys Leu Leu Glu Gly His Ser Asn
    930                 935                 940

Lys
945
```

<210> 5
<211> 510
<212> PRT
<213> Artificial Sequence

<220>
<223> SLO [79-571] fragment w/ leader

<400> 5

```
Met His His His His His His Gly Ser Gly Glu Asn Leu Tyr Phe Gln
1               5                   10                  15


Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
            20                  25                  30


Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
        35                  40                  45


Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
    50                  55                  60


Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
65                  70                  75                  80


Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn
                85                  90                  95


Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
            100                 105                 110


Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys
        115                 120                 125


Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
    130                 135                 140


Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
145                 150                 155                 160


Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
                165                 170                 175


Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
            180                 185                 190


Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
        195                 200                 205


Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
    210                 215                 220


Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
225                 230                 235                 240


Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
                245                 250                 255
```

```
Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
            260                 265                 270

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
        275                 280                 285

Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe
    290                 295                 300

Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser
305                 310                 315                 320

Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
                325                 330                 335

Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg
            340                 345                 350

Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
        355                 360                 365

Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
    370                 375                 380

Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
385                 390                 395                 400

Ser Gly Lys Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln Tyr
                405                 410                 415

Glu Ile Leu Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu Val
            420                 425                 430

Ile Thr Lys Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser Pro
        435                 440                 445

Phe Ser Thr Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg Ile
    450                 455                 460

Met Ala Arg Glu Cys Thr Gly Leu Ala Trp Glu Trp Trp Arg Lys Val
465                 470                 475                 480

Ile Asp Glu Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn Ile
                485                 490                 495

Ser Gly Ser Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys
            500                 505                 510
```

<210> 6
<211> 493
<212> PRT
<213> Streptococcus pyogenes

<400> 6

Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys Lys
1 5 10 15

Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile Asn
20 25 30

Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala Lys
35 40 45

Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys Lys
50 55 60

Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn Thr
65 70 75 80

Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr Tyr
85 90 95

Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys Pro
100 105 110

Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp Leu
115 120 125

Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr Tyr
130 135 140

Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His Asp
145 150 155 160

Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr Glu
165 170 175

Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val Asn
180 185 190

Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile Ser
195 200 205

Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe Tyr
210 215 220

Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp Lys
225 230 235 240

Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu Ala
245 250 255

Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe Val
260 265 270

```
Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe Ser
        275                 280                 285


Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser Asp
    290                 295                 300


Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp Ala
305                 310                 315                 320


Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg Asn
                325                 330                 335


Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr Pro
            340                 345                 350


Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly Val
        355                 360                 365


Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr Ser
    370                 375                 380


Gly Lys Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln Tyr Glu
385                 390                 395                 400


Ile Leu Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu Val Ile
                405                 410                 415


Thr Lys Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser Pro Phe
            420                 425                 430


Ser Thr Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg Ile Met
        435                 440                 445


Ala Arg Glu Cys Thr Gly Leu Ala Trp Glu Trp Trp Arg Lys Val Ile
    450                 455                 460


Asp Glu Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn Ile Ser
465                 470                 475                 480


Gly Ser Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys
                485                 490


<210>  7
<211>  510
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  SLO [79-571 fragment] - W535 mutant w/ leader

<400>  7

Met His His His His His His Gly Ser Gly Glu Asn Leu Tyr Phe Gln
```

```
1               5                   10                  15

Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
            20                  25                  30

Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
        35                  40                  45

Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
    50                  55                  60

Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
65                  70                  75                  80

Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn
                85                  90                  95

Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
            100                 105                 110

Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys
        115                 120                 125

Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
    130                 135                 140

Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
145                 150                 155                 160

Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
                165                 170                 175

Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
            180                 185                 190

Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
        195                 200                 205

Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
    210                 215                 220

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
225                 230                 235                 240

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
                245                 250                 255

Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
            260                 265                 270

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
        275                 280                 285
```

```
Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe
    290                 295                 300

Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser
305                 310                 315                 320

Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
                325                 330                 335

Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg
            340                 345                 350

Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
        355                 360                 365

Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
    370                 375                 380

Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
385                 390                 395                 400

Ser Gly Lys Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln Tyr
                405                 410                 415

Glu Ile Leu Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu Val
            420                 425                 430

Ile Thr Lys Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser Pro
        435                 440                 445

Phe Ser Thr Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg Ile
    450                 455                 460

Met Ala Arg Glu Cys Thr Gly Leu Ala Ala Glu Trp Trp Arg Lys Val
465                 470                 475                 480

Ile Asp Glu Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn Ile
                485                 490                 495

Ser Gly Ser Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys
            500                 505                 510
```

<210> 8
<211> 493
<212> PRT
<213> Artificial Sequence

<220>
<223> SLO [79-571 fragment] - W535 mutant w/o leader

<400> 8

```
Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys Lys
```

```
1               5                   10                  15

Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile Asn
            20                  25                  30

Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala Lys
        35                  40                  45

Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys Lys
    50                  55                  60

Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn Thr
65                  70                  75                  80

Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr Tyr
                85                  90                  95

Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys Pro
            100                 105                 110

Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp Leu
        115                 120                 125

Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr Tyr
    130                 135                 140

Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His Asp
145                 150                 155                 160

Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr Glu
                165                 170                 175

Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val Asn
            180                 185                 190

Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile Ser
        195                 200                 205

Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe Tyr
    210                 215                 220

Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp Lys
225                 230                 235                 240

Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu Ala
                245                 250                 255

Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe Val
            260                 265                 270

Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe Ser
        275                 280                 285
```

```
Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser Asp
    290                 295                 300

Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp Ala
305                 310                 315                 320

Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg Asn
                325                 330                 335

Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr Pro
            340                 345                 350

Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly Val
        355                 360                 365

Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr Ser
    370                 375                 380

Gly Lys Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln Tyr Glu
385                 390                 395                 400

Ile Leu Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu Val Ile
                405                 410                 415

Thr Lys Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser Pro Phe
            420                 425                 430

Ser Thr Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg Ile Met
        435                 440                 445

Ala Arg Glu Cys Thr Gly Leu Ala Ala Glu Trp Trp Arg Lys Val Ile
    450                 455                 460

Asp Glu Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn Ile Ser
465                 470                 475                 480

Gly Ser Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys
                485                 490
```

<210> 9
<211> 834
<212> PRT
<213> Artificial Sequence

<220>
<223> SpyAD [33-849 fragment] WT w/ leader

<400> 9

```
Met His His His His His His Gly Ser Gly Glu Asn Leu Tyr Phe Gln
1               5                   10                  15

Gly Gln Val Lys Ala Asp Asp Arg Ala Ser Gly Glu Thr Lys Ala Ser
```

```
            20                  25                  30

Asn Thr His Asp Asp Ser Leu Pro Lys Pro Glu Thr Ile Gln Glu Ala
        35                  40                  45

Lys Ala Thr Ile Asp Ala Val Glu Lys Thr Leu Ser Gln Gln Lys Ala
    50                  55                  60

Glu Leu Thr Glu Leu Ala Thr Ala Leu Thr Lys Thr Thr Ala Glu Ile
65                  70                  75                  80

Asn His Leu Lys Glu Gln Gln Asp Asn Glu Gln Lys Ala Leu Thr Ser
                85                  90                  95

Ala Gln Glu Ile Tyr Thr Asn Thr Leu Ala Ser Ser Glu Glu Thr Leu
            100                 105                 110

Leu Ala Gln Gly Ala Glu His Gln Arg Glu Leu Thr Ala Thr Glu Thr
        115                 120                 125

Glu Leu His Asn Ala Gln Ala Asp Gln His Ser Lys Glu Thr Ala Leu
    130                 135                 140

Ser Glu Gln Lys Ala Ser Ile Ser Ala Glu Thr Thr Arg Ala Gln Asp
145                 150                 155                 160

Leu Val Glu Gln Val Lys Thr Ser Glu Gln Asn Ile Ala Lys Leu Asn
                165                 170                 175

Ala Met Ile Ser Asn Pro Asp Ala Ile Thr Lys Ala Ala Gln Thr Ala
            180                 185                 190

Asn Asp Asn Thr Lys Ala Leu Ser Ser Glu Leu Glu Lys Ala Lys Ala
        195                 200                 205

Asp Leu Glu Asn Gln Lys Ala Lys Val Lys Lys Gln Leu Thr Glu Glu
    210                 215                 220

Leu Ala Ala Gln Lys Ala Ala Leu Ala Glu Lys Glu Ala Glu Leu Ser
225                 230                 235                 240

Arg Leu Lys Ser Ser Ala Pro Ser Thr Gln Asp Ser Ile Val Gly Asn
                245                 250                 255

Asn Thr Met Lys Ala Pro Gln Gly Tyr Pro Leu Glu Glu Leu Lys Lys
            260                 265                 270

Leu Glu Ala Ser Gly Tyr Ile Gly Ser Ala Ser Tyr Asn Asn Tyr Tyr
        275                 280                 285

Lys Glu His Ala Asp Gln Ile Ile Ala Lys Ala Ser Pro Gly Asn Gln
    290                 295                 300
```

```
Leu Asn Gln Tyr Gln Asp Ile Pro Ala Asp Arg Asn Arg Phe Val Asp
305                 310                 315                 320

Pro Asp Asn Leu Thr Pro Glu Val Gln Asn Glu Leu Ala Gln Phe Ala
                325                 330                 335

Ala His Met Ile Asn Ser Val Arg Arg Gln Leu Gly Leu Pro Pro Val
            340                 345                 350

Thr Val Thr Ala Gly Ser Gln Glu Phe Ala Arg Leu Leu Ser Thr Ser
        355                 360                 365

Tyr Lys Lys Thr His Gly Asn Thr Arg Pro Ser Phe Val Tyr Gly Gln
    370                 375                 380

Pro Gly Val Ser Gly His Tyr Gly Val Gly Pro His Asp Lys Thr Ile
385                 390                 395                 400

Ile Glu Asp Ser Ala Gly Ala Ser Gly Leu Ile Arg Asn Asp Asp Asn
                405                 410                 415

Met Tyr Glu Asn Ile Gly Ala Phe Asn Asp Val His Thr Val Asn Gly
            420                 425                 430

Ile Lys Arg Gly Ile Tyr Asp Ser Ile Lys Tyr Met Leu Phe Thr Asp
        435                 440                 445

His Leu His Gly Asn Thr Tyr Gly His Ala Ile Asn Phe Leu Arg Val
    450                 455                 460

Asp Lys His Asn Pro Asn Ala Pro Val Tyr Leu Gly Phe Ser Thr Ser
465                 470                 475                 480

Asn Val Gly Ser Leu Asn Glu His Phe Val Met Phe Pro Glu Ser Asn
                485                 490                 495

Ile Ala Asn His Gln Arg Phe Asn Lys Thr Pro Ile Lys Ala Val Gly
            500                 505                 510

Ser Thr Lys Asp Tyr Ala Gln Arg Val Gly Thr Val Ser Asp Thr Ile
        515                 520                 525

Ala Ala Ile Lys Gly Lys Val Ser Ser Leu Glu Asn Arg Leu Ser Ala
    530                 535                 540

Ile His Gln Glu Ala Asp Ile Met Ala Ala Gln Ala Lys Val Ser Gln
545                 550                 555                 560

Leu Gln Gly Lys Leu Ala Ser Thr Leu Lys Gln Ser Asp Ser Leu Asn
                565                 570                 575
```

```
Leu Gln Val Arg Gln Leu Asn Asp Thr Lys Gly Ser Leu Arg Thr Glu
            580                 585                 590

Leu Leu Ala Ala Lys Ala Lys Gln Ala Gln Leu Glu Ala Thr Arg Asp
        595                 600                 605

Gln Ser Leu Ala Lys Leu Ala Ser Leu Lys Ala Ala Leu His Gln Thr
    610                 615                 620

Glu Ala Leu Ala Glu Gln Ala Ala Ala Arg Val Thr Ala Leu Val Ala
625                 630                 635                 640

Lys Lys Ala His Leu Gln Tyr Leu Arg Asp Phe Lys Leu Asn Pro Asn
                645                 650                 655

Arg Leu Gln Val Ile Arg Glu Arg Ile Asp Asn Thr Lys Gln Asp Leu
            660                 665                 670

Ala Lys Thr Thr Ser Ser Leu Leu Asn Ala Gln Glu Ala Leu Ala Ala
        675                 680                 685

Leu Gln Ala Lys Gln Ser Ser Leu Glu Ala Thr Ile Ala Thr Thr Glu
    690                 695                 700

His Gln Leu Thr Leu Leu Lys Thr Leu Ala Asn Glu Lys Glu Tyr Arg
705                 710                 715                 720

His Leu Asp Glu Asp Ile Ala Thr Val Pro Asp Leu Gln Val Ala Pro
                725                 730                 735

Pro Leu Thr Gly Val Lys Pro Leu Ser Tyr Ser Lys Ile Asp Thr Thr
            740                 745                 750

Pro Leu Val Gln Glu Met Val Lys Glu Thr Lys Gln Leu Leu Glu Ala
        755                 760                 765

Ser Ala Arg Leu Ala Ala Glu Asn Thr Ser Leu Val Ala Glu Ala Leu
    770                 775                 780

Val Gly Gln Thr Ser Glu Met Val Ala Ser Asn Ala Ile Val Ser Lys
785                 790                 795                 800

Ile Thr Ser Ser Ile Thr Gln Pro Ser Ser Lys Thr Ser Tyr Gly Ser
                805                 810                 815

Gly Ser Ser Thr Thr Ser Asn Leu Ile Ser Asp Val Asp Glu Ser Thr
            820                 825                 830

Gln Arg
```

<210> 10
<211> 817
<212> PRT
<213> Streptococcus pyogenes

<400> 10

Gln Val Lys Ala Asp Asp Arg Ala Ser Gly Glu Thr Lys Ala Ser Asn
1 5 10 15

Thr His Asp Asp Ser Leu Pro Lys Pro Glu Thr Ile Gln Glu Ala Lys
20 25 30

Ala Thr Ile Asp Ala Val Glu Lys Thr Leu Ser Gln Gln Lys Ala Glu
35 40 45

Leu Thr Glu Leu Ala Thr Ala Leu Thr Lys Thr Thr Ala Glu Ile Asn
50 55 60

His Leu Lys Glu Gln Gln Asp Asn Glu Gln Lys Ala Leu Thr Ser Ala
65 70 75 80

Gln Glu Ile Tyr Thr Asn Thr Leu Ala Ser Ser Glu Glu Thr Leu Leu
85 90 95

Ala Gln Gly Ala Glu His Gln Arg Glu Leu Thr Ala Thr Glu Thr Glu
100 105 110

Leu His Asn Ala Gln Ala Asp Gln His Ser Lys Glu Thr Ala Leu Ser
115 120 125

Glu Gln Lys Ala Ser Ile Ser Ala Glu Thr Thr Arg Ala Gln Asp Leu
130 135 140

Val Glu Gln Val Lys Thr Ser Glu Gln Asn Ile Ala Lys Leu Asn Ala
145 150 155 160

Met Ile Ser Asn Pro Asp Ala Ile Thr Lys Ala Ala Gln Thr Ala Asn
165 170 175

Asp Asn Thr Lys Ala Leu Ser Ser Glu Leu Glu Lys Ala Lys Ala Asp
180 185 190

Leu Glu Asn Gln Lys Ala Lys Val Lys Lys Gln Leu Thr Glu Glu Leu
195 200 205

Ala Ala Gln Lys Ala Ala Leu Ala Glu Lys Glu Ala Glu Leu Ser Arg
210 215 220

Leu Lys Ser Ser Ala Pro Ser Thr Gln Asp Ser Ile Val Gly Asn Asn
225 230 235 240

Thr Met Lys Ala Pro Gln Gly Tyr Pro Leu Glu Glu Leu Lys Lys Leu
245 250 255

```
Glu Ala Ser Gly Tyr Ile Gly Ser Ala Ser Tyr Asn Asn Tyr Tyr Lys
            260                 265                 270

Glu His Ala Asp Gln Ile Ile Ala Lys Ala Ser Pro Gly Asn Gln Leu
        275                 280                 285

Asn Gln Tyr Gln Asp Ile Pro Ala Asp Arg Asn Arg Phe Val Asp Pro
    290                 295                 300

Asp Asn Leu Thr Pro Glu Val Gln Asn Glu Leu Ala Gln Phe Ala Ala
305                 310                 315                 320

His Met Ile Asn Ser Val Arg Arg Gln Leu Gly Leu Pro Pro Val Thr
                325                 330                 335

Val Thr Ala Gly Ser Gln Glu Phe Ala Arg Leu Leu Ser Thr Ser Tyr
            340                 345                 350

Lys Lys Thr His Gly Asn Thr Arg Pro Ser Phe Val Tyr Gly Gln Pro
        355                 360                 365

Gly Val Ser Gly His Tyr Gly Val Gly Pro His Asp Lys Thr Ile Ile
    370                 375                 380

Glu Asp Ser Ala Gly Ala Ser Gly Leu Ile Arg Asn Asp Asp Asn Met
385                 390                 395                 400

Tyr Glu Asn Ile Gly Ala Phe Asn Asp Val His Thr Val Asn Gly Ile
                405                 410                 415

Lys Arg Gly Ile Tyr Asp Ser Ile Lys Tyr Met Leu Phe Thr Asp His
            420                 425                 430

Leu His Gly Asn Thr Tyr Gly His Ala Ile Asn Phe Leu Arg Val Asp
        435                 440                 445

Lys His Asn Pro Asn Ala Pro Val Tyr Leu Gly Phe Ser Thr Ser Asn
    450                 455                 460

Val Gly Ser Leu Asn Glu His Phe Val Met Phe Pro Glu Ser Asn Ile
465                 470                 475                 480

Ala Asn His Gln Arg Phe Asn Lys Thr Pro Ile Lys Ala Val Gly Ser
                485                 490                 495

Thr Lys Asp Tyr Ala Gln Arg Val Gly Thr Val Ser Asp Thr Ile Ala
            500                 505                 510

Ala Ile Lys Gly Lys Val Ser Ser Leu Glu Asn Arg Leu Ser Ala Ile
        515                 520                 525
```

```
His Gln Glu Ala Asp Ile Met Ala Ala Gln Ala Lys Val Ser Gln Leu
    530                 535                 540

Gln Gly Lys Leu Ala Ser Thr Leu Lys Gln Ser Asp Ser Leu Asn Leu
545                 550                 555                 560

Gln Val Arg Gln Leu Asn Asp Thr Lys Gly Ser Leu Arg Thr Glu Leu
                565                 570                 575

Leu Ala Ala Lys Ala Lys Gln Ala Gln Leu Glu Ala Thr Arg Asp Gln
            580                 585                 590

Ser Leu Ala Lys Leu Ala Ser Leu Lys Ala Ala Leu His Gln Thr Glu
        595                 600                 605

Ala Leu Ala Glu Gln Ala Ala Ala Arg Val Thr Ala Leu Val Ala Lys
    610                 615                 620

Lys Ala His Leu Gln Tyr Leu Arg Asp Phe Lys Leu Asn Pro Asn Arg
625                 630                 635                 640

Leu Gln Val Ile Arg Glu Arg Ile Asp Asn Thr Lys Gln Asp Leu Ala
                645                 650                 655

Lys Thr Thr Ser Ser Leu Leu Asn Ala Gln Glu Ala Leu Ala Ala Leu
            660                 665                 670

Gln Ala Lys Gln Ser Ser Leu Glu Ala Thr Ile Ala Thr Thr Glu His
        675                 680                 685

Gln Leu Thr Leu Leu Lys Thr Leu Ala Asn Glu Lys Glu Tyr Arg His
    690                 695                 700

Leu Asp Glu Asp Ile Ala Thr Val Pro Asp Leu Gln Val Ala Pro Pro
705                 710                 715                 720

Leu Thr Gly Val Lys Pro Leu Ser Tyr Ser Lys Ile Asp Thr Thr Pro
                725                 730                 735

Leu Val Gln Glu Met Val Lys Glu Thr Lys Gln Leu Leu Glu Ala Ser
            740                 745                 750

Ala Arg Leu Ala Ala Glu Asn Thr Ser Leu Val Ala Glu Ala Leu Val
        755                 760                 765

Gly Gln Thr Ser Glu Met Val Ala Ser Asn Ala Ile Val Ser Lys Ile
    770                 775                 780

Thr Ser Ser Ile Thr Gln Pro Ser Ser Lys Thr Ser Tyr Gly Ser Gly
785                 790                 795                 800
```

Ser Ser Thr Thr Ser Asn Leu Ile Ser Asp Val Asp Glu Ser Thr Gln
805 810 815

Arg

<210> 11
<211> 834
<212> PRT
<213> Artificial Sequence

<220>
<223> SpyAD [33-849 fragment] nnAA (e.g. pAMF) K64/K287/K386/K657 mutant w/ leader

<220>
<221> MOD_RES
<222> (49)..(49)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (272)..(272)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (371)..(371)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (642)..(642)
<223> Xaa = nnAA (e.g pAMF)

<400> 11

Met His His His His His His Gly Ser Gly Glu Asn Leu Tyr Phe Gln
1 5 10 15

Gly Gln Val Lys Ala Asp Asp Arg Ala Ser Gly Glu Thr Lys Ala Ser
20 25 30

Asn Thr His Asp Asp Ser Leu Pro Lys Pro Glu Thr Ile Gln Glu Ala
35 40 45

Xaa Ala Thr Ile Asp Ala Val Glu Lys Thr Leu Ser Gln Gln Lys Ala
50 55 60

Glu Leu Thr Glu Leu Ala Thr Ala Leu Thr Lys Thr Thr Ala Glu Ile
65 70 75 80

Asn His Leu Lys Glu Gln Gln Asp Asn Glu Gln Lys Ala Leu Thr Ser
85 90 95

Ala Gln Glu Ile Tyr Thr Asn Thr Leu Ala Ser Ser Glu Glu Thr Leu
100 105 110

Leu Ala Gln Gly Ala Glu His Gln Arg Glu Leu Thr Ala Thr Glu Thr

```
        115                 120                 125

Glu Leu His Asn Ala Gln Ala Asp Gln His Ser Lys Glu Thr Ala Leu
    130                 135                 140

Ser Glu Gln Lys Ala Ser Ile Ser Ala Glu Thr Thr Arg Ala Gln Asp
145                 150                 155                 160

Leu Val Glu Gln Val Lys Thr Ser Glu Gln Asn Ile Ala Lys Leu Asn
                165                 170                 175

Ala Met Ile Ser Asn Pro Asp Ala Ile Thr Lys Ala Ala Gln Thr Ala
            180                 185                 190

Asn Asp Asn Thr Lys Ala Leu Ser Ser Glu Leu Glu Lys Ala Lys Ala
        195                 200                 205

Asp Leu Glu Asn Gln Lys Ala Lys Val Lys Lys Gln Leu Thr Glu Glu
    210                 215                 220

Leu Ala Ala Gln Lys Ala Ala Leu Ala Glu Lys Glu Ala Glu Leu Ser
225                 230                 235                 240

Arg Leu Lys Ser Ser Ala Pro Ser Thr Gln Asp Ser Ile Val Gly Asn
                245                 250                 255

Asn Thr Met Lys Ala Pro Gln Gly Tyr Pro Leu Glu Glu Leu Lys Xaa
            260                 265                 270

Leu Glu Ala Ser Gly Tyr Ile Gly Ser Ala Ser Tyr Asn Asn Tyr Tyr
        275                 280                 285

Lys Glu His Ala Asp Gln Ile Ile Ala Lys Ala Ser Pro Gly Asn Gln
    290                 295                 300

Leu Asn Gln Tyr Gln Asp Ile Pro Ala Asp Arg Asn Arg Phe Val Asp
305                 310                 315                 320

Pro Asp Asn Leu Thr Pro Glu Val Gln Asn Glu Leu Ala Gln Phe Ala
                325                 330                 335

Ala His Met Ile Asn Ser Val Arg Arg Gln Leu Gly Leu Pro Pro Val
            340                 345                 350

Thr Val Thr Ala Gly Ser Gln Glu Phe Ala Arg Leu Leu Ser Thr Ser
        355                 360                 365

Tyr Lys Xaa Thr His Gly Asn Thr Arg Pro Ser Phe Val Tyr Gly Gln
    370                 375                 380

Pro Gly Val Ser Gly His Tyr Gly Val Gly Pro His Asp Lys Thr Ile
385                 390                 395                 400
```

```
Ile Glu Asp Ser Ala Gly Ala Ser Gly Leu Ile Arg Asn Asp Asp Asn
                405                 410                 415

Met Tyr Glu Asn Ile Gly Ala Phe Asn Asp Val His Thr Val Asn Gly
            420                 425                 430

Ile Lys Arg Gly Ile Tyr Asp Ser Ile Lys Tyr Met Leu Phe Thr Asp
        435                 440                 445

His Leu His Gly Asn Thr Tyr Gly His Ala Ile Asn Phe Leu Arg Val
    450                 455                 460

Asp Lys His Asn Pro Asn Ala Pro Val Tyr Leu Gly Phe Ser Thr Ser
465                 470                 475                 480

Asn Val Gly Ser Leu Asn Glu His Phe Val Met Phe Pro Glu Ser Asn
                485                 490                 495

Ile Ala Asn His Gln Arg Phe Asn Lys Thr Pro Ile Lys Ala Val Gly
            500                 505                 510

Ser Thr Lys Asp Tyr Ala Gln Arg Val Gly Thr Val Ser Asp Thr Ile
        515                 520                 525

Ala Ala Ile Lys Gly Lys Val Ser Ser Leu Glu Asn Arg Leu Ser Ala
    530                 535                 540

Ile His Gln Glu Ala Asp Ile Met Ala Ala Gln Ala Lys Val Ser Gln
545                 550                 555                 560

Leu Gln Gly Lys Leu Ala Ser Thr Leu Lys Gln Ser Asp Ser Leu Asn
                565                 570                 575

Leu Gln Val Arg Gln Leu Asn Asp Thr Lys Gly Ser Leu Arg Thr Glu
            580                 585                 590

Leu Leu Ala Ala Lys Ala Lys Gln Ala Gln Leu Glu Ala Thr Arg Asp
        595                 600                 605

Gln Ser Leu Ala Lys Leu Ala Ser Leu Lys Ala Ala Leu His Gln Thr
    610                 615                 620

Glu Ala Leu Ala Glu Gln Ala Ala Ala Arg Val Thr Ala Leu Val Ala
625                 630                 635                 640

Lys Xaa Ala His Leu Gln Tyr Leu Arg Asp Phe Lys Leu Asn Pro Asn
                645                 650                 655

Arg Leu Gln Val Ile Arg Glu Arg Ile Asp Asn Thr Lys Gln Asp Leu
            660                 665                 670
```

```
Ala Lys Thr Thr Ser Ser Leu Leu Asn Ala Gln Glu Ala Leu Ala Ala
        675                 680                 685

Leu Gln Ala Lys Gln Ser Ser Leu Glu Ala Thr Ile Ala Thr Thr Glu
    690                 695                 700

His Gln Leu Thr Leu Leu Lys Thr Leu Ala Asn Glu Lys Glu Tyr Arg
705                 710                 715                 720

His Leu Asp Glu Asp Ile Ala Thr Val Pro Asp Leu Gln Val Ala Pro
                725                 730                 735

Pro Leu Thr Gly Val Lys Pro Leu Ser Tyr Ser Lys Ile Asp Thr Thr
            740                 745                 750

Pro Leu Val Gln Glu Met Val Lys Glu Thr Lys Gln Leu Leu Glu Ala
        755                 760                 765

Ser Ala Arg Leu Ala Ala Glu Asn Thr Ser Leu Val Ala Glu Ala Leu
    770                 775                 780

Val Gly Gln Thr Ser Glu Met Val Ala Ser Asn Ala Ile Val Ser Lys
785                 790                 795                 800

Ile Thr Ser Ser Ile Thr Gln Pro Ser Ser Lys Thr Ser Tyr Gly Ser
                805                 810                 815

Gly Ser Ser Thr Thr Ser Asn Leu Ile Ser Asp Val Asp Glu Ser Thr
            820                 825                 830

Gln Arg
```

<210> 12
<211> 817
<212> PRT
<213> Artificial Sequence

<220>
<223> SpyAD [33-849 fragment] nnAA (e.g. pAMF) K64/K287/K386/K657 mutant w/o leader

<220>
<221> MOD_RES
<222> (31)..(31)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> misc_feature
<222> (32)..(32)
<223> Xaa can be any naturally occurring amino acid

<220>
<221> MOD_RES
<222> (255)..(255)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (354)..(354)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (625)..(625)
<223> Xaa = nnAA (e.g pAMF)

<400> 12

```
Gln Val Lys Ala Asp Asp Arg Ala Ser Gly Glu Thr Lys Ala Ser Asn
1               5                   10                  15


Thr His Asp Asp Ser Leu Pro Lys Pro Glu Thr Ile Gln Glu Ala Xaa
            20                  25                  30


Ala Thr Ile Asp Ala Val Glu Lys Thr Leu Ser Gln Gln Lys Ala Glu
        35                  40                  45


Leu Thr Glu Leu Ala Thr Ala Leu Thr Lys Thr Thr Ala Glu Ile Asn
    50                  55                  60


His Leu Lys Glu Gln Gln Asp Asn Glu Gln Lys Ala Leu Thr Ser Ala
65                  70                  75                  80


Gln Glu Ile Tyr Thr Asn Thr Leu Ala Ser Ser Glu Glu Thr Leu Leu
                85                  90                  95


Ala Gln Gly Ala Glu His Gln Arg Glu Leu Thr Ala Thr Glu Thr Glu
            100                 105                 110


Leu His Asn Ala Gln Ala Asp Gln His Ser Lys Glu Thr Ala Leu Ser
        115                 120                 125


Glu Gln Lys Ala Ser Ile Ser Ala Glu Thr Thr Arg Ala Gln Asp Leu
    130                 135                 140


Val Glu Gln Val Lys Thr Ser Glu Gln Asn Ile Ala Lys Leu Asn Ala
145                 150                 155                 160


Met Ile Ser Asn Pro Asp Ala Ile Thr Lys Ala Ala Gln Thr Ala Asn
                165                 170                 175


Asp Asn Thr Lys Ala Leu Ser Ser Glu Leu Glu Lys Ala Lys Ala Asp
            180                 185                 190


Leu Glu Asn Gln Lys Ala Lys Val Lys Lys Gln Leu Thr Glu Glu Leu
        195                 200                 205


Ala Ala Gln Lys Ala Ala Leu Ala Glu Lys Glu Ala Glu Leu Ser Arg
    210                 215                 220
```

```
Leu Lys Ser Ser Ala Pro Ser Thr Gln Asp Ser Ile Val Gly Asn Asn
225                 230                 235                 240

Thr Met Lys Ala Pro Gln Gly Tyr Pro Leu Glu Glu Leu Lys Xaa Leu
                245                 250                 255

Glu Ala Ser Gly Tyr Ile Gly Ser Ala Ser Tyr Asn Asn Tyr Tyr Lys
            260                 265                 270

Glu His Ala Asp Gln Ile Ile Ala Lys Ala Ser Pro Gly Asn Gln Leu
        275                 280                 285

Asn Gln Tyr Gln Asp Ile Pro Ala Asp Arg Asn Arg Phe Val Asp Pro
    290                 295                 300

Asp Asn Leu Thr Pro Glu Val Gln Asn Glu Leu Ala Gln Phe Ala Ala
305                 310                 315                 320

His Met Ile Asn Ser Val Arg Arg Gln Leu Gly Leu Pro Pro Val Thr
                325                 330                 335

Val Thr Ala Gly Ser Gln Glu Phe Ala Arg Leu Leu Ser Thr Ser Tyr
            340                 345                 350

Lys Xaa Thr His Gly Asn Thr Arg Pro Ser Phe Val Tyr Gly Gln Pro
        355                 360                 365

Gly Val Ser Gly His Tyr Gly Val Gly Pro His Asp Lys Thr Ile Ile
    370                 375                 380

Glu Asp Ser Ala Gly Ala Ser Gly Leu Ile Arg Asn Asp Asp Asn Met
385                 390                 395                 400

Tyr Glu Asn Ile Gly Ala Phe Asn Asp Val His Thr Val Asn Gly Ile
                405                 410                 415

Lys Arg Gly Ile Tyr Asp Ser Ile Lys Tyr Met Leu Phe Thr Asp His
            420                 425                 430

Leu His Gly Asn Thr Tyr Gly His Ala Ile Asn Phe Leu Arg Val Asp
        435                 440                 445

Lys His Asn Pro Asn Ala Pro Val Tyr Leu Gly Phe Ser Thr Ser Asn
    450                 455                 460

Val Gly Ser Leu Asn Glu His Phe Val Met Phe Pro Glu Ser Asn Ile
465                 470                 475                 480

Ala Asn His Gln Arg Phe Asn Lys Thr Pro Ile Lys Ala Val Gly Ser
                485                 490                 495

Thr Lys Asp Tyr Ala Gln Arg Val Gly Thr Val Ser Asp Thr Ile Ala
```

```
            500                 505                 510

Ala Ile Lys Gly Lys Val Ser Ser Leu Glu Asn Arg Leu Ser Ala Ile
        515                 520                 525

His Gln Glu Ala Asp Ile Met Ala Ala Gln Ala Lys Val Ser Gln Leu
    530                 535                 540

Gln Gly Lys Leu Ala Ser Thr Leu Lys Gln Ser Asp Ser Leu Asn Leu
545                 550                 555                 560

Gln Val Arg Gln Leu Asn Asp Thr Lys Gly Ser Leu Arg Thr Glu Leu
                565                 570                 575

Leu Ala Ala Lys Ala Lys Gln Ala Gln Leu Glu Ala Thr Arg Asp Gln
            580                 585                 590

Ser Leu Ala Lys Leu Ala Ser Leu Lys Ala Ala Leu His Gln Thr Glu
        595                 600                 605

Ala Leu Ala Glu Gln Ala Ala Ala Arg Val Thr Ala Leu Val Ala Lys
    610                 615                 620

Xaa Ala His Leu Gln Tyr Leu Arg Asp Phe Lys Leu Asn Pro Asn Arg
625                 630                 635                 640

Leu Gln Val Ile Arg Glu Arg Ile Asp Asn Thr Lys Gln Asp Leu Ala
                645                 650                 655

Lys Thr Thr Ser Ser Leu Leu Asn Ala Gln Glu Ala Leu Ala Ala Leu
            660                 665                 670

Gln Ala Lys Gln Ser Ser Leu Glu Ala Thr Ile Ala Thr Thr Glu His
        675                 680                 685

Gln Leu Thr Leu Leu Lys Thr Leu Ala Asn Glu Lys Glu Tyr Arg His
    690                 695                 700

Leu Asp Glu Asp Ile Ala Thr Val Pro Asp Leu Gln Val Ala Pro Pro
705                 710                 715                 720

Leu Thr Gly Val Lys Pro Leu Ser Tyr Ser Lys Ile Asp Thr Thr Pro
                725                 730                 735

Leu Val Gln Glu Met Val Lys Glu Thr Lys Gln Leu Leu Glu Ala Ser
            740                 745                 750

Ala Arg Leu Ala Ala Glu Asn Thr Ser Leu Val Ala Glu Ala Leu Val
        755                 760                 765

Gly Gln Thr Ser Glu Met Val Ala Ser Asn Ala Ile Val Ser Lys Ile
    770                 775                 780
```

Thr Ser Ser Ile Thr Gln Pro Ser Ser Lys Thr Ser Tyr Gly Ser Gly
785 790 795 800

Ser Ser Thr Thr Ser Asn Leu Ile Ser Asp Val Asp Glu Ser Thr Gln
805 810 815

Arg

<210> 13
<211> 427
<212> PRT
<213> Artificial Sequence

<220>
<223> ADI [full length] WT w/ leader

<400> 13

Met His His His His His His Gly Ser Gly Glu Asn Leu Tyr Phe Gln
1 5 10 15

Gly Thr Ala Gln Thr Pro Ile His Val Tyr Ser Glu Ile Gly Lys Leu
20 25 30

Lys Lys Val Leu Leu His Arg Pro Gly Lys Glu Ile Glu Asn Leu Met
35 40 45

Pro Asp Tyr Leu Glu Arg Leu Leu Phe Asp Asp Ile Pro Phe Leu Glu
50 55 60

Asp Ala Gln Lys Glu His Asp Ala Phe Ala Gln Ala Leu Arg Asp Glu
65 70 75 80

Gly Ile Glu Val Leu Tyr Leu Glu Thr Leu Ala Ala Glu Ser Leu Val
85 90 95

Thr Pro Glu Ile Arg Glu Ala Phe Ile Asp Glu Tyr Leu Ser Glu Ala
100 105 110

Asn Ile Arg Gly Arg Ala Thr Lys Lys Ala Ile Arg Glu Leu Leu Met
115 120 125

Ala Ile Glu Asp Asn Gln Glu Leu Ile Glu Lys Thr Met Ala Gly Val
130 135 140

Gln Lys Ser Glu Leu Pro Glu Ile Pro Ala Ser Glu Lys Gly Leu Thr
145 150 155 160

Asp Leu Val Glu Ser Asn Tyr Pro Phe Ala Ile Asp Pro Met Pro Asn
165 170 175

Leu Tyr Phe Thr Arg Asp Pro Phe Ala Thr Ile Gly Thr Gly Val Ser

```
            180                 185                 190

Leu Asn His Met Phe Ser Glu Thr Arg Asn Arg Glu Thr Leu Tyr Gly
        195                 200                 205

Lys Tyr Ile Phe Thr His His Pro Ile Tyr Gly Gly Gly Lys Val Pro
    210                 215                 220

Met Val Tyr Asp Arg Asn Glu Thr Thr Arg Ile Glu Gly Gly Asp Glu
225                 230                 235                 240

Leu Val Leu Ser Lys Asp Val Leu Ala Val Gly Ile Ser Gln Arg Thr
                245                 250                 255

Asp Ala Ala Ser Ile Glu Lys Leu Leu Val Asn Ile Phe Lys Gln Asn
            260                 265                 270

Leu Gly Phe Lys Lys Val Leu Ala Phe Glu Phe Ala Asn Asn Arg Lys
        275                 280                 285

Phe Met His Leu Asp Thr Val Phe Thr Met Val Asp Tyr Asp Lys Phe
    290                 295                 300

Thr Ile His Pro Glu Ile Glu Gly Asp Leu Arg Val Tyr Ser Val Thr
305                 310                 315                 320

Tyr Asp Asn Glu Glu Leu His Ile Val Glu Glu Lys Gly Asp Leu Ala
                325                 330                 335

Glu Leu Leu Ala Ala Asn Leu Gly Val Glu Lys Val Asp Leu Ile Arg
            340                 345                 350

Cys Gly Gly Asp Asn Leu Val Ala Ala Gly Arg Glu Gln Trp Asn Asp
        355                 360                 365

Gly Ser Asn Thr Leu Thr Ile Ala Pro Gly Val Val Val Val Tyr Asn
    370                 375                 380

Arg Asn Thr Ile Thr Asn Ala Ile Leu Glu Ser Lys Gly Leu Lys Leu
385                 390                 395                 400

Ile Lys Ile His Gly Ser Glu Leu Val Arg Gly Arg Gly Gly Pro Arg
                405                 410                 415

Cys Met Ser Met Pro Phe Glu Arg Glu Asp Ile
            420                 425
```

<210> 14
<211> 410
<212> PRT
<213> Streptococcus pyogenes

<400> 14

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

Lys Val Leu Leu His Arg Pro Gly Lys Glu Ile Glu Asn Leu Met Pro
            20                  25                  30

Asp Tyr Leu Glu Arg Leu Leu Phe Asp Asp Ile Pro Phe Leu Glu Asp
        35                  40                  45

Ala Gln Lys Glu His Asp Ala Phe Ala Gln Ala Leu Arg Asp Glu Gly
    50                  55                  60

Ile Glu Val Leu Tyr Leu Glu Thr Leu Ala Ala Glu Ser Leu Val Thr
65                  70                  75                  80

Pro Glu Ile Arg Glu Ala Phe Ile Asp Glu Tyr Leu Ser Glu Ala Asn
                85                  90                  95

Ile Arg Gly Arg Ala Thr Lys Lys Ala Ile Arg Glu Leu Leu Met Ala
            100                 105                 110

Ile Glu Asp Asn Gln Glu Leu Ile Glu Lys Thr Met Ala Gly Val Gln
        115                 120                 125

Lys Ser Glu Leu Pro Glu Ile Pro Ala Ser Glu Lys Gly Leu Thr Asp
    130                 135                 140

Leu Val Glu Ser Asn Tyr Pro Phe Ala Ile Asp Pro Met Pro Asn Leu
145                 150                 155                 160

Tyr Phe Thr Arg Asp Pro Phe Ala Thr Ile Gly Thr Gly Val Ser Leu
                165                 170                 175

Asn His Met Phe Ser Glu Thr Arg Asn Arg Glu Thr Leu Tyr Gly Lys
            180                 185                 190

Tyr Ile Phe Thr His His Pro Ile Tyr Gly Gly Gly Lys Val Pro Met
        195                 200                 205

Val Tyr Asp Arg Asn Glu Thr Thr Arg Ile Glu Gly Gly Asp Glu Leu
    210                 215                 220

Val Leu Ser Lys Asp Val Leu Ala Val Gly Ile Ser Gln Arg Thr Asp
225                 230                 235                 240

Ala Ala Ser Ile Glu Lys Leu Leu Val Asn Ile Phe Lys Gln Asn Leu
                245                 250                 255

Gly Phe Lys Lys Val Leu Ala Phe Glu Phe Ala Asn Asn Arg Lys Phe
            260                 265                 270
```

```
Met His Leu Asp Thr Val Phe Thr Met Val Asp Tyr Asp Lys Phe Thr
        275                 280                 285

Ile His Pro Glu Ile Glu Gly Asp Leu Arg Val Tyr Ser Val Thr Tyr
    290                 295                 300

Asp Asn Glu Glu Leu His Ile Val Glu Glu Lys Gly Asp Leu Ala Glu
305                 310                 315                 320

Leu Leu Ala Ala Asn Leu Gly Val Glu Lys Val Asp Leu Ile Arg Cys
                325                 330                 335

Gly Gly Asp Asn Leu Val Ala Ala Gly Arg Glu Gln Trp Asn Asp Gly
            340                 345                 350

Ser Asn Thr Leu Thr Ile Ala Pro Gly Val Val Val Val Tyr Asn Arg
        355                 360                 365

Asn Thr Ile Thr Asn Ala Ile Leu Glu Ser Lys Gly Leu Lys Leu Ile
    370                 375                 380

Lys Ile His Gly Ser Glu Leu Val Arg Gly Arg Gly Gly Pro Arg Cys
385                 390                 395                 400

Met Ser Met Pro Phe Glu Arg Glu Asp Ile
                405                 410
```

<210> 15
<211> 427
<212> PRT
<213> Artificial Sequence

<220>
<223> ADI [full length] D277A mut w/ leader

<400> 15

```
Met His His His His His His Gly Ser Gly Glu Asn Leu Tyr Phe Gln
1               5                   10                  15

Gly Thr Ala Gln Thr Pro Ile His Val Tyr Ser Glu Ile Gly Lys Leu
            20                  25                  30

Lys Lys Val Leu Leu His Arg Pro Gly Lys Glu Ile Glu Asn Leu Met
        35                  40                  45

Pro Asp Tyr Leu Glu Arg Leu Leu Phe Asp Asp Ile Pro Phe Leu Glu
    50                  55                  60

Asp Ala Gln Lys Glu His Asp Ala Phe Ala Gln Ala Leu Arg Asp Glu
65                  70                  75                  80

Gly Ile Glu Val Leu Tyr Leu Glu Thr Leu Ala Ala Glu Ser Leu Val
                85                  90                  95
```

```
Thr Pro Glu Ile Arg Glu Ala Phe Ile Asp Glu Tyr Leu Ser Glu Ala
            100                 105                 110

Asn Ile Arg Gly Arg Ala Thr Lys Lys Ala Ile Arg Glu Leu Leu Met
        115                 120                 125

Ala Ile Glu Asp Asn Gln Glu Leu Ile Glu Lys Thr Met Ala Gly Val
    130                 135                 140

Gln Lys Ser Glu Leu Pro Glu Ile Pro Ala Ser Glu Lys Gly Leu Thr
145                 150                 155                 160

Asp Leu Val Glu Ser Asn Tyr Pro Phe Ala Ile Asp Pro Met Pro Asn
                165                 170                 175

Leu Tyr Phe Thr Arg Asp Pro Phe Ala Thr Ile Gly Thr Gly Val Ser
            180                 185                 190

Leu Asn His Met Phe Ser Glu Thr Arg Asn Arg Glu Thr Leu Tyr Gly
        195                 200                 205

Lys Tyr Ile Phe Thr His His Pro Ile Tyr Gly Gly Gly Lys Val Pro
    210                 215                 220

Met Val Tyr Asp Arg Asn Glu Thr Thr Arg Ile Glu Gly Gly Asp Glu
225                 230                 235                 240

Leu Val Leu Ser Lys Asp Val Leu Ala Val Gly Ile Ser Gln Arg Thr
                245                 250                 255

Asp Ala Ala Ser Ile Glu Lys Leu Leu Val Asn Ile Phe Lys Gln Asn
            260                 265                 270

Leu Gly Phe Lys Lys Val Leu Ala Phe Glu Phe Ala Asn Asn Arg Lys
        275                 280                 285

Phe Met His Leu Ala Thr Val Phe Thr Met Val Asp Tyr Asp Lys Phe
    290                 295                 300

Thr Ile His Pro Glu Ile Glu Gly Asp Leu Arg Val Tyr Ser Val Thr
305                 310                 315                 320

Tyr Asp Asn Glu Glu Leu His Ile Val Glu Glu Lys Gly Asp Leu Ala
                325                 330                 335

Glu Leu Leu Ala Ala Asn Leu Gly Val Glu Lys Val Asp Leu Ile Arg
            340                 345                 350

Cys Gly Gly Asp Asn Leu Val Ala Ala Gly Arg Glu Gln Trp Asn Asp
        355                 360                 365
```

```
Gly Ser Asn Thr Leu Thr Ile Ala Pro Gly Val Val Val Val Tyr Asn
    370                 375                 380

Arg Asn Thr Ile Thr Asn Ala Ile Leu Glu Ser Lys Gly Leu Lys Leu
385                 390                 395                 400

Ile Lys Ile His Gly Ser Glu Leu Val Arg Gly Arg Gly Gly Pro Arg
                405                 410                 415

Cys Met Ser Met Pro Phe Glu Arg Glu Asp Ile
            420                 425
```

<210> 16
<211> 410
<212> PRT
<213> Artificial Sequence

<220>
<223> ADI [full length] D277A mut w/o leader

<400> 16

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

Lys Val Leu Leu His Arg Pro Gly Lys Glu Ile Glu Asn Leu Met Pro
            20                  25                  30

Asp Tyr Leu Glu Arg Leu Leu Phe Asp Asp Ile Pro Phe Leu Glu Asp
        35                  40                  45

Ala Gln Lys Glu His Asp Ala Phe Ala Gln Ala Leu Arg Asp Glu Gly
    50                  55                  60

Ile Glu Val Leu Tyr Leu Glu Thr Leu Ala Ala Glu Ser Leu Val Thr
65                  70                  75                  80

Pro Glu Ile Arg Glu Ala Phe Ile Asp Glu Tyr Leu Ser Glu Ala Asn
                85                  90                  95

Ile Arg Gly Arg Ala Thr Lys Lys Ala Ile Arg Glu Leu Leu Met Ala
            100                 105                 110

Ile Glu Asp Asn Gln Glu Leu Ile Glu Lys Thr Met Ala Gly Val Gln
        115                 120                 125

Lys Ser Glu Leu Pro Glu Ile Pro Ala Ser Glu Lys Gly Leu Thr Asp
    130                 135                 140

Leu Val Glu Ser Asn Tyr Pro Phe Ala Ile Asp Pro Met Pro Asn Leu
145                 150                 155                 160

Tyr Phe Thr Arg Asp Pro Phe Ala Thr Ile Gly Thr Gly Val Ser Leu
                165                 170                 175
```

```
Asn His Met Phe Ser Glu Thr Arg Asn Arg Glu Thr Leu Tyr Gly Lys
            180                 185                 190

Tyr Ile Phe Thr His His Pro Ile Tyr Gly Gly Gly Lys Val Pro Met
        195                 200                 205

Val Tyr Asp Arg Asn Glu Thr Thr Arg Ile Glu Gly Gly Asp Glu Leu
    210                 215                 220

Val Leu Ser Lys Asp Val Leu Ala Val Gly Ile Ser Gln Arg Thr Asp
225                 230                 235                 240

Ala Ala Ser Ile Glu Lys Leu Leu Val Asn Ile Phe Lys Gln Asn Leu
                245                 250                 255

Gly Phe Lys Lys Val Leu Ala Phe Glu Phe Ala Asn Asn Arg Lys Phe
            260                 265                 270

Met His Leu Ala Thr Val Phe Thr Met Val Asp Tyr Asp Lys Phe Thr
        275                 280                 285

Ile His Pro Glu Ile Glu Gly Asp Leu Arg Val Tyr Ser Val Thr Tyr
    290                 295                 300

Asp Asn Glu Glu Leu His Ile Val Glu Glu Lys Gly Asp Leu Ala Glu
305                 310                 315                 320

Leu Leu Ala Ala Asn Leu Gly Val Glu Lys Val Asp Leu Ile Arg Cys
                325                 330                 335

Gly Gly Asp Asn Leu Val Ala Ala Gly Arg Glu Gln Trp Asn Asp Gly
            340                 345                 350

Ser Asn Thr Leu Thr Ile Ala Pro Gly Val Val Val Val Tyr Asn Arg
        355                 360                 365

Asn Thr Ile Thr Asn Ala Ile Leu Glu Ser Lys Gly Leu Lys Leu Ile
    370                 375                 380

Lys Ile His Gly Ser Glu Leu Val Arg Gly Arg Gly Gly Pro Arg Cys
385                 390                 395                 400

Met Ser Met Pro Phe Glu Arg Glu Asp Ile
                405                 410
```

<210> 17
<211> 427
<212> PRT
<213> Artificial Sequence

<220>
<223> ADI [full length] WT nnAA (e.g. pAMF) K15/K193/K316 w/ leader

<220>
<221> MOD_RES
<222> (31)..(31)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (209)..(209)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (332)..(332)
<223> Xaa = nnAA (e.g pAMF)

<400> 17

Met His His His His His His Gly Ser Gly Glu Asn Leu Tyr Phe Gln
1   5   10   15

Gly Thr Ala Gln Thr Pro Ile His Val Tyr Ser Glu Ile Gly Xaa Leu
            20   25   30

Lys Lys Val Leu Leu His Arg Pro Gly Lys Glu Ile Glu Asn Leu Met
        35   40   45

Pro Asp Tyr Leu Glu Arg Leu Leu Phe Asp Asp Ile Pro Phe Leu Glu
    50   55   60

Asp Ala Gln Lys Glu His Asp Ala Phe Ala Gln Ala Leu Arg Asp Glu
65   70   75   80

Gly Ile Glu Val Leu Tyr Leu Glu Thr Leu Ala Ala Glu Ser Leu Val
                85   90   95

Thr Pro Glu Ile Arg Glu Ala Phe Ile Asp Glu Tyr Leu Ser Glu Ala
            100   105   110

Asn Ile Arg Gly Arg Ala Thr Lys Lys Ala Ile Arg Glu Leu Leu Met
        115   120   125

Ala Ile Glu Asp Asn Gln Glu Leu Ile Glu Lys Thr Met Ala Gly Val
    130   135   140

Gln Lys Ser Glu Leu Pro Glu Ile Pro Ala Ser Glu Lys Gly Leu Thr
145   150   155   160

Asp Leu Val Glu Ser Asn Tyr Pro Phe Ala Ile Asp Pro Met Pro Asn
                165   170   175

Leu Tyr Phe Thr Arg Asp Pro Phe Ala Thr Ile Gly Thr Gly Val Ser
            180   185   190

Leu Asn His Met Phe Ser Glu Thr Arg Asn Arg Glu Thr Leu Tyr Gly
        195   200   205

```
Xaa Tyr Ile Phe Thr His His Pro Ile Tyr Gly Gly Gly Lys Val Pro
    210                 215                 220

Met Val Tyr Asp Arg Asn Glu Thr Thr Arg Ile Glu Gly Gly Asp Glu
225                 230                 235                 240

Leu Val Leu Ser Lys Asp Val Leu Ala Val Gly Ile Ser Gln Arg Thr
                245                 250                 255

Asp Ala Ala Ser Ile Glu Lys Leu Leu Val Asn Ile Phe Lys Gln Asn
            260                 265                 270

Leu Gly Phe Lys Lys Val Leu Ala Phe Glu Phe Ala Asn Asn Arg Lys
        275                 280                 285

Phe Met His Leu Asp Thr Val Phe Thr Met Val Asp Tyr Asp Lys Phe
    290                 295                 300

Thr Ile His Pro Glu Ile Glu Gly Asp Leu Arg Val Tyr Ser Val Thr
305                 310                 315                 320

Tyr Asp Asn Glu Glu Leu His Ile Val Glu Glu Xaa Gly Asp Leu Ala
                325                 330                 335

Glu Leu Leu Ala Ala Asn Leu Gly Val Glu Lys Val Asp Leu Ile Arg
            340                 345                 350

Cys Gly Gly Asp Asn Leu Val Ala Ala Gly Arg Glu Gln Trp Asn Asp
        355                 360                 365

Gly Ser Asn Thr Leu Thr Ile Ala Pro Gly Val Val Val Val Tyr Asn
    370                 375                 380

Arg Asn Thr Ile Thr Asn Ala Ile Leu Glu Ser Lys Gly Leu Lys Leu
385                 390                 395                 400

Ile Lys Ile His Gly Ser Glu Leu Val Arg Gly Arg Gly Gly Pro Arg
                405                 410                 415

Cys Met Ser Met Pro Phe Glu Arg Glu Asp Ile
            420                 425


<210>  18
<211>  410
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  ADI [full length] WT nnAA (e.g. pAMF) K15/K193/K316 w/o leader


<220>
<221>  MOD_RES
<222>  (14)..(14)
```

<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (192)..(192)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (315)..(315)
<223> Xaa = nnAA (e.g pAMF)

<400> 18

```
Thr Ala Gln Thr Pro Ile His Val Tyr Ser Glu Ile Gly Xaa Leu Lys
1               5                   10                  15


Lys Val Leu Leu His Arg Pro Gly Lys Glu Ile Glu Asn Leu Met Pro
            20                  25                  30


Asp Tyr Leu Glu Arg Leu Leu Phe Asp Asp Ile Pro Phe Leu Glu Asp
        35                  40                  45


Ala Gln Lys Glu His Asp Ala Phe Ala Gln Ala Leu Arg Asp Glu Gly
    50                  55                  60


Ile Glu Val Leu Tyr Leu Glu Thr Leu Ala Ala Glu Ser Leu Val Thr
65                  70                  75                  80


Pro Glu Ile Arg Glu Ala Phe Ile Asp Glu Tyr Leu Ser Glu Ala Asn
                85                  90                  95


Ile Arg Gly Arg Ala Thr Lys Lys Ala Ile Arg Glu Leu Leu Met Ala
            100                 105                 110


Ile Glu Asp Asn Gln Glu Leu Ile Glu Lys Thr Met Ala Gly Val Gln
        115                 120                 125


Lys Ser Glu Leu Pro Glu Ile Pro Ala Ser Glu Lys Gly Leu Thr Asp
    130                 135                 140


Leu Val Glu Ser Asn Tyr Pro Phe Ala Ile Asp Pro Met Pro Asn Leu
145                 150                 155                 160


Tyr Phe Thr Arg Asp Pro Phe Ala Thr Ile Gly Thr Gly Val Ser Leu
                165                 170                 175


Asn His Met Phe Ser Glu Thr Arg Asn Arg Glu Thr Leu Tyr Gly Xaa
            180                 185                 190


Tyr Ile Phe Thr His His Pro Ile Tyr Gly Gly Gly Lys Val Pro Met
        195                 200                 205


Val Tyr Asp Arg Asn Glu Thr Thr Arg Ile Glu Gly Gly Asp Glu Leu
    210                 215                 220
```

```
Val Leu Ser Lys Asp Val Leu Ala Val Gly Ile Ser Gln Arg Thr Asp
225                 230                 235                 240


Ala Ala Ser Ile Glu Lys Leu Leu Val Asn Ile Phe Lys Gln Asn Leu
                245                 250                 255


Gly Phe Lys Lys Val Leu Ala Phe Glu Phe Ala Asn Asn Arg Lys Phe
            260                 265                 270


Met His Leu Asp Thr Val Phe Thr Met Val Asp Tyr Asp Lys Phe Thr
        275                 280                 285


Ile His Pro Glu Ile Glu Gly Asp Leu Arg Val Tyr Ser Val Thr Tyr
    290                 295                 300


Asp Asn Glu Glu Leu His Ile Val Glu Glu Xaa Gly Asp Leu Ala Glu
305                 310                 315                 320


Leu Leu Ala Ala Asn Leu Gly Val Glu Lys Val Asp Leu Ile Arg Cys
                325                 330                 335


Gly Gly Asp Asn Leu Val Ala Ala Gly Arg Glu Gln Trp Asn Asp Gly
            340                 345                 350


Ser Asn Thr Leu Thr Ile Ala Pro Gly Val Val Val Val Tyr Asn Arg
        355                 360                 365


Asn Thr Ile Thr Asn Ala Ile Leu Glu Ser Lys Gly Leu Lys Leu Ile
    370                 375                 380


Lys Ile His Gly Ser Glu Leu Val Arg Gly Arg Gly Gly Pro Arg Cys
385                 390                 395                 400


Met Ser Met Pro Phe Glu Arg Glu Asp Ile
                405                 410


<210>  19
<211>  427
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  ADI [full length] D277A nnAA (e.g. pAMF) K15/K193/K316 w/ leader


<220>
<221>  MOD_RES
<222>  (31)..(31)
<223>  Xaa = nnAA (e.g pAMF)

<220>
<221>  MOD_RES
<222>  (209)..(209)
<223>  Xaa = nnAA (e.g pAMF)

<220>
```

<221> MOD_RES
<222> (332)..(332)
<223> Xaa = nnAA (e.g pAMF)

<400> 19

```
Met His His His His His His Gly Ser Gly Glu Asn Leu Tyr Phe Gln
1               5                   10                  15


Gly Thr Ala Gln Thr Pro Ile His Val Tyr Ser Glu Ile Gly Xaa Leu
            20                  25                  30


Lys Lys Val Leu Leu His Arg Pro Gly Lys Glu Ile Glu Asn Leu Met
        35                  40                  45


Pro Asp Tyr Leu Glu Arg Leu Leu Phe Asp Asp Ile Pro Phe Leu Glu
    50                  55                  60


Asp Ala Gln Lys Glu His Asp Ala Phe Ala Gln Ala Leu Arg Asp Glu
65                  70                  75                  80


Gly Ile Glu Val Leu Tyr Leu Glu Thr Leu Ala Ala Glu Ser Leu Val
                85                  90                  95


Thr Pro Glu Ile Arg Glu Ala Phe Ile Asp Glu Tyr Leu Ser Glu Ala
            100                 105                 110


Asn Ile Arg Gly Arg Ala Thr Lys Lys Ala Ile Arg Glu Leu Leu Met
        115                 120                 125


Ala Ile Glu Asp Asn Gln Glu Leu Ile Glu Lys Thr Met Ala Gly Val
    130                 135                 140


Gln Lys Ser Glu Leu Pro Glu Ile Pro Ala Ser Glu Lys Gly Leu Thr
145                 150                 155                 160


Asp Leu Val Glu Ser Asn Tyr Pro Phe Ala Ile Asp Pro Met Pro Asn
                165                 170                 175


Leu Tyr Phe Thr Arg Asp Pro Phe Ala Thr Ile Gly Thr Gly Val Ser
            180                 185                 190


Leu Asn His Met Phe Ser Glu Thr Arg Asn Arg Glu Thr Leu Tyr Gly
        195                 200                 205


Xaa Tyr Ile Phe Thr His His Pro Ile Tyr Gly Gly Gly Lys Val Pro
    210                 215                 220


Met Val Tyr Asp Arg Asn Glu Thr Thr Arg Ile Glu Gly Gly Asp Glu
225                 230                 235                 240


Leu Val Leu Ser Lys Asp Val Leu Ala Val Gly Ile Ser Gln Arg Thr
                245                 250                 255
```

```
Asp Ala Ala Ser Ile Glu Lys Leu Leu Val Asn Ile Phe Lys Gln Asn
            260                 265                 270

Leu Gly Phe Lys Lys Val Leu Ala Phe Glu Phe Ala Asn Asn Arg Lys
        275                 280                 285

Phe Met His Leu Ala Thr Val Phe Thr Met Val Asp Tyr Asp Lys Phe
    290                 295                 300

Thr Ile His Pro Glu Ile Glu Gly Asp Leu Arg Val Tyr Ser Val Thr
305                 310                 315                 320

Tyr Asp Asn Glu Glu Leu His Ile Val Glu Glu Xaa Gly Asp Leu Ala
                325                 330                 335

Glu Leu Leu Ala Ala Asn Leu Gly Val Glu Lys Val Asp Leu Ile Arg
            340                 345                 350

Cys Gly Gly Asp Asn Leu Val Ala Ala Gly Arg Glu Gln Trp Asn Asp
        355                 360                 365

Gly Ser Asn Thr Leu Thr Ile Ala Pro Gly Val Val Val Val Tyr Asn
    370                 375                 380

Arg Asn Thr Ile Thr Asn Ala Ile Leu Glu Ser Lys Gly Leu Lys Leu
385                 390                 395                 400

Ile Lys Ile His Gly Ser Glu Leu Val Arg Gly Arg Gly Gly Pro Arg
                405                 410                 415

Cys Met Ser Met Pro Phe Glu Arg Glu Asp Ile
            420                 425


<210>  20
<211>  410
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  ADI [full length] D277A nnAA (e.g. pAMF) K15/K193/K316 w/o leader


<220>
<221>  MOD_RES
<222>  (14)..(14)
<223>  Xaa = nnAA (e.g pAMF)

<220>
<221>  MOD_RES
<222>  (192)..(192)
<223>  Xaa = nnAA (e.g pAMF)

<220>
<221>  MOD_RES
<222>  (315)..(315)
<223>  Xaa = nnAA (e.g pAMF)
```

<400> 20

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

Lys Val Leu Leu His Arg Pro Gly Lys Glu Ile Glu Asn Leu Met Pro
20 25 30

Asp Tyr Leu Glu Arg Leu Leu Phe Asp Asp Ile Pro Phe Leu Glu Asp
35 40 45

Ala Gln Lys Glu His Asp Ala Phe Ala Gln Ala Leu Arg Asp Glu Gly
50 55 60

Ile Glu Val Leu Tyr Leu Glu Thr Leu Ala Ala Glu Ser Leu Val Thr
65 70 75 80

Pro Glu Ile Arg Glu Ala Phe Ile Asp Glu Tyr Leu Ser Glu Ala Asn
85 90 95

Ile Arg Gly Arg Ala Thr Lys Lys Ala Ile Arg Glu Leu Leu Met Ala
100 105 110

Ile Glu Asp Asn Gln Glu Leu Ile Glu Lys Thr Met Ala Gly Val Gln
115 120 125

Lys Ser Glu Leu Pro Glu Ile Pro Ala Ser Glu Lys Gly Leu Thr Asp
130 135 140

Leu Val Glu Ser Asn Tyr Pro Phe Ala Ile Asp Pro Met Pro Asn Leu
145 150 155 160

Tyr Phe Thr Arg Asp Pro Phe Ala Thr Ile Gly Thr Gly Val Ser Leu
165 170 175

Asn His Met Phe Ser Glu Thr Arg Asn Arg Glu Thr Leu Tyr Gly Xaa
180 185 190

Tyr Ile Phe Thr His His Pro Ile Tyr Gly Gly Gly Lys Val Pro Met
195 200 205

Val Tyr Asp Arg Asn Glu Thr Thr Arg Ile Glu Gly Gly Asp Glu Leu
210 215 220

Val Leu Ser Lys Asp Val Leu Ala Val Gly Ile Ser Gln Arg Thr Asp
225 230 235 240

Ala Ala Ser Ile Glu Lys Leu Leu Val Asn Ile Phe Lys Gln Asn Leu
245 250 255

Gly Phe Lys Lys Val Leu Ala Phe Glu Phe Ala Asn Asn Arg Lys Phe
260 265 270

```
Met His Leu Ala Thr Val Phe Thr Met Val Asp Tyr Asp Lys Phe Thr
        275                 280                 285

Ile His Pro Glu Ile Glu Gly Asp Leu Arg Val Tyr Ser Val Thr Tyr
    290                 295                 300

Asp Asn Glu Glu Leu His Ile Val Glu Glu Xaa Gly Asp Leu Ala Glu
305                 310                 315                 320

Leu Leu Ala Ala Asn Leu Gly Val Glu Lys Val Asp Leu Ile Arg Cys
                325                 330                 335

Gly Gly Asp Asn Leu Val Ala Ala Gly Arg Glu Gln Trp Asn Asp Gly
            340                 345                 350

Ser Asn Thr Leu Thr Ile Ala Pro Gly Val Val Val Val Tyr Asn Arg
        355                 360                 365

Asn Thr Ile Thr Asn Ala Ile Leu Glu Ser Lys Gly Leu Lys Leu Ile
    370                 375                 380

Lys Ile His Gly Ser Glu Leu Val Arg Gly Arg Gly Gly Pro Arg Cys
385                 390                 395                 400

Met Ser Met Pro Phe Glu Arg Glu Asp Ile
                405                 410
```

<210> 21
<211> 184
<212> PRT
<213> Artificial Sequence

<220>
<223> hFerritin [full length] WT w/ leader

<400> 21

```
Met His His His His His His Gly Ser Gly Ser Ser Gln Ile Arg Gln
1               5                   10                  15

Asn Tyr Ser Thr Asp Val Glu Ala Ala Val Asn Ser Leu Val Asn Leu
            20                  25                  30

Tyr Leu Gln Ala Ser Tyr Thr Tyr Leu Ser Leu Gly Phe Tyr Phe Asp
        35                  40                  45

Arg Asp Asp Val Ala Leu Glu Gly Val Ser His Phe Phe Arg Glu Leu
    50                  55                  60

Ala Glu Glu Lys Arg Glu Gly Tyr Glu Arg Leu Leu Lys Met Gln Asn
65                  70                  75                  80

Gln Arg Gly Gly Arg Ala Leu Phe Gln Asp Ile Lys Lys Pro Ala Glu
                85                  90                  95
```

```
Asp Glu Trp Gly Lys Thr Pro Asp Ala Met Lys Ala Ala Met Ala Leu
            100                 105                 110


Glu Lys Lys Leu Asn Gln Ala Leu Leu Asp Leu His Ala Leu Gly Ser
        115                 120                 125


Ala Arg Thr Asp Pro His Leu Cys Asp Phe Leu Glu Thr His Phe Leu
    130                 135                 140


Asp Glu Glu Val Lys Leu Ile Lys Lys Met Gly Asp His Leu Thr Asn
145                 150                 155                 160


Leu His Arg Leu Gly Gly Pro Glu Ala Gly Leu Gly Glu Tyr Leu Phe
                165                 170                 175


Glu Arg Leu Thr Leu Lys His Asp
            180


<210>  22
<211>  174
<212>  PRT
<213>  Homo sapiens

<400>  22

Ser Ser Gln Ile Arg Gln Asn Tyr Ser Thr Asp Val Glu Ala Ala Val
1               5                   10                  15


Asn Ser Leu Val Asn Leu Tyr Leu Gln Ala Ser Tyr Thr Tyr Leu Ser
            20                  25                  30


Leu Gly Phe Tyr Phe Asp Arg Asp Asp Val Ala Leu Glu Gly Val Ser
        35                  40                  45


His Phe Phe Arg Glu Leu Ala Glu Glu Lys Arg Glu Gly Tyr Glu Arg
    50                  55                  60


Leu Leu Lys Met Gln Asn Gln Arg Gly Gly Arg Ala Leu Phe Gln Asp
65                  70                  75                  80


Ile Lys Lys Pro Ala Glu Asp Glu Trp Gly Lys Thr Pro Asp Ala Met
                85                  90                  95


Lys Ala Ala Met Ala Leu Glu Lys Lys Leu Asn Gln Ala Leu Leu Asp
            100                 105                 110


Leu His Ala Leu Gly Ser Ala Arg Thr Asp Pro His Leu Cys Asp Phe
        115                 120                 125


Leu Glu Thr His Phe Leu Asp Glu Glu Val Lys Leu Ile Lys Lys Met
    130                 135                 140
```

Gly Asp His Leu Thr Asn Leu His Arg Leu Gly Gly Pro Glu Ala Gly
145 150 155 160

Leu Gly Glu Tyr Leu Phe Glu Arg Leu Thr Leu Lys His Asp
165 170

<210> 23
<211> 184
<212> PRT
<213> Artificial Sequence

<220>
<223> hFerritin [full length] nnAA (e.g. pAMF) mutant I5 w/ leader

<220>
<221> MOD_RES
<222> (14)..(14)
<223> Xaa = nnAA (e.g pAMF)

<400> 23

Met His His His His His His Gly Ser Gly Ser Ser Gln Xaa Arg Gln
1 5 10 15

Asn Tyr Ser Thr Asp Val Glu Ala Ala Val Asn Ser Leu Val Asn Leu
20 25 30

Tyr Leu Gln Ala Ser Tyr Thr Tyr Leu Ser Leu Gly Phe Tyr Phe Asp
35 40 45

Arg Asp Asp Val Ala Leu Glu Gly Val Ser His Phe Phe Arg Glu Leu
50 55 60

Ala Glu Glu Lys Arg Glu Gly Tyr Glu Arg Leu Leu Lys Met Gln Asn
65 70 75 80

Gln Arg Gly Gly Arg Ala Leu Phe Gln Asp Ile Lys Lys Pro Ala Glu
85 90 95

Asp Glu Trp Gly Lys Thr Pro Asp Ala Met Lys Ala Ala Met Ala Leu
100 105 110

Glu Lys Lys Leu Asn Gln Ala Leu Leu Asp Leu His Ala Leu Gly Ser
115 120 125

Ala Arg Thr Asp Pro His Leu Cys Asp Phe Leu Glu Thr His Phe Leu
130 135 140

Asp Glu Glu Val Lys Leu Ile Lys Lys Met Gly Asp His Leu Thr Asn
145 150 155 160

Leu His Arg Leu Gly Gly Pro Glu Ala Gly Leu Gly Glu Tyr Leu Phe
165 170 175

Glu Arg Leu Thr Leu Lys His Asp

180

<210> 24
<211> 174
<212> PRT
<213> Artificial Sequence

<220>
<223> hFerritin [full length] nnAA (e.g. pAMF) mutant I5 w/o leader

<220>
<221> MOD_RES
<222> (4)..(4)
<223> Xaa = nnAA (e.g pAMF)

<400> 24

Ser Ser Gln Xaa Arg Gln Asn Tyr Ser Thr Asp Val Glu Ala Ala Val
1 5 10 15

Asn Ser Leu Val Asn Leu Tyr Leu Gln Ala Ser Tyr Thr Tyr Leu Ser
20 25 30

Leu Gly Phe Tyr Phe Asp Arg Asp Asp Val Ala Leu Glu Gly Val Ser
35 40 45

His Phe Phe Arg Glu Leu Ala Glu Glu Lys Arg Glu Gly Tyr Glu Arg
50 55 60

Leu Leu Lys Met Gln Asn Gln Arg Gly Gly Arg Ala Leu Phe Gln Asp
65 70 75 80

Ile Lys Lys Pro Ala Glu Asp Glu Trp Gly Lys Thr Pro Asp Ala Met
85 90 95

Lys Ala Ala Met Ala Leu Glu Lys Lys Leu Asn Gln Ala Leu Leu Asp
100 105 110

Leu His Ala Leu Gly Ser Ala Arg Thr Asp Pro His Leu Cys Asp Phe
115 120 125

Leu Glu Thr His Phe Leu Asp Glu Glu Val Lys Leu Ile Lys Lys Met
130 135 140

Gly Asp His Leu Thr Asn Leu His Arg Leu Gly Gly Pro Glu Ala Gly
145 150 155 160

Leu Gly Glu Tyr Leu Phe Glu Arg Leu Thr Leu Lys His Asp
165 170

<210> 25
<211> 536
<212> PRT
<213> Artificial Sequence

<220>

<223> eCRM - nnAA (e.g. pAMF) mutant

<220>
<221> MOD_RES
<222> (34)..(34)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (213)..(213)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (245)..(245)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (265)..(265)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (386)..(386)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (527)..(527)
<223> Xaa = nnAA (e.g pAMF)

<400> 25

Met Gly Ala Asp Asp Val Val Asp Ser Ser Lys Ser Phe Val Met Glu
1   5   10   15

Asn Phe Ser Ser Tyr His Gly Thr Lys Pro Gly Tyr Val Asp Ser Ile
            20   25   30

Gln Xaa Gly Ile Gln Lys Pro Lys Ser Gly Thr Gln Gly Asn Tyr Asp
        35   40   45

Asp Asp Trp Lys Glu Phe Tyr Ser Thr Asp Asn Lys Tyr Asp Ala Ala
    50   55   60

Gly Tyr Ser Val Asp Asn Glu Asn Pro Leu Ser Gly Lys Ala Gly Gly
65   70   75   80

Val Val Lys Val Thr Tyr Pro Gly Leu Thr Lys Val Leu Ala Leu Lys
                85   90   95

Val Asp Asn Ala Glu Thr Ile Lys Lys Glu Leu Gly Leu Ser Leu Thr
            100   105   110

Glu Pro Leu Met Glu Gln Val Gly Thr Glu Glu Phe Ile Lys Arg Phe
        115   120   125

Gly Asp Gly Ala Ser Arg Val Val Leu Ser Leu Pro Phe Ala Glu Gly
    130   135   140

```
Ser Ser Ser Val Glu Tyr Ile Asn Asn Trp Glu Gln Ala Lys Ala Leu
145                 150                 155                 160

Ser Val Glu Leu Glu Ile Asn Phe Glu Thr Arg Gly Lys Arg Gly Gln
                165                 170                 175

Asp Ala Met Tyr Glu Tyr Met Ala Gln Ala Cys Ala Gly Asn Arg Val
            180                 185                 190

Arg Asn Ser Val Gly Ser Ser Leu Ser Cys Ile Asn Leu Asp Trp Asp
        195                 200                 205

Val Ile Arg Asp Xaa Thr Lys Thr Lys Ile Glu Ser Leu Lys Glu His
    210                 215                 220

Gly Pro Ile Lys Asn Lys Met Ser Glu Ser Pro Asn Lys Thr Val Ser
225                 230                 235                 240

Glu Glu Lys Ala Xaa Gln Tyr Leu Glu Glu Phe His Gln Thr Ala Leu
                245                 250                 255

Glu His Pro Glu Leu Ser Glu Leu Xaa Thr Val Thr Gly Thr Asn Pro
            260                 265                 270

Val Phe Ala Gly Ala Asn Tyr Ala Ala Trp Ala Val Asn Val Ala Gln
        275                 280                 285

Val Ile Asp Ser Glu Thr Ala Asp Asn Leu Glu Lys Thr Thr Ala Ala
    290                 295                 300

Leu Ser Ile Leu Pro Gly Ile Gly Ser Val Met Gly Ile Ala Asp Gly
305                 310                 315                 320

Ala Val His His Asn Thr Glu Glu Ile Val Ala Gln Ser Ile Ala Leu
                325                 330                 335

Ser Ser Leu Met Val Ala Gln Ala Ile Pro Leu Val Gly Glu Leu Val
            340                 345                 350

Asp Ile Gly Phe Ala Ala Tyr Asn Phe Val Glu Ser Ile Ile Asn Leu
        355                 360                 365

Phe Gln Val Val His Asn Ser Tyr Asn Arg Pro Ala Tyr Ser Pro Gly
    370                 375                 380

His Xaa Thr Gln Pro Phe Leu His Asp Gly Tyr Ala Val Ser Trp Asn
385                 390                 395                 400

Thr Val Glu Asp Ser Ile Ile Arg Thr Gly Phe Gln Gly Glu Ser Gly
                405                 410                 415
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```
His Asp Ile Lys Ile Thr Ala Glu Asn Thr Pro Leu Pro Ile Ala Gly
            420                 425                 430

Val Leu Leu Pro Thr Ile Pro Gly Lys Leu Asp Val Asn Lys Ser Lys
        435                 440                 445

Thr His Ile Ser Val Asn Gly Arg Lys Ile Arg Met Arg Cys Arg Ala
    450                 455                 460

Ile Asp Gly Asp Val Thr Phe Cys Arg Pro Lys Ser Pro Val Tyr Val
465                 470                 475                 480

Gly Asn Gly Val His Ala Asn Leu His Val Ala Phe His Arg Ser Ser
                485                 490                 495

Ser Glu Lys Ile His Ser Asn Glu Ile Ser Ser Asp Ser Ile Gly Val
            500                 505                 510

Leu Gly Tyr Gln Lys Thr Val Asp His Thr Lys Val Asn Ser Xaa Leu
        515                 520                 525

Ser Leu Phe Phe Glu Ile Lys Ser
    530                 535


<210>  26
<211>  427
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  ADI (mut2) [full length] D277A nnAA (e.g. pAMF) mutant w/ leader


<220>
<221>  MOD_RES
<222>  (139)..(139)
<223>  Xaa = nnAA (e.g pAMF)

<220>
<221>  MOD_RES
<222>  (263)..(263)
<223>  Xaa = nnAA (e.g pAMF)

<220>
<221>  MOD_RES
<222>  (332)..(332)
<223>  Xaa = nnAA (e.g pAMF)

<400>  26

Met His His His His His His Gly Ser Gly Glu Asn Leu Tyr Phe Gln
1               5                   10                  15

Gly Thr Ala Gln Thr Pro Ile His Val Tyr Ser Glu Ile Gly Lys Leu
            20                  25                  30

Lys Lys Val Leu Leu His Arg Pro Gly Lys Glu Ile Glu Asn Leu Met
        35                  40                  45
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```
Pro Asp Tyr Leu Glu Arg Leu Leu Phe Asp Asp Ile Pro Phe Leu Glu
    50                  55                  60

Asp Ala Gln Lys Glu His Asp Ala Phe Ala Gln Ala Leu Arg Asp Glu
65                  70                  75                  80

Gly Ile Glu Val Leu Tyr Leu Glu Thr Leu Ala Ala Glu Ser Leu Val
                85                  90                  95

Thr Pro Glu Ile Arg Glu Ala Phe Ile Asp Glu Tyr Leu Ser Glu Ala
            100                 105                 110

Asn Ile Arg Gly Arg Ala Thr Lys Lys Ala Ile Arg Glu Leu Leu Met
        115                 120                 125

Ala Ile Glu Asp Asn Gln Glu Leu Ile Glu Xaa Thr Met Ala Gly Val
    130                 135                 140

Gln Lys Ser Glu Leu Pro Glu Ile Pro Ala Ser Glu Lys Gly Leu Thr
145                 150                 155                 160

Asp Leu Val Glu Ser Asn Tyr Pro Phe Ala Ile Asp Pro Met Pro Asn
                165                 170                 175

Leu Tyr Phe Thr Arg Asp Pro Phe Ala Thr Ile Gly Thr Gly Val Ser
            180                 185                 190

Leu Asn His Met Phe Ser Glu Thr Arg Asn Arg Glu Thr Leu Tyr Gly
        195                 200                 205

Lys Tyr Ile Phe Thr His His Pro Ile Tyr Gly Gly Gly Lys Val Pro
    210                 215                 220

Met Val Tyr Asp Arg Asn Glu Thr Thr Arg Ile Glu Gly Gly Asp Glu
225                 230                 235                 240

Leu Val Leu Ser Lys Asp Val Leu Ala Val Gly Ile Ser Gln Arg Thr
                245                 250                 255

Asp Ala Ala Ser Ile Glu Xaa Leu Leu Val Asn Ile Phe Lys Gln Asn
            260                 265                 270

Leu Gly Phe Lys Lys Val Leu Ala Phe Glu Phe Ala Asn Asn Arg Lys
        275                 280                 285

Phe Met His Leu Ala Thr Val Phe Thr Met Val Asp Tyr Asp Lys Phe
    290                 295                 300

Thr Ile His Pro Glu Ile Glu Gly Asp Leu Arg Val Tyr Ser Val Thr
305                 310                 315                 320
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```
Tyr Asp Asn Glu Glu Leu His Ile Val Glu Glu Xaa Gly Asp Leu Ala
                325                 330                 335


Glu Leu Leu Ala Ala Asn Leu Gly Val Glu Lys Val Asp Leu Ile Arg
            340                 345                 350


Cys Gly Gly Asp Asn Leu Val Ala Ala Gly Arg Glu Gln Trp Asn Asp
        355                 360                 365


Gly Ser Asn Thr Leu Thr Ile Ala Pro Gly Val Val Val Val Tyr Asn
    370                 375                 380


Arg Asn Thr Ile Thr Asn Ala Ile Leu Glu Ser Lys Gly Leu Lys Leu
385                 390                 395                 400


Ile Lys Ile His Gly Ser Glu Leu Val Arg Gly Arg Gly Gly Pro Arg
                405                 410                 415


Cys Met Ser Met Pro Phe Glu Arg Glu Asp Ile
            420                 425


<210>  27
<211>  411
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  ADI (mut2) [full length] D277A nnAA (e.g. pAMF) mutant w/o
       leader, with G overhang from cleavage


<220>
<221>  MOD_RES
<222>  (123)..(123)
<223>  Xaa = nnAA (e.g pAMF)

<220>
<221>  MOD_RES
<222>  (247)..(247)
<223>  Xaa = nnAA (e.g pAMF)

<220>
<221>  MOD_RES
<222>  (316)..(316)
<223>  Xaa = nnAA (e.g pAMF)

<400>  27

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


Lys Lys Val Leu Leu His Arg Pro Gly Lys Glu Ile Glu Asn Leu Met
            20                  25                  30


Pro Asp Tyr Leu Glu Arg Leu Leu Phe Asp Asp Ile Pro Phe Leu Glu
        35                  40                  45


Asp Ala Gln Lys Glu His Asp Ala Phe Ala Gln Ala Leu Arg Asp Glu
```

```
    50                  55                  60

Gly Ile Glu Val Leu Tyr Leu Glu Thr Leu Ala Ala Glu Ser Leu Val
65                  70                  75                  80

Thr Pro Glu Ile Arg Glu Ala Phe Ile Asp Glu Tyr Leu Ser Glu Ala
                85                  90                  95

Asn Ile Arg Gly Arg Ala Thr Lys Lys Ala Ile Arg Glu Leu Leu Met
            100                 105                 110

Ala Ile Glu Asp Asn Gln Glu Leu Ile Glu Xaa Thr Met Ala Gly Val
        115                 120                 125

Gln Lys Ser Glu Leu Pro Glu Ile Pro Ala Ser Glu Lys Gly Leu Thr
    130                 135                 140

Asp Leu Val Glu Ser Asn Tyr Pro Phe Ala Ile Asp Pro Met Pro Asn
145                 150                 155                 160

Leu Tyr Phe Thr Arg Asp Pro Phe Ala Thr Ile Gly Thr Gly Val Ser
                165                 170                 175

Leu Asn His Met Phe Ser Glu Thr Arg Asn Arg Glu Thr Leu Tyr Gly
            180                 185                 190

Lys Tyr Ile Phe Thr His His Pro Ile Tyr Gly Gly Gly Lys Val Pro
        195                 200                 205

Met Val Tyr Asp Arg Asn Glu Thr Thr Arg Ile Glu Gly Gly Asp Glu
    210                 215                 220

Leu Val Leu Ser Lys Asp Val Leu Ala Val Gly Ile Ser Gln Arg Thr
225                 230                 235                 240

Asp Ala Ala Ser Ile Glu Xaa Leu Leu Val Asn Ile Phe Lys Gln Asn
                245                 250                 255

Leu Gly Phe Lys Lys Val Leu Ala Phe Glu Phe Ala Asn Asn Arg Lys
            260                 265                 270

Phe Met His Leu Ala Thr Val Phe Thr Met Val Asp Tyr Asp Lys Phe
        275                 280                 285

Thr Ile His Pro Glu Ile Glu Gly Asp Leu Arg Val Tyr Ser Val Thr
    290                 295                 300

Tyr Asp Asn Glu Glu Leu His Ile Val Glu Glu Xaa Gly Asp Leu Ala
305                 310                 315                 320

Glu Leu Leu Ala Ala Asn Leu Gly Val Glu Lys Val Asp Leu Ile Arg
                325                 330                 335
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```
Cys Gly Gly Asp Asn Leu Val Ala Ala Gly Arg Glu Gln Trp Asn Asp
            340                 345                 350


Gly Ser Asn Thr Leu Thr Ile Ala Pro Gly Val Val Val Val Tyr Asn
        355                 360                 365


Arg Asn Thr Ile Thr Asn Ala Ile Leu Glu Ser Lys Gly Leu Lys Leu
    370                 375                 380


Ile Lys Ile His Gly Ser Glu Leu Val Arg Gly Arg Gly Gly Pro Arg
385                 390                 395                 400


Cys Met Ser Met Pro Phe Glu Arg Glu Asp Ile
                405                 410


<210>  28
<211>  157
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Sfb1 repeat region with leader

<400>  28

Met His His His His His His Gly Ser Gly Val Glu Thr Glu Asp Thr
1               5                   10                  15


Lys Glu Pro Gly Val Leu Met Gly Gly Gln Ser Glu Ser Val Glu Phe
            20                  25                  30


Thr Lys Asp Thr Gln Thr Gly Met Ser Gly Gln Thr Thr Pro Gln Val
        35                  40                  45


Glu Thr Glu Asp Thr Lys Glu Pro Gly Val Leu Met Gly Gly Gln Ser
    50                  55                  60


Glu Ser Val Glu Phe Thr Lys Asp Thr Gln Thr Gly Met Ser Gly Gln
65                  70                  75                  80


Thr Ala Ser Gln Val Glu Thr Glu Asp Thr Lys Glu Pro Gly Val Leu
                85                  90                  95


Met Gly Gly Gln Ser Glu Ser Val Glu Phe Thr Lys Asp Thr Gln Thr
            100                 105                 110


Gly Met Ser Gly Gln Thr Thr Pro Gln Val Glu Thr Glu Asp Thr Lys
        115                 120                 125


Glu Pro Gly Val Leu Met Gly Gly Gln Ser Glu Ser Val Glu Phe Thr
    130                 135                 140


Lys Asp Thr Gln Thr Gly Met Ser Gly Phe Ser Glu Thr
```

145 150 155

<210> 29
<211> 946
<212> PRT
<213> Artificial Sequence

<220>
<223> C5a [90-1035 fragment] WT w/o leader, with G overhang from cleavage

<400> 29

Gly Lys Thr Ala Asp Thr Pro Val Thr Ser Lys Ala Thr Ile Arg Asp
1 5 10 15

Leu Asn Asp Pro Ser Gln Val Lys Thr Leu Gln Glu Lys Ala Gly Lys
20 25 30

Gly Ala Gly Thr Val Val Ala Val Ile Asp Ala Gly Phe Asp Lys Asn
35 40 45

His Glu Ala Trp Arg Leu Thr Asp Lys Thr Lys Ala Arg Tyr Gln Ser
50 55 60

Lys Glu Asp Leu Glu Lys Ala Lys Lys Glu His Gly Ile Thr Tyr Gly
65 70 75 80

Glu Trp Val Asn Asp Lys Val Ala Tyr Tyr His Asp Tyr Ser Lys Asp
85 90 95

Gly Lys Thr Ala Val Asp Gln Glu His Gly Thr His Val Ser Gly Ile
100 105 110

Leu Ser Gly Asn Ala Pro Ser Glu Thr Lys Glu Pro Tyr Arg Leu Glu
115 120 125

Gly Ala Met Pro Glu Ala Gln Leu Leu Leu Met Arg Val Glu Ile Val
130 135 140

Asn Gly Leu Ala Asp Tyr Ala Arg Asn Tyr Ala Gln Ala Ile Arg Asp
145 150 155 160

Ala Val Asn Leu Gly Ala Lys Val Ile Asn Met Ser Phe Gly Asn Ala
165 170 175

Ala Leu Ala Tyr Ala Asn Leu Pro Asp Glu Thr Lys Lys Ala Phe Asp
180 185 190

Tyr Ala Lys Ser Lys Gly Val Ser Ile Val Thr Ser Ala Gly Asn Asp
195 200 205

Ser Ser Phe Gly Gly Lys Thr Arg Leu Pro Leu Ala Asp His Pro Asp
210 215 220

```
Tyr Gly Val Val Gly Thr Pro Ala Ala Ala Asp Ser Thr Leu Thr Val
225                 230                 235                 240

Ala Ser Tyr Ser Pro Asp Lys Gln Leu Thr Glu Thr Ala Thr Val Lys
                245                 250                 255

Thr Ala Asp Lys Gln Asp Lys Glu Met Pro Val Leu Ser Thr Asn Arg
            260                 265                 270

Phe Glu Pro Asn Lys Ala Tyr Asp Tyr Ala Tyr Ala Asn Arg Gly Met
        275                 280                 285

Lys Glu Asp Asp Phe Lys Asp Val Lys Gly Lys Ile Ala Leu Ile Glu
    290                 295                 300

Arg Gly Asp Ile Asp Phe Lys Asp Lys Ile Ala Asn Ala Lys Lys Ala
305                 310                 315                 320

Gly Ala Val Gly Val Leu Ile Tyr Asp Asn Gln Asp Lys Gly Phe Pro
                325                 330                 335

Ile Glu Leu Pro Asn Val Asp Gln Met Pro Ala Ala Phe Ile Ser Arg
            340                 345                 350

Lys Asp Gly Leu Leu Leu Lys Asp Asn Pro Lys Lys Thr Ile Thr Phe
        355                 360                 365

Asn Ala Thr Pro Lys Val Leu Pro Thr Ala Ser Gly Thr Lys Leu Ser
    370                 375                 380

Arg Phe Ser Ser Trp Gly Leu Thr Ala Asp Gly Asn Ile Lys Pro Asp
385                 390                 395                 400

Ile Ala Ala Pro Gly Gln Asp Ile Leu Ser Ser Val Ala Asn Asn Lys
                405                 410                 415

Tyr Ala Lys Leu Ser Gly Thr Ser Met Ser Ala Pro Leu Val Ala Gly
            420                 425                 430

Ile Met Gly Leu Leu Gln Lys Gln Tyr Glu Thr Gln Tyr Pro Asp Met
        435                 440                 445

Thr Pro Ser Glu Arg Leu Asp Leu Ala Lys Lys Val Leu Met Ser Ser
    450                 455                 460

Ala Thr Ala Leu Tyr Asp Glu Asp Glu Lys Ala Tyr Phe Ser Pro Arg
465                 470                 475                 480

Gln Gln Gly Ala Gly Ala Val Asp Ala Lys Lys Ala Ser Ala Ala Thr
                485                 490                 495
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```
Met Tyr Val Thr Asp Lys Asp Asn Thr Ser Ser Lys Val His Leu Asn
            500                 505                 510

Asn Val Ser Asp Thr Phe Glu Val Thr Val Thr Val His Asn Lys Ser
        515                 520                 525

Asp Lys Pro Gln Glu Leu Tyr Tyr Gln Ala Thr Val Gln Thr Asp Lys
    530                 535                 540

Val Asp Gly Lys His Phe Ala Leu Ala Pro Lys Ala Leu Tyr Glu Thr
545                 550                 555                 560

Ser Trp Gln Lys Ile Thr Ile Pro Ala Asn Ser Ser Lys Gln Val Thr
                565                 570                 575

Val Pro Ile Asp Ala Ser Arg Phe Ser Lys Asp Leu Leu Ala Gln Met
            580                 585                 590

Lys Asn Gly Tyr Phe Leu Glu Gly Phe Val Arg Phe Lys Gln Asp Pro
        595                 600                 605

Thr Lys Glu Glu Leu Met Ser Ile Pro Tyr Ile Gly Phe Arg Gly Asp
    610                 615                 620

Phe Gly Asn Leu Ser Ala Leu Glu Lys Pro Ile Tyr Asp Ser Lys Asp
625                 630                 635                 640

Gly Ser Ser Tyr Tyr His Glu Ala Asn Ser Asp Ala Lys Asp Gln Leu
                645                 650                 655

Asp Gly Asp Gly Leu Gln Phe Tyr Ala Leu Lys Asn Asn Phe Thr Ala
            660                 665                 670

Leu Thr Thr Glu Ser Asn Pro Trp Thr Ile Ile Lys Ala Val Lys Glu
        675                 680                 685

Gly Val Glu Asn Ile Glu Asp Ile Glu Ser Ser Glu Ile Thr Glu Thr
    690                 695                 700

Ile Phe Ala Gly Thr Phe Ala Lys Gln Asp Asp Asp Ser His Tyr Tyr
705                 710                 715                 720

Ile His Arg His Ala Asn Gly Lys Pro Tyr Ala Ala Ile Ser Pro Asn
                725                 730                 735

Gly Asp Gly Asn Arg Asp Tyr Val Gln Phe Gln Gly Thr Phe Leu Arg
            740                 745                 750

Asn Ala Lys Asn Leu Val Ala Glu Val Leu Asp Lys Glu Gly Asn Val
        755                 760                 765

Val Trp Thr Ser Glu Val Thr Glu Gln Val Val Lys Asn Tyr Asn Asn
```

```
    770                 775                 780

Asp Leu Ala Ser Thr Leu Gly Ser Thr Arg Phe Glu Lys Thr Arg Trp
785                 790                 795                 800

Asp Gly Lys Asp Lys Asp Gly Lys Val Val Ala Asn Gly Thr Tyr Thr
                805                 810                 815

Tyr Arg Val Arg Tyr Thr Pro Ile Ser Ser Gly Ala Lys Glu Gln His
            820                 825                 830

Thr Asp Phe Asp Val Ile Val Asp Asn Thr Thr Pro Glu Val Ala Thr
        835                 840                 845

Ser Ala Thr Phe Ser Thr Glu Asp Arg Arg Leu Thr Leu Ala Ser Lys
    850                 855                 860

Pro Lys Thr Ser Gln Pro Val Tyr Arg Glu Arg Ile Ala Tyr Thr Tyr
865                 870                 875                 880

Met Asp Glu Asp Leu Pro Thr Thr Glu Tyr Ile Ser Pro Asn Glu Asp
                885                 890                 895

Gly Thr Phe Thr Leu Pro Glu Glu Ala Glu Thr Met Glu Gly Ala Thr
            900                 905                 910

Val Pro Leu Lys Met Ser Asp Phe Thr Tyr Val Val Glu Asp Met Ala
        915                 920                 925

Gly Asn Ile Thr Tyr Thr Pro Val Thr Lys Leu Leu Glu Gly His Ser
    930                 935                 940

Asn Lys
945


<210>  30
<211>  946
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  C5a [90-1035 fragment] D131A/S513A w/o leader, with G overhang
       from cleavage

<400>  30

Gly Lys Thr Ala Asp Thr Pro Val Thr Ser Lys Ala Thr Ile Arg Asp
1               5                   10                  15

Leu Asn Asp Pro Ser Gln Val Lys Thr Leu Gln Glu Lys Ala Gly Lys
            20                  25                  30

Gly Ala Gly Thr Val Val Ala Val Ile Ala Ala Gly Phe Asp Lys Asn
        35                  40                  45
```

```
His Glu Ala Trp Arg Leu Thr Asp Lys Thr Lys Ala Arg Tyr Gln Ser
    50                  55                  60

Lys Glu Asp Leu Glu Lys Ala Lys Lys Glu His Gly Ile Thr Tyr Gly
65                  70                  75                  80

Glu Trp Val Asn Asp Lys Val Ala Tyr Tyr His Asp Tyr Ser Lys Asp
                85                  90                  95

Gly Lys Thr Ala Val Asp Gln Glu His Gly Thr His Val Ser Gly Ile
            100                 105                 110

Leu Ser Gly Asn Ala Pro Ser Glu Thr Lys Glu Pro Tyr Arg Leu Glu
        115                 120                 125

Gly Ala Met Pro Glu Ala Gln Leu Leu Leu Met Arg Val Glu Ile Val
    130                 135                 140

Asn Gly Leu Ala Asp Tyr Ala Arg Asn Tyr Ala Gln Ala Ile Arg Asp
145                 150                 155                 160

Ala Val Asn Leu Gly Ala Lys Val Ile Asn Met Ser Phe Gly Asn Ala
                165                 170                 175

Ala Leu Ala Tyr Ala Asn Leu Pro Asp Glu Thr Lys Lys Ala Phe Asp
            180                 185                 190

Tyr Ala Lys Ser Lys Gly Val Ser Ile Val Thr Ser Ala Gly Asn Asp
        195                 200                 205

Ser Ser Phe Gly Gly Lys Thr Arg Leu Pro Leu Ala Asp His Pro Asp
    210                 215                 220

Tyr Gly Val Val Gly Thr Pro Ala Ala Ala Asp Ser Thr Leu Thr Val
225                 230                 235                 240

Ala Ser Tyr Ser Pro Asp Lys Gln Leu Thr Glu Thr Ala Thr Val Lys
                245                 250                 255

Thr Ala Asp Lys Gln Asp Lys Glu Met Pro Val Leu Ser Thr Asn Arg
            260                 265                 270

Phe Glu Pro Asn Lys Ala Tyr Asp Tyr Ala Tyr Ala Asn Arg Gly Met
        275                 280                 285

Lys Glu Asp Asp Phe Lys Asp Val Lys Gly Lys Ile Ala Leu Ile Glu
    290                 295                 300

Arg Gly Asp Ile Asp Phe Lys Asp Lys Ile Ala Asn Ala Lys Lys Ala
305                 310                 315                 320
```

```
Gly Ala Val Gly Val Leu Ile Tyr Asp Asn Gln Asp Lys Gly Phe Pro
                325                 330                 335

Ile Glu Leu Pro Asn Val Asp Gln Met Pro Ala Ala Phe Ile Ser Arg
            340                 345                 350

Lys Asp Gly Leu Leu Leu Lys Asp Asn Pro Lys Lys Thr Ile Thr Phe
        355                 360                 365

Asn Ala Thr Pro Lys Val Leu Pro Thr Ala Ser Gly Thr Lys Leu Ser
    370                 375                 380

Arg Phe Ser Ser Trp Gly Leu Thr Ala Asp Gly Asn Ile Lys Pro Asp
385                 390                 395                 400

Ile Ala Ala Pro Gly Gln Asp Ile Leu Ser Ser Val Ala Asn Asn Lys
                405                 410                 415

Tyr Ala Lys Leu Ser Gly Thr Ala Met Ser Ala Pro Leu Val Ala Gly
            420                 425                 430

Ile Met Gly Leu Leu Gln Lys Gln Tyr Glu Thr Gln Tyr Pro Asp Met
        435                 440                 445

Thr Pro Ser Glu Arg Leu Asp Leu Ala Lys Lys Val Leu Met Ser Ser
    450                 455                 460

Ala Thr Ala Leu Tyr Asp Glu Asp Glu Lys Ala Tyr Phe Ser Pro Arg
465                 470                 475                 480

Gln Gln Gly Ala Gly Ala Val Asp Ala Lys Lys Ala Ser Ala Ala Thr
                485                 490                 495

Met Tyr Val Thr Asp Lys Asp Asn Thr Ser Ser Lys Val His Leu Asn
            500                 505                 510

Asn Val Ser Asp Thr Phe Glu Val Thr Val Thr Val His Asn Lys Ser
        515                 520                 525

Asp Lys Pro Gln Glu Leu Tyr Tyr Gln Ala Thr Val Gln Thr Asp Lys
    530                 535                 540

Val Asp Gly Lys His Phe Ala Leu Ala Pro Lys Ala Leu Tyr Glu Thr
545                 550                 555                 560

Ser Trp Gln Lys Ile Thr Ile Pro Ala Asn Ser Ser Lys Gln Val Thr
                565                 570                 575

Val Pro Ile Asp Ala Ser Arg Phe Ser Lys Asp Leu Leu Ala Gln Met
            580                 585                 590

Lys Asn Gly Tyr Phe Leu Glu Gly Phe Val Arg Phe Lys Gln Asp Pro
```

```
        595                 600                 605

Thr Lys Glu Glu Leu Met Ser Ile Pro Tyr Ile Gly Phe Arg Gly Asp
    610                 615                 620

Phe Gly Asn Leu Ser Ala Leu Glu Lys Pro Ile Tyr Asp Ser Lys Asp
625                 630                 635                 640

Gly Ser Ser Tyr Tyr His Glu Ala Asn Ser Asp Ala Lys Asp Gln Leu
                645                 650                 655

Asp Gly Asp Gly Leu Gln Phe Tyr Ala Leu Lys Asn Asn Phe Thr Ala
            660                 665                 670

Leu Thr Thr Glu Ser Asn Pro Trp Thr Ile Ile Lys Ala Val Lys Glu
        675                 680                 685

Gly Val Glu Asn Ile Glu Asp Ile Glu Ser Ser Glu Ile Thr Glu Thr
    690                 695                 700

Ile Phe Ala Gly Thr Phe Ala Lys Gln Asp Asp Asp Ser His Tyr Tyr
705                 710                 715                 720

Ile His Arg His Ala Asn Gly Lys Pro Tyr Ala Ala Ile Ser Pro Asn
                725                 730                 735

Gly Asp Gly Asn Arg Asp Tyr Val Gln Phe Gln Gly Thr Phe Leu Arg
            740                 745                 750

Asn Ala Lys Asn Leu Val Ala Glu Val Leu Asp Lys Glu Gly Asn Val
        755                 760                 765

Val Trp Thr Ser Glu Val Thr Glu Gln Val Val Lys Asn Tyr Asn Asn
    770                 775                 780

Asp Leu Ala Ser Thr Leu Gly Ser Thr Arg Phe Glu Lys Thr Arg Trp
785                 790                 795                 800

Asp Gly Lys Asp Lys Asp Gly Lys Val Val Ala Asn Gly Thr Tyr Thr
                805                 810                 815

Tyr Arg Val Arg Tyr Thr Pro Ile Ser Ser Gly Ala Lys Glu Gln His
            820                 825                 830

Thr Asp Phe Asp Val Ile Val Asp Asn Thr Thr Pro Glu Val Ala Thr
        835                 840                 845

Ser Ala Thr Phe Ser Thr Glu Asp Arg Arg Leu Thr Leu Ala Ser Lys
    850                 855                 860

Pro Lys Thr Ser Gln Pro Val Tyr Arg Glu Arg Ile Ala Tyr Thr Tyr
865                 870                 875                 880
```

```
Met Asp Glu Asp Leu Pro Thr Thr Glu Tyr Ile Ser Pro Asn Glu Asp
                885                 890                 895


Gly Thr Phe Thr Leu Pro Glu Glu Ala Glu Thr Met Glu Gly Ala Thr
            900                 905                 910


Val Pro Leu Lys Met Ser Asp Phe Thr Tyr Val Val Glu Asp Met Ala
        915                 920                 925


Gly Asn Ile Thr Tyr Thr Pro Val Thr Lys Leu Leu Glu Gly His Ser
    930                 935                 940


Asn Lys
945
```

<210> 31
<211> 494
<212> PRT
<213> Artificial Sequence

<220>
<223> SLO [79-571 fragment] WT w/o leader, with G overhang from cleavage

<400> 31

```
Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
1               5                   10                  15


Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
            20                  25                  30


Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
        35                  40                  45


Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
    50                  55                  60


Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn
65                  70                  75                  80


Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
                85                  90                  95


Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys
            100                 105                 110


Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
        115                 120                 125


Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
    130                 135                 140
```

```
Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
145                 150                 155                 160

Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
                165                 170                 175

Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
            180                 185                 190

Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
        195                 200                 205

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
    210                 215                 220

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
225                 230                 235                 240

Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
                245                 250                 255

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
            260                 265                 270

Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe
        275                 280                 285

Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser
    290                 295                 300

Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
305                 310                 315                 320

Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg
                325                 330                 335

Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
            340                 345                 350

Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
        355                 360                 365

Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
    370                 375                 380

Ser Gly Lys Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln Tyr
385                 390                 395                 400

Glu Ile Leu Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu Val
                405                 410                 415

Ile Thr Lys Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser Pro
```

```
            420                 425                 430

Phe Ser Thr Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg Ile
        435                 440                 445

Met Ala Arg Glu Cys Thr Gly Leu Ala Trp Glu Trp Trp Arg Lys Val
    450                 455                 460

Ile Asp Glu Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn Ile
465                 470                 475                 480

Ser Gly Ser Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys
                485                 490
```

<210> 32
<211> 494
<212> PRT
<213> Artificial Sequence

<220>
<223> SLO [79-571 fragment] W535A w/o leader, with G overhang from cleavage

<400> 32

```
Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
1               5                   10                  15

Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
            20                  25                  30

Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
        35                  40                  45

Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
    50                  55                  60

Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn
65                  70                  75                  80

Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
                85                  90                  95

Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys
            100                 105                 110

Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
        115                 120                 125

Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
    130                 135                 140

Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
145                 150                 155                 160
```

```
Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
                165                 170                 175

Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
            180                 185                 190

Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
        195                 200                 205

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
    210                 215                 220

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
225                 230                 235                 240

Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
                245                 250                 255

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
            260                 265                 270

Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe
        275                 280                 285

Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser
    290                 295                 300

Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
305                 310                 315                 320

Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg
                325                 330                 335

Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
            340                 345                 350

Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
        355                 360                 365

Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
    370                 375                 380

Ser Gly Lys Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln Tyr
385                 390                 395                 400

Glu Ile Leu Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu Val
                405                 410                 415

Ile Thr Lys Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser Pro
            420                 425                 430
```

Phe Ser Thr Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg Ile
        435   440   445

Met Ala Arg Glu Cys Thr Gly Leu Ala Ala Glu Trp Trp Arg Lys Val
    450   455   460

Ile Asp Glu Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn Ile
465   470   475   480

Ser Gly Ser Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys
                485   490

<210> 33
<211> 818
<212> PRT
<213> Artificial Sequence

<220>
<223> SpyAD [33-849 fragment] WT w/o leader, with G overhang from
       cleavage

<400> 33

Gly Gln Val Lys Ala Asp Asp Arg Ala Ser Gly Glu Thr Lys Ala Ser
1   5   10   15

Asn Thr His Asp Asp Ser Leu Pro Lys Pro Glu Thr Ile Gln Glu Ala
            20   25   30

Lys Ala Thr Ile Asp Ala Val Glu Lys Thr Leu Ser Gln Gln Lys Ala
        35   40   45

Glu Leu Thr Glu Leu Ala Thr Ala Leu Thr Lys Thr Thr Ala Glu Ile
    50   55   60

Asn His Leu Lys Glu Gln Gln Asp Asn Glu Gln Lys Ala Leu Thr Ser
65   70   75   80

Ala Gln Glu Ile Tyr Thr Asn Thr Leu Ala Ser Ser Glu Glu Thr Leu
                85   90   95

Leu Ala Gln Gly Ala Glu His Gln Arg Glu Leu Thr Ala Thr Glu Thr
            100   105   110

Glu Leu His Asn Ala Gln Ala Asp Gln His Ser Lys Glu Thr Ala Leu
        115   120   125

Ser Glu Gln Lys Ala Ser Ile Ser Ala Glu Thr Thr Arg Ala Gln Asp
    130   135   140

Leu Val Glu Gln Val Lys Thr Ser Glu Gln Asn Ile Ala Lys Leu Asn
145   150   155   160

Ala Met Ile Ser Asn Pro Asp Ala Ile Thr Lys Ala Ala Gln Thr Ala
                165   170   175

```
Asn Asp Asn Thr Lys Ala Leu Ser Ser Glu Leu Glu Lys Ala Lys Ala
            180                 185                 190

Asp Leu Glu Asn Gln Lys Ala Lys Val Lys Lys Gln Leu Thr Glu Glu
        195                 200                 205

Leu Ala Ala Gln Lys Ala Ala Leu Ala Glu Lys Glu Ala Glu Leu Ser
    210                 215                 220

Arg Leu Lys Ser Ser Ala Pro Ser Thr Gln Asp Ser Ile Val Gly Asn
225                 230                 235                 240

Asn Thr Met Lys Ala Pro Gln Gly Tyr Pro Leu Glu Glu Leu Lys Lys
                245                 250                 255

Leu Glu Ala Ser Gly Tyr Ile Gly Ser Ala Ser Tyr Asn Asn Tyr Tyr
            260                 265                 270

Lys Glu His Ala Asp Gln Ile Ile Ala Lys Ala Ser Pro Gly Asn Gln
        275                 280                 285

Leu Asn Gln Tyr Gln Asp Ile Pro Ala Asp Arg Asn Arg Phe Val Asp
    290                 295                 300

Pro Asp Asn Leu Thr Pro Glu Val Gln Asn Glu Leu Ala Gln Phe Ala
305                 310                 315                 320

Ala His Met Ile Asn Ser Val Arg Arg Gln Leu Gly Leu Pro Pro Val
                325                 330                 335

Thr Val Thr Ala Gly Ser Gln Glu Phe Ala Arg Leu Leu Ser Thr Ser
            340                 345                 350

Tyr Lys Lys Thr His Gly Asn Thr Arg Pro Ser Phe Val Tyr Gly Gln
        355                 360                 365

Pro Gly Val Ser Gly His Tyr Gly Val Gly Pro His Asp Lys Thr Ile
    370                 375                 380

Ile Glu Asp Ser Ala Gly Ala Ser Gly Leu Ile Arg Asn Asp Asp Asn
385                 390                 395                 400

Met Tyr Glu Asn Ile Gly Ala Phe Asn Asp Val His Thr Val Asn Gly
                405                 410                 415

Ile Lys Arg Gly Ile Tyr Asp Ser Ile Lys Tyr Met Leu Phe Thr Asp
            420                 425                 430

His Leu His Gly Asn Thr Tyr Gly His Ala Ile Asn Phe Leu Arg Val
        435                 440                 445
```

```
Asp Lys His Asn Pro Asn Ala Pro Val Tyr Leu Gly Phe Ser Thr Ser
    450                 455                 460

Asn Val Gly Ser Leu Asn Glu His Phe Val Met Phe Pro Glu Ser Asn
465                 470                 475                 480

Ile Ala Asn His Gln Arg Phe Asn Lys Thr Pro Ile Lys Ala Val Gly
                485                 490                 495

Ser Thr Lys Asp Tyr Ala Gln Arg Val Gly Thr Val Ser Asp Thr Ile
            500                 505                 510

Ala Ala Ile Lys Gly Lys Val Ser Ser Leu Glu Asn Arg Leu Ser Ala
        515                 520                 525

Ile His Gln Glu Ala Asp Ile Met Ala Ala Gln Ala Lys Val Ser Gln
    530                 535                 540

Leu Gln Gly Lys Leu Ala Ser Thr Leu Lys Gln Ser Asp Ser Leu Asn
545                 550                 555                 560

Leu Gln Val Arg Gln Leu Asn Asp Thr Lys Gly Ser Leu Arg Thr Glu
                565                 570                 575

Leu Leu Ala Ala Lys Ala Lys Gln Ala Gln Leu Glu Ala Thr Arg Asp
            580                 585                 590

Gln Ser Leu Ala Lys Leu Ala Ser Leu Lys Ala Ala Leu His Gln Thr
        595                 600                 605

Glu Ala Leu Ala Glu Gln Ala Ala Ala Arg Val Thr Ala Leu Val Ala
    610                 615                 620

Lys Lys Ala His Leu Gln Tyr Leu Arg Asp Phe Lys Leu Asn Pro Asn
625                 630                 635                 640

Arg Leu Gln Val Ile Arg Glu Arg Ile Asp Asn Thr Lys Gln Asp Leu
                645                 650                 655

Ala Lys Thr Thr Ser Ser Leu Leu Asn Ala Gln Glu Ala Leu Ala Ala
            660                 665                 670

Leu Gln Ala Lys Gln Ser Ser Leu Glu Ala Thr Ile Ala Thr Thr Glu
        675                 680                 685

His Gln Leu Thr Leu Leu Lys Thr Leu Ala Asn Glu Lys Glu Tyr Arg
    690                 695                 700

His Leu Asp Glu Asp Ile Ala Thr Val Pro Asp Leu Gln Val Ala Pro
705                 710                 715                 720
```

Pro Leu Thr Gly Val Lys Pro Leu Ser Tyr Ser Lys Ile Asp Thr Thr
725 730 735

Pro Leu Val Gln Glu Met Val Lys Glu Thr Lys Gln Leu Leu Glu Ala
740 745 750

Ser Ala Arg Leu Ala Ala Glu Asn Thr Ser Leu Val Ala Glu Ala Leu
755 760 765

Val Gly Gln Thr Ser Glu Met Val Ala Ser Asn Ala Ile Val Ser Lys
770 775 780

Ile Thr Ser Ser Ile Thr Gln Pro Ser Ser Lys Thr Ser Tyr Gly Ser
785 790 795 800

Gly Ser Ser Thr Thr Ser Asn Leu Ile Ser Asp Val Asp Glu Ser Thr
805 810 815

Gln Arg

<210> 34
<211> 818
<212> PRT
<213> Artificial Sequence

<220>
<223> SpyAD [33-849 fragment] nnAA (e.g. pAMF) mutant w/o leader, with G overhang from cleavage

<220>
<221> MOD_RES
<222> (33)..(33)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (256)..(256)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (355)..(355)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (626)..(626)
<223> Xaa = nnAA (e.g pAMF)

<400> 34

Gly Gln Val Lys Ala Asp Asp Arg Ala Ser Gly Glu Thr Lys Ala Ser
1 5 10 15

Asn Thr His Asp Asp Ser Leu Pro Lys Pro Glu Thr Ile Gln Glu Ala
20 25 30

Xaa Ala Thr Ile Asp Ala Val Glu Lys Thr Leu Ser Gln Gln Lys Ala

```
        35                  40                  45

Glu Leu Thr Glu Leu Ala Thr Ala Leu Thr Lys Thr Thr Ala Glu Ile
    50                  55                  60

Asn His Leu Lys Glu Gln Gln Asp Asn Glu Gln Lys Ala Leu Thr Ser
65                  70                  75                  80

Ala Gln Glu Ile Tyr Thr Asn Thr Leu Ala Ser Ser Glu Glu Thr Leu
                85                  90                  95

Leu Ala Gln Gly Ala Glu His Gln Arg Glu Leu Thr Ala Thr Glu Thr
            100                 105                 110

Glu Leu His Asn Ala Gln Ala Asp Gln His Ser Lys Glu Thr Ala Leu
        115                 120                 125

Ser Glu Gln Lys Ala Ser Ile Ser Ala Glu Thr Thr Arg Ala Gln Asp
    130                 135                 140

Leu Val Glu Gln Val Lys Thr Ser Glu Gln Asn Ile Ala Lys Leu Asn
145                 150                 155                 160

Ala Met Ile Ser Asn Pro Asp Ala Ile Thr Lys Ala Ala Gln Thr Ala
                165                 170                 175

Asn Asp Asn Thr Lys Ala Leu Ser Ser Glu Leu Glu Lys Ala Lys Ala
            180                 185                 190

Asp Leu Glu Asn Gln Lys Ala Lys Val Lys Lys Gln Leu Thr Glu Glu
        195                 200                 205

Leu Ala Ala Gln Lys Ala Ala Leu Ala Glu Lys Glu Ala Glu Leu Ser
    210                 215                 220

Arg Leu Lys Ser Ser Ala Pro Ser Thr Gln Asp Ser Ile Val Gly Asn
225                 230                 235                 240

Asn Thr Met Lys Ala Pro Gln Gly Tyr Pro Leu Glu Glu Leu Lys Xaa
                245                 250                 255

Leu Glu Ala Ser Gly Tyr Ile Gly Ser Ala Ser Tyr Asn Asn Tyr Tyr
            260                 265                 270

Lys Glu His Ala Asp Gln Ile Ile Ala Lys Ala Ser Pro Gly Asn Gln
        275                 280                 285

Leu Asn Gln Tyr Gln Asp Ile Pro Ala Asp Arg Asn Arg Phe Val Asp
    290                 295                 300

Pro Asp Asn Leu Thr Pro Glu Val Gln Asn Glu Leu Ala Gln Phe Ala
305                 310                 315                 320
```

```
Ala His Met Ile Asn Ser Val Arg Arg Gln Leu Gly Leu Pro Pro Val
                325                 330                 335

Thr Val Thr Ala Gly Ser Gln Glu Phe Ala Arg Leu Leu Ser Thr Ser
            340                 345                 350

Tyr Lys Xaa Thr His Gly Asn Thr Arg Pro Ser Phe Val Tyr Gly Gln
        355                 360                 365

Pro Gly Val Ser Gly His Tyr Gly Val Gly Pro His Asp Lys Thr Ile
    370                 375                 380

Ile Glu Asp Ser Ala Gly Ala Ser Gly Leu Ile Arg Asn Asp Asp Asn
385                 390                 395                 400

Met Tyr Glu Asn Ile Gly Ala Phe Asn Asp Val His Thr Val Asn Gly
                405                 410                 415

Ile Lys Arg Gly Ile Tyr Asp Ser Ile Lys Tyr Met Leu Phe Thr Asp
            420                 425                 430

His Leu His Gly Asn Thr Tyr Gly His Ala Ile Asn Phe Leu Arg Val
        435                 440                 445

Asp Lys His Asn Pro Asn Ala Pro Val Tyr Leu Gly Phe Ser Thr Ser
    450                 455                 460

Asn Val Gly Ser Leu Asn Glu His Phe Val Met Phe Pro Glu Ser Asn
465                 470                 475                 480

Ile Ala Asn His Gln Arg Phe Asn Lys Thr Pro Ile Lys Ala Val Gly
                485                 490                 495

Ser Thr Lys Asp Tyr Ala Gln Arg Val Gly Thr Val Ser Asp Thr Ile
            500                 505                 510

Ala Ala Ile Lys Gly Lys Val Ser Ser Leu Glu Asn Arg Leu Ser Ala
        515                 520                 525

Ile His Gln Glu Ala Asp Ile Met Ala Ala Gln Ala Lys Val Ser Gln
    530                 535                 540

Leu Gln Gly Lys Leu Ala Ser Thr Leu Lys Gln Ser Asp Ser Leu Asn
545                 550                 555                 560

Leu Gln Val Arg Gln Leu Asn Asp Thr Lys Gly Ser Leu Arg Thr Glu
                565                 570                 575

Leu Leu Ala Ala Lys Ala Lys Gln Ala Gln Leu Glu Ala Thr Arg Asp
            580                 585                 590
```

```
Gln Ser Leu Ala Lys Leu Ala Ser Leu Lys Ala Ala Leu His Gln Thr
        595                 600                 605

Glu Ala Leu Ala Glu Gln Ala Ala Ala Arg Val Thr Ala Leu Val Ala
    610                 615                 620

Lys Xaa Ala His Leu Gln Tyr Leu Arg Asp Phe Lys Leu Asn Pro Asn
625                 630                 635                 640

Arg Leu Gln Val Ile Arg Glu Arg Ile Asp Asn Thr Lys Gln Asp Leu
                645                 650                 655

Ala Lys Thr Thr Ser Ser Leu Leu Asn Ala Gln Glu Ala Leu Ala Ala
            660                 665                 670

Leu Gln Ala Lys Gln Ser Ser Leu Glu Ala Thr Ile Ala Thr Thr Glu
        675                 680                 685

His Gln Leu Thr Leu Leu Lys Thr Leu Ala Asn Glu Lys Glu Tyr Arg
    690                 695                 700

His Leu Asp Glu Asp Ile Ala Thr Val Pro Asp Leu Gln Val Ala Pro
705                 710                 715                 720

Pro Leu Thr Gly Val Lys Pro Leu Ser Tyr Ser Lys Ile Asp Thr Thr
                725                 730                 735

Pro Leu Val Gln Glu Met Val Lys Glu Thr Lys Gln Leu Leu Glu Ala
            740                 745                 750

Ser Ala Arg Leu Ala Ala Glu Asn Thr Ser Leu Val Ala Glu Ala Leu
        755                 760                 765

Val Gly Gln Thr Ser Glu Met Val Ala Ser Asn Ala Ile Val Ser Lys
    770                 775                 780

Ile Thr Ser Ser Ile Thr Gln Pro Ser Ser Lys Thr Ser Tyr Gly Ser
785                 790                 795                 800

Gly Ser Ser Thr Thr Ser Asn Leu Ile Ser Asp Val Asp Glu Ser Thr
                805                 810                 815

Gln Arg
```

<210> 35
<211> 411
<212> PRT
<213> Artificial Sequence

<220>
<223> ADI [full length] WT w/o leader, with G overhang from cleavage

<400> 35

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

Lys Lys Val Leu Leu His Arg Pro Gly Lys Glu Ile Glu Asn Leu Met
            20                  25                  30

Pro Asp Tyr Leu Glu Arg Leu Leu Phe Asp Asp Ile Pro Phe Leu Glu
        35                  40                  45

Asp Ala Gln Lys Glu His Asp Ala Phe Ala Gln Ala Leu Arg Asp Glu
    50                  55                  60

Gly Ile Glu Val Leu Tyr Leu Glu Thr Leu Ala Ala Glu Ser Leu Val
65                  70                  75                  80

Thr Pro Glu Ile Arg Glu Ala Phe Ile Asp Glu Tyr Leu Ser Glu Ala
                85                  90                  95

Asn Ile Arg Gly Arg Ala Thr Lys Lys Ala Ile Arg Glu Leu Leu Met
            100                 105                 110

Ala Ile Glu Asp Asn Gln Glu Leu Ile Glu Lys Thr Met Ala Gly Val
        115                 120                 125

Gln Lys Ser Glu Leu Pro Glu Ile Pro Ala Ser Glu Lys Gly Leu Thr
    130                 135                 140

Asp Leu Val Glu Ser Asn Tyr Pro Phe Ala Ile Asp Pro Met Pro Asn
145                 150                 155                 160

Leu Tyr Phe Thr Arg Asp Pro Phe Ala Thr Ile Gly Thr Gly Val Ser
                165                 170                 175

Leu Asn His Met Phe Ser Glu Thr Arg Asn Arg Glu Thr Leu Tyr Gly
            180                 185                 190

Lys Tyr Ile Phe Thr His His Pro Ile Tyr Gly Gly Gly Lys Val Pro
        195                 200                 205

Met Val Tyr Asp Arg Asn Glu Thr Thr Arg Ile Glu Gly Gly Asp Glu
    210                 215                 220

Leu Val Leu Ser Lys Asp Val Leu Ala Val Gly Ile Ser Gln Arg Thr
225                 230                 235                 240

Asp Ala Ala Ser Ile Glu Lys Leu Leu Val Asn Ile Phe Lys Gln Asn
                245                 250                 255

Leu Gly Phe Lys Lys Val Leu Ala Phe Glu Phe Ala Asn Asn Arg Lys
            260                 265                 270
```

```
Phe Met His Leu Asp Thr Val Phe Thr Met Val Asp Tyr Asp Lys Phe
        275                 280                 285

Thr Ile His Pro Glu Ile Glu Gly Asp Leu Arg Val Tyr Ser Val Thr
    290                 295                 300

Tyr Asp Asn Glu Glu Leu His Ile Val Glu Glu Lys Gly Asp Leu Ala
305                 310                 315                 320

Glu Leu Leu Ala Ala Asn Leu Gly Val Glu Lys Val Asp Leu Ile Arg
                325                 330                 335

Cys Gly Gly Asp Asn Leu Val Ala Ala Gly Arg Glu Gln Trp Asn Asp
            340                 345                 350

Gly Ser Asn Thr Leu Thr Ile Ala Pro Gly Val Val Val Val Tyr Asn
        355                 360                 365

Arg Asn Thr Ile Thr Asn Ala Ile Leu Glu Ser Lys Gly Leu Lys Leu
    370                 375                 380

Ile Lys Ile His Gly Ser Glu Leu Val Arg Gly Arg Gly Gly Pro Arg
385                 390                 395                 400

Cys Met Ser Met Pro Phe Glu Arg Glu Asp Ile
                405                 410
```

<210> 36
<211> 411
<212> PRT
<213> Artificial Sequence

<220>
<223> DI [full length] D277A w/o leader, with G overhang from cleavage

<400> 36

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

Lys Lys Val Leu Leu His Arg Pro Gly Lys Glu Ile Glu Asn Leu Met
            20                  25                  30

Pro Asp Tyr Leu Glu Arg Leu Leu Phe Asp Asp Ile Pro Phe Leu Glu
        35                  40                  45

Asp Ala Gln Lys Glu His Asp Ala Phe Ala Gln Ala Leu Arg Asp Glu
    50                  55                  60

Gly Ile Glu Val Leu Tyr Leu Glu Thr Leu Ala Ala Glu Ser Leu Val
65                  70                  75                  80

Thr Pro Glu Ile Arg Glu Ala Phe Ile Asp Glu Tyr Leu Ser Glu Ala
                85                  90                  95
```

```
Asn Ile Arg Gly Arg Ala Thr Lys Lys Ala Ile Arg Glu Leu Leu Met
            100                 105                 110

Ala Ile Glu Asp Asn Gln Glu Leu Ile Glu Lys Thr Met Ala Gly Val
        115                 120                 125

Gln Lys Ser Glu Leu Pro Glu Ile Pro Ala Ser Glu Lys Gly Leu Thr
    130                 135                 140

Asp Leu Val Glu Ser Asn Tyr Pro Phe Ala Ile Asp Pro Met Pro Asn
145                 150                 155                 160

Leu Tyr Phe Thr Arg Asp Pro Phe Ala Thr Ile Gly Thr Gly Val Ser
                165                 170                 175

Leu Asn His Met Phe Ser Glu Thr Arg Asn Arg Glu Thr Leu Tyr Gly
            180                 185                 190

Lys Tyr Ile Phe Thr His His Pro Ile Tyr Gly Gly Gly Lys Val Pro
        195                 200                 205

Met Val Tyr Asp Arg Asn Glu Thr Thr Arg Ile Glu Gly Gly Asp Glu
    210                 215                 220

Leu Val Leu Ser Lys Asp Val Leu Ala Val Gly Ile Ser Gln Arg Thr
225                 230                 235                 240

Asp Ala Ala Ser Ile Glu Lys Leu Leu Val Asn Ile Phe Lys Gln Asn
                245                 250                 255

Leu Gly Phe Lys Lys Val Leu Ala Phe Glu Phe Ala Asn Asn Arg Lys
            260                 265                 270

Phe Met His Leu Ala Thr Val Phe Thr Met Val Asp Tyr Asp Lys Phe
        275                 280                 285

Thr Ile His Pro Glu Ile Glu Gly Asp Leu Arg Val Tyr Ser Val Thr
    290                 295                 300

Tyr Asp Asn Glu Glu Leu His Ile Val Glu Glu Lys Gly Asp Leu Ala
305                 310                 315                 320

Glu Leu Leu Ala Ala Asn Leu Gly Val Glu Lys Val Asp Leu Ile Arg
                325                 330                 335

Cys Gly Gly Asp Asn Leu Val Ala Ala Gly Arg Glu Gln Trp Asn Asp
            340                 345                 350

Gly Ser Asn Thr Leu Thr Ile Ala Pro Gly Val Val Val Val Tyr Asn
        355                 360                 365
```

Arg Asn Thr Ile Thr Asn Ala Ile Leu Glu Ser Lys Gly Leu Lys Leu
    370   375   380

Ile Lys Ile His Gly Ser Glu Leu Val Arg Gly Arg Gly Gly Pro Arg
385   390   395   400

Cys Met Ser Met Pro Phe Glu Arg Glu Asp Ile
                405   410

<210> 37
<211> 411
<212> PRT
<213> Artificial Sequence

<220>
<223> ADI [full length] nnAA (e.g. pAMF) mutant w/o leader, with G
       overhang from cleavage

<220>
<221> MOD_RES
<222> (15)..(15)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (193)..(193)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (316)..(316)
<223> Xaa = nnAA (e.g pAMF)

<400> 37

Gly Thr Ala Gln Thr Pro Ile His Val Tyr Ser Glu Ile Gly Xaa Leu
1   5   10   15

Lys Lys Val Leu Leu His Arg Pro Gly Lys Glu Ile Glu Asn Leu Met
            20   25   30

Pro Asp Tyr Leu Glu Arg Leu Leu Phe Asp Asp Ile Pro Phe Leu Glu
        35   40   45

Asp Ala Gln Lys Glu His Asp Ala Phe Ala Gln Ala Leu Arg Asp Glu
    50   55   60

Gly Ile Glu Val Leu Tyr Leu Glu Thr Leu Ala Ala Glu Ser Leu Val
65   70   75   80

Thr Pro Glu Ile Arg Glu Ala Phe Ile Asp Glu Tyr Leu Ser Glu Ala
                85   90   95

Asn Ile Arg Gly Arg Ala Thr Lys Lys Ala Ile Arg Glu Leu Leu Met
            100   105   110

Ala Ile Glu Asp Asn Gln Glu Leu Ile Glu Lys Thr Met Ala Gly Val

```
        115                 120                 125

Gln Lys Ser Glu Leu Pro Glu Ile Pro Ala Ser Glu Lys Gly Leu Thr
    130                 135                 140

Asp Leu Val Glu Ser Asn Tyr Pro Phe Ala Ile Asp Pro Met Pro Asn
145                 150                 155                 160

Leu Tyr Phe Thr Arg Asp Pro Phe Ala Thr Ile Gly Thr Gly Val Ser
                165                 170                 175

Leu Asn His Met Phe Ser Glu Thr Arg Asn Arg Glu Thr Leu Tyr Gly
            180                 185                 190

Xaa Tyr Ile Phe Thr His His Pro Ile Tyr Gly Gly Gly Lys Val Pro
        195                 200                 205

Met Val Tyr Asp Arg Asn Glu Thr Thr Arg Ile Glu Gly Gly Asp Glu
    210                 215                 220

Leu Val Leu Ser Lys Asp Val Leu Ala Val Gly Ile Ser Gln Arg Thr
225                 230                 235                 240

Asp Ala Ala Ser Ile Glu Lys Leu Leu Val Asn Ile Phe Lys Gln Asn
                245                 250                 255

Leu Gly Phe Lys Lys Val Leu Ala Phe Glu Phe Ala Asn Asn Arg Lys
            260                 265                 270

Phe Met His Leu Asp Thr Val Phe Thr Met Val Asp Tyr Asp Lys Phe
        275                 280                 285

Thr Ile His Pro Glu Ile Glu Gly Asp Leu Arg Val Tyr Ser Val Thr
    290                 295                 300

Tyr Asp Asn Glu Glu Leu His Ile Val Glu Glu Xaa Gly Asp Leu Ala
305                 310                 315                 320

Glu Leu Leu Ala Ala Asn Leu Gly Val Glu Lys Val Asp Leu Ile Arg
                325                 330                 335

Cys Gly Gly Asp Asn Leu Val Ala Ala Gly Arg Glu Gln Trp Asn Asp
            340                 345                 350

Gly Ser Asn Thr Leu Thr Ile Ala Pro Gly Val Val Val Val Tyr Asn
        355                 360                 365

Arg Asn Thr Ile Thr Asn Ala Ile Leu Glu Ser Lys Gly Leu Lys Leu
    370                 375                 380

Ile Lys Ile His Gly Ser Glu Leu Val Arg Gly Arg Gly Gly Pro Arg
385                 390                 395                 400
```

Cys Met Ser Met Pro Phe Glu Arg Glu Asp Ile
405 410

<210> 38
<211> 411
<212> PRT
<213> Artificial Sequence

<220>
<223> ADI (mut5) [full length] D277A nnAA (e.g. pAMF) mutant w/o leader, with G overhang from cleavage

<220>
<221> MOD_RES
<222> (15)..(15)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (193)..(193)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (316)..(316)
<223> Xaa = nnAA (e.g pAMF)

<400> 38

Gly Thr Ala Gln Thr Pro Ile His Val Tyr Ser Glu Ile Gly Xaa Leu
1 5 10 15

Lys Lys Val Leu Leu His Arg Pro Gly Lys Glu Ile Glu Asn Leu Met
20 25 30

Pro Asp Tyr Leu Glu Arg Leu Leu Phe Asp Asp Ile Pro Phe Leu Glu
35 40 45

Asp Ala Gln Lys Glu His Asp Ala Phe Ala Gln Ala Leu Arg Asp Glu
50 55 60

Gly Ile Glu Val Leu Tyr Leu Glu Thr Leu Ala Ala Glu Ser Leu Val
65 70 75 80

Thr Pro Glu Ile Arg Glu Ala Phe Ile Asp Glu Tyr Leu Ser Glu Ala
85 90 95

Asn Ile Arg Gly Arg Ala Thr Lys Lys Ala Ile Arg Glu Leu Leu Met
100 105 110

Ala Ile Glu Asp Asn Gln Glu Leu Ile Glu Lys Thr Met Ala Gly Val
115 120 125

Gln Lys Ser Glu Leu Pro Glu Ile Pro Ala Ser Glu Lys Gly Leu Thr
130 135 140

```
Asp Leu Val Glu Ser Asn Tyr Pro Phe Ala Ile Asp Pro Met Pro Asn
145                 150                 155                 160

Leu Tyr Phe Thr Arg Asp Pro Phe Ala Thr Ile Gly Thr Gly Val Ser
                165                 170                 175

Leu Asn His Met Phe Ser Glu Thr Arg Asn Arg Glu Thr Leu Tyr Gly
            180                 185                 190

Xaa Tyr Ile Phe Thr His His Pro Ile Tyr Gly Gly Gly Lys Val Pro
        195                 200                 205

Met Val Tyr Asp Arg Asn Glu Thr Thr Arg Ile Glu Gly Gly Asp Glu
    210                 215                 220

Leu Val Leu Ser Lys Asp Val Leu Ala Val Gly Ile Ser Gln Arg Thr
225                 230                 235                 240

Asp Ala Ala Ser Ile Glu Lys Leu Leu Val Asn Ile Phe Lys Gln Asn
                245                 250                 255

Leu Gly Phe Lys Lys Val Leu Ala Phe Glu Phe Ala Asn Asn Arg Lys
            260                 265                 270

Phe Met His Leu Ala Thr Val Phe Thr Met Val Asp Tyr Asp Lys Phe
        275                 280                 285

Thr Ile His Pro Glu Ile Glu Gly Asp Leu Arg Val Tyr Ser Val Thr
    290                 295                 300

Tyr Asp Asn Glu Glu Leu His Ile Val Glu Glu Xaa Gly Asp Leu Ala
305                 310                 315                 320

Glu Leu Leu Ala Ala Asn Leu Gly Val Glu Lys Val Asp Leu Ile Arg
                325                 330                 335

Cys Gly Gly Asp Asn Leu Val Ala Ala Gly Arg Glu Gln Trp Asn Asp
            340                 345                 350

Gly Ser Asn Thr Leu Thr Ile Ala Pro Gly Val Val Val Val Tyr Asn
        355                 360                 365

Arg Asn Thr Ile Thr Asn Ala Ile Leu Glu Ser Lys Gly Leu Lys Leu
    370                 375                 380

Ile Lys Ile His Gly Ser Glu Leu Val Arg Gly Arg Gly Gly Pro Arg
385                 390                 395                 400

Cys Met Ser Met Pro Phe Glu Arg Glu Asp Ile
                405                 410
```

<210> 39

<211> 184
<212> PRT
<213> Artificial Sequence

<220>
<223> hFL [full length] WT w/ leader

<400> 39

Met His His His His His His Gly Ser Gly Ser Ser Gln Ile Arg Gln
1   5   10   15

Asn Tyr Ser Thr Asp Val Glu Ala Ala Val Asn Ser Leu Val Asn Leu
            20   25   30

Tyr Leu Gln Ala Ser Tyr Thr Tyr Leu Ser Leu Gly Phe Tyr Phe Asp
        35   40   45

Arg Asp Asp Val Ala Leu Glu Gly Val Ser His Phe Phe Arg Glu Leu
    50   55   60

Ala Glu Glu Lys Arg Glu Gly Tyr Glu Arg Leu Leu Lys Met Gln Asn
65   70   75   80

Gln Arg Gly Gly Arg Ala Leu Phe Gln Asp Ile Lys Lys Pro Ala Glu
                85   90   95

Asp Glu Trp Gly Lys Thr Pro Asp Ala Met Lys Ala Ala Met Ala Leu
            100   105   110

Glu Lys Lys Leu Asn Gln Ala Leu Leu Asp Leu His Ala Leu Gly Ser
        115   120   125

Ala Arg Thr Asp Pro His Leu Cys Asp Phe Leu Glu Thr His Phe Leu
    130   135   140

Asp Glu Glu Val Lys Leu Ile Lys Lys Met Gly Asp His Leu Thr Asn
145   150   155   160

Leu His Arg Leu Gly Gly Pro Glu Ala Gly Leu Gly Glu Tyr Leu Phe
                165   170   175

Glu Arg Leu Thr Leu Lys His Asp
            180

<210> 40
<211> 174
<212> PRT
<213> Homo sapiens

<400> 40

Ser Ser Gln Ile Arg Gln Asn Tyr Ser Thr Asp Val Glu Ala Ala Val
1   5   10   15

Asn Ser Leu Val Asn Leu Tyr Leu Gln Ala Ser Tyr Thr Tyr Leu Ser

```
            20                  25                  30

Leu Gly Phe Tyr Phe Asp Arg Asp Asp Val Ala Leu Glu Gly Val Ser
        35                  40                  45

His Phe Phe Arg Glu Leu Ala Glu Glu Lys Arg Glu Gly Tyr Glu Arg
    50                  55                  60

Leu Leu Lys Met Gln Asn Gln Arg Gly Gly Arg Ala Leu Phe Gln Asp
65                  70                  75                  80

Ile Lys Lys Pro Ala Glu Asp Glu Trp Gly Lys Thr Pro Asp Ala Met
                85                  90                  95

Lys Ala Ala Met Ala Leu Glu Lys Lys Leu Asn Gln Ala Leu Leu Asp
            100                 105                 110

Leu His Ala Leu Gly Ser Ala Arg Thr Asp Pro His Leu Cys Asp Phe
        115                 120                 125

Leu Glu Thr His Phe Leu Asp Glu Glu Val Lys Leu Ile Lys Lys Met
    130                 135                 140

Gly Asp His Leu Thr Asn Leu His Arg Leu Gly Gly Pro Glu Ala Gly
145                 150                 155                 160

Leu Gly Glu Tyr Leu Phe Glu Arg Leu Thr Leu Lys His Asp
                165                 170


<210>  41
<211>  184
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  hFL [full length] nnAA (e.g. pAMF) mutant w/ leader


<220>
<221>  MOD_RES
<222>  (14)..(14)
<223>  Xaa = nnAA (e.g pAMF)

<400>  41

Met His His His His His His Gly Ser Gly Ser Ser Gln Xaa Arg Gln
1               5                   10                  15

Asn Tyr Ser Thr Asp Val Glu Ala Ala Val Asn Ser Leu Val Asn Leu
            20                  25                  30

Tyr Leu Gln Ala Ser Tyr Thr Tyr Leu Ser Leu Gly Phe Tyr Phe Asp
        35                  40                  45

Arg Asp Asp Val Ala Leu Glu Gly Val Ser His Phe Phe Arg Glu Leu
    50                  55                  60
```

```
Ala Glu Glu Lys Arg Glu Gly Tyr Glu Arg Leu Leu Lys Met Gln Asn
65                  70                  75                  80


Gln Arg Gly Gly Arg Ala Leu Phe Gln Asp Ile Lys Lys Pro Ala Glu
                85                  90                  95


Asp Glu Trp Gly Lys Thr Pro Asp Ala Met Lys Ala Ala Met Ala Leu
            100                 105                 110


Glu Lys Lys Leu Asn Gln Ala Leu Leu Asp Leu His Ala Leu Gly Ser
        115                 120                 125


Ala Arg Thr Asp Pro His Leu Cys Asp Phe Leu Glu Thr His Phe Leu
    130                 135                 140


Asp Glu Glu Val Lys Leu Ile Lys Lys Met Gly Asp His Leu Thr Asn
145                 150                 155                 160


Leu His Arg Leu Gly Gly Pro Glu Ala Gly Leu Gly Glu Tyr Leu Phe
                165                 170                 175


Glu Arg Leu Thr Leu Lys His Asp
            180


<210>  42
<211>  174
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  hFL [full length] nnAA (e.g. pAMF) mutant w/o leader


<220>
<221>  MOD_RES
<222>  (4)..(4)
<223>  Xaa = nnAA (e.g pAMF)

<400>  42

Ser Ser Gln Xaa Arg Gln Asn Tyr Ser Thr Asp Val Glu Ala Ala Val
1               5                   10                  15


Asn Ser Leu Val Asn Leu Tyr Leu Gln Ala Ser Tyr Thr Tyr Leu Ser
            20                  25                  30


Leu Gly Phe Tyr Phe Asp Arg Asp Asp Val Ala Leu Glu Gly Val Ser
        35                  40                  45


His Phe Phe Arg Glu Leu Ala Glu Glu Lys Arg Glu Gly Tyr Glu Arg
    50                  55                  60


Leu Leu Lys Met Gln Asn Gln Arg Gly Gly Arg Ala Leu Phe Gln Asp
65                  70                  75                  80
```

```
Ile Lys Lys Pro Ala Glu Asp Glu Trp Gly Lys Thr Pro Asp Ala Met
                85                  90                  95


Lys Ala Ala Met Ala Leu Glu Lys Lys Leu Asn Gln Ala Leu Leu Asp
            100                 105                 110


Leu His Ala Leu Gly Ser Ala Arg Thr Asp Pro His Leu Cys Asp Phe
        115                 120                 125


Leu Glu Thr His Phe Leu Asp Glu Glu Val Lys Leu Ile Lys Lys Met
    130                 135                 140


Gly Asp His Leu Thr Asn Leu His Arg Leu Gly Gly Pro Glu Ala Gly
145                 150                 155                 160


Leu Gly Glu Tyr Leu Phe Glu Arg Leu Thr Leu Lys His Asp
                165                 170


<210>  43
<211>  535
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  eCRM-nnAA (e.g. pAMF) mutant


<220>
<221>  MOD_RES
<222>  (33)..(33)
<223>  Xaa = nnAA (e.g pAMF)

<220>
<221>  MOD_RES
<222>  (212)..(212)
<223>  Xaa = nnAA (e.g pAMF)

<220>
<221>  MOD_RES
<222>  (244)..(244)
<223>  Xaa = nnAA (e.g pAMF)

<220>
<221>  MOD_RES
<222>  (264)..(264)
<223>  Xaa = nnAA (e.g pAMF)

<220>
<221>  MOD_RES
<222>  (385)..(385)
<223>  Xaa = nnAA (e.g pAMF)

<220>
<221>  MOD_RES
<222>  (526)..(526)
<223>  Xaa = nnAA (e.g pAMF)

<400>  43

Gly Ala Asp Asp Val Val Asp Ser Ser Lys Ser Phe Val Met Glu Asn
1               5                   10                  15
```

```
Phe Ser Ser Tyr His Gly Thr Lys Pro Gly Tyr Val Asp Ser Ile Gln
            20                  25                  30

Xaa Gly Ile Gln Lys Pro Lys Ser Gly Thr Gln Gly Asn Tyr Asp Asp
        35                  40                  45

Asp Trp Lys Glu Phe Tyr Ser Thr Asp Asn Lys Tyr Asp Ala Ala Gly
    50                  55                  60

Tyr Ser Val Asp Asn Glu Asn Pro Leu Ser Gly Lys Ala Gly Gly Val
65                  70                  75                  80

Val Lys Val Thr Tyr Pro Gly Leu Thr Lys Val Leu Ala Leu Lys Val
                85                  90                  95

Asp Asn Ala Glu Thr Ile Lys Lys Glu Leu Gly Leu Ser Leu Thr Glu
            100                 105                 110

Pro Leu Met Glu Gln Val Gly Thr Glu Glu Phe Ile Lys Arg Phe Gly
        115                 120                 125

Asp Gly Ala Ser Arg Val Val Leu Ser Leu Pro Phe Ala Glu Gly Ser
    130                 135                 140

Ser Ser Val Glu Tyr Ile Asn Asn Trp Glu Gln Ala Lys Ala Leu Ser
145                 150                 155                 160

Val Glu Leu Glu Ile Asn Phe Glu Thr Arg Gly Lys Arg Gly Gln Asp
                165                 170                 175

Ala Met Tyr Glu Tyr Met Ala Gln Ala Cys Ala Gly Asn Arg Val Arg
            180                 185                 190

Asn Ser Val Gly Ser Ser Leu Ser Cys Ile Asn Leu Asp Trp Asp Val
        195                 200                 205

Ile Arg Asp Xaa Thr Lys Thr Lys Ile Glu Ser Leu Lys Glu His Gly
    210                 215                 220

Pro Ile Lys Asn Lys Met Ser Glu Ser Pro Asn Lys Thr Val Ser Glu
225                 230                 235                 240

Glu Lys Ala Xaa Gln Tyr Leu Glu Glu Phe His Gln Thr Ala Leu Glu
                245                 250                 255

His Pro Glu Leu Ser Glu Leu Xaa Thr Val Thr Gly Thr Asn Pro Val
            260                 265                 270

Phe Ala Gly Ala Asn Tyr Ala Ala Trp Ala Val Asn Val Ala Gln Val
        275                 280                 285
```

```
Ile Asp Ser Glu Thr Ala Asp Asn Leu Glu Lys Thr Thr Ala Ala Leu
    290                 295                 300

Ser Ile Leu Pro Gly Ile Gly Ser Val Met Gly Ile Ala Asp Gly Ala
305                 310                 315                 320

Val His His Asn Thr Glu Glu Ile Val Ala Gln Ser Ile Ala Leu Ser
                325                 330                 335

Ser Leu Met Val Ala Gln Ala Ile Pro Leu Val Gly Glu Leu Val Asp
            340                 345                 350

Ile Gly Phe Ala Ala Tyr Asn Phe Val Glu Ser Ile Ile Asn Leu Phe
        355                 360                 365

Gln Val Val His Asn Ser Tyr Asn Arg Pro Ala Tyr Ser Pro Gly His
    370                 375                 380

Xaa Thr Gln Pro Phe Leu His Asp Gly Tyr Ala Val Ser Trp Asn Thr
385                 390                 395                 400

Val Glu Asp Ser Ile Ile Arg Thr Gly Phe Gln Gly Glu Ser Gly His
                405                 410                 415

Asp Ile Lys Ile Thr Ala Glu Asn Thr Pro Leu Pro Ile Ala Gly Val
            420                 425                 430

Leu Leu Pro Thr Ile Pro Gly Lys Leu Asp Val Asn Lys Ser Lys Thr
        435                 440                 445

His Ile Ser Val Asn Gly Arg Lys Ile Arg Met Arg Cys Arg Ala Ile
    450                 455                 460

Asp Gly Asp Val Thr Phe Cys Arg Pro Lys Ser Pro Val Tyr Val Gly
465                 470                 475                 480

Asn Gly Val His Ala Asn Leu His Val Ala Phe His Arg Ser Ser Ser
                485                 490                 495

Glu Lys Ile His Ser Asn Glu Ile Ser Ser Asp Ser Ile Gly Val Leu
            500                 505                 510

Gly Tyr Gln Lys Thr Val Asp His Thr Lys Val Asn Ser Xaa Leu Ser
        515                 520                 525

Leu Phe Phe Glu Ile Lys Ser
    530                 535
```

<210> 44
<211> 483
<212> PRT
<213> Artificial Sequence

<220>
<223> SLO(deltaC11) W535A w/o leader, with G overhang from cleavage

<400> 44

Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
1   5   10   15

Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
            20   25   30

Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
        35   40   45

Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
    50   55   60

Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn
65   70   75   80

Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
                85   90   95

Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys
            100   105   110

Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
        115   120   125

Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
    130   135   140

Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
145   150   155   160

Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
                165   170   175

Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
            180   185   190

Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
        195   200   205

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
    210   215   220

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
225   230   235   240

Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
                245   250   255

```
Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
            260                 265                 270

Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe
        275                 280                 285

Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser
    290                 295                 300

Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
305                 310                 315                 320

Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg
                325                 330                 335

Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
            340                 345                 350

Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
        355                 360                 365

Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
    370                 375                 380

Ser Gly Lys Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln Tyr
385                 390                 395                 400

Glu Ile Leu Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu Val
                405                 410                 415

Ile Thr Lys Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser Pro
            420                 425                 430

Phe Ser Thr Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg Ile
        435                 440                 445

Met Ala Arg Glu Cys Thr Gly Leu Ala Ala Glu Trp Trp Arg Lys Val
    450                 455                 460

Ile Asp Glu Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn Ile
465                 470                 475                 480

Ser Gly Ser
```

<210> 45
<211> 473
<212> PRT
<213> Artificial Sequence

<220>
<223> SLO(deltaC21) fragment W535A w/o leader, with G overhang from cleavage

<400> 45

```
Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
1               5                   10                  15

Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
            20                  25                  30

Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
        35                  40                  45

Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
    50                  55                  60

Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn
65                  70                  75                  80

Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
                85                  90                  95

Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys
            100                 105                 110

Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
        115                 120                 125

Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
    130                 135                 140

Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
145                 150                 155                 160

Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
                165                 170                 175

Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
            180                 185                 190

Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
        195                 200                 205

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
    210                 215                 220

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
225                 230                 235                 240

Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
                245                 250                 255

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
            260                 265                 270
```

Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe
        275   280   285

Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser
    290   295   300

Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
305   310   315   320

Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg
                325   330   335

Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
            340   345   350

Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
        355   360   365

Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
    370   375   380

Ser Gly Lys Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln Tyr
385   390   395   400

Glu Ile Leu Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu Val
                405   410   415

Ile Thr Lys Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser Pro
            420   425   430

Phe Ser Thr Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg Ile
        435   440   445

Met Ala Arg Glu Cys Thr Gly Leu Ala Ala Glu Trp Trp Arg Lys Val
    450   455   460

Ile Asp Glu Arg Asp Val Lys Leu Ser
465   470

<210> 46
<211> 463
<212> PRT
<213> Artificial Sequence

<220>
<223> SLO(deltaC31) fragment W535A w/o leader, with G overhang from
       cleavage

<400> 46

Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
1   5   10   15

Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile

```
            20                  25                  30

Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
        35                  40                  45

Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
    50                  55                  60

Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn
65                  70                  75                  80

Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
                85                  90                  95

Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys
            100                 105                 110

Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
        115                 120                 125

Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
    130                 135                 140

Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
145                 150                 155                 160

Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
                165                 170                 175

Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
            180                 185                 190

Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
        195                 200                 205

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
    210                 215                 220

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
225                 230                 235                 240

Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
                245                 250                 255

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
            260                 265                 270

Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe
        275                 280                 285

Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser
    290                 295                 300
```

```
Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
305                 310                 315                 320


Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg
                325                 330                 335


Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
            340                 345                 350


Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
        355                 360                 365


Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
    370                 375                 380


Ser Gly Lys Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln Tyr
385                 390                 395                 400


Glu Ile Leu Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu Val
                405                 410                 415


Ile Thr Lys Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser Pro
            420                 425                 430


Phe Ser Thr Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg Ile
        435                 440                 445


Met Ala Arg Glu Cys Thr Gly Leu Ala Ala Glu Trp Trp Arg Lys
    450                 455                 460


<210>  47
<211>  453
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  SLO(deltaC41) fragment W535A w/o leader, with G overhang from
       cleavage

<400>  47

Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
1               5                   10                  15


Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
            20                  25                  30


Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
        35                  40                  45


Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
    50                  55                  60
```

```
Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn
65                  70                  75                  80

Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
                85                  90                  95

Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys
            100                 105                 110

Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
        115                 120                 125

Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
    130                 135                 140

Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
145                 150                 155                 160

Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
                165                 170                 175

Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
            180                 185                 190

Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
        195                 200                 205

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
    210                 215                 220

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
225                 230                 235                 240

Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
                245                 250                 255

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
            260                 265                 270

Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe
        275                 280                 285

Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser
    290                 295                 300

Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
305                 310                 315                 320

Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg
                325                 330                 335

Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
```

```
            340                 345                 350

Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
        355                 360                 365

Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
    370                 375                 380

Ser Gly Lys Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln Tyr
385                 390                 395                 400

Glu Ile Leu Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu Val
                405                 410                 415

Ile Thr Lys Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser Pro
            420                 425                 430

Phe Ser Thr Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg Ile
        435                 440                 445

Met Ala Arg Glu Cys
    450
```

<210> 48
<211> 443
<212> PRT
<213> Artificial Sequence

<220>
<223> SLO(deltaC51) fragment W535A w/o leader, with G overhang from cleavage

<400> 48

```
Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
1               5                   10                  15

Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
            20                  25                  30

Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
        35                  40                  45

Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
    50                  55                  60

Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn
65                  70                  75                  80

Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
                85                  90                  95

Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys
            100                 105                 110
```

```
Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
        115                 120                 125

Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
    130                 135                 140

Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
145                 150                 155                 160

Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
                165                 170                 175

Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
            180                 185                 190

Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
        195                 200                 205

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
    210                 215                 220

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
225                 230                 235                 240

Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
                245                 250                 255

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
            260                 265                 270

Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe
        275                 280                 285

Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser
    290                 295                 300

Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
305                 310                 315                 320

Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg
                325                 330                 335

Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
            340                 345                 350

Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
        355                 360                 365

Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
    370                 375                 380
```

Ser Gly Lys Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln Tyr
385 390 395 400

Glu Ile Leu Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu Val
405 410 415

Ile Thr Lys Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser Pro
420 425 430

Phe Ser Thr Val Ile Pro Leu Gly Ala Asn Ser
435 440

<210> 49
<211> 433
<212> PRT
<213> Artificial Sequence

<220>
<223> SLO(deltaC61) fragment W535A w/o leader, with G overhang from cleavage

<400> 49

Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
1 5 10 15

Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
20 25 30

Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
35 40 45

Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
50 55 60

Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn
65 70 75 80

Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
85 90 95

Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys
100 105 110

Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
115 120 125

Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
130 135 140

Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
145 150 155 160

Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
165 170 175

```
Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
            180                 185                 190

Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
        195                 200                 205

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
    210                 215                 220

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
225                 230                 235                 240

Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
                245                 250                 255

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
            260                 265                 270

Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe
        275                 280                 285

Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser
    290                 295                 300

Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
305                 310                 315                 320

Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg
                325                 330                 335

Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
            340                 345                 350

Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
        355                 360                 365

Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
    370                 375                 380

Ser Gly Lys Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln Tyr
385                 390                 395                 400

Glu Ile Leu Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu Val
                405                 410                 415

Ile Thr Lys Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser Pro
            420                 425                 430

Phe
```

<210> 50
<211> 423
<212> PRT
<213> Artificial Sequence

<220>
<223> SLO(deltaC71) fragment W535A w/o leader, with G overhang from cleavage

<400> 50

Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
1 5 10 15

Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
20 25 30

Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
35 40 45

Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
50 55 60

Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn
65 70 75 80

Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
85 90 95

Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys
100 105 110

Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
115 120 125

Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
130 135 140

Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
145 150 155 160

Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
165 170 175

Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
180 185 190

Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
195 200 205

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
210 215 220

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp

225 230 235 240

Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
245 250 255

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
260 265 270

Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe
275 280 285

Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser
290 295 300

Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
305 310 315 320

Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg
325 330 335

Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
340 345 350

Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
355 360 365

Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
370 375 380

Ser Gly Lys Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln Tyr
385 390 395 400

Glu Ile Leu Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu Val
405 410 415

Ile Thr Lys Arg Arg Trp Asp
420

<210> 51
<211> 413
<212> PRT
<213> Artificial Sequence

<220>
<223> SLO(deltaC81) fragment W535A w/o leader, with G overhang from cleavage

<400> 51

Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
1 5 10 15

Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
20 25 30

```
Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
        35                  40                  45

Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
    50                  55                  60

Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn
65                  70                  75                  80

Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
                85                  90                  95

Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys
            100                 105                 110

Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
        115                 120                 125

Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
    130                 135                 140

Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
145                 150                 155                 160

Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
                165                 170                 175

Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
            180                 185                 190

Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
        195                 200                 205

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
    210                 215                 220

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
225                 230                 235                 240

Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
                245                 250                 255

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
            260                 265                 270

Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe
        275                 280                 285

Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser
    290                 295                 300
```

```
Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
305                 310                 315                 320


Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg
                325                 330                 335


Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
            340                 345                 350


Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
        355                 360                 365


Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
    370                 375                 380


Ser Gly Lys Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln Tyr
385                 390                 395                 400


Glu Ile Leu Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly
                405                 410


<210>  52
<211>  403
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  SLO(deltaC91) fragment W535A w/o leader, with G overhang from
       cleavage

<400>  52

Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
1               5                   10                  15


Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
            20                  25                  30


Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
        35                  40                  45


Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
    50                  55                  60


Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn
65                  70                  75                  80


Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
                85                  90                  95


Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys
            100                 105                 110


Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
        115                 120                 125
```

```
Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
    130                 135                 140

Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
145                 150                 155                 160

Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
                165                 170                 175

Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
            180                 185                 190

Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
        195                 200                 205

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
    210                 215                 220

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
225                 230                 235                 240

Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
                245                 250                 255

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
            260                 265                 270

Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe
        275                 280                 285

Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser
    290                 295                 300

Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
305                 310                 315                 320

Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg
                325                 330                 335

Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
            340                 345                 350

Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
        355                 360                 365

Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
    370                 375                 380

Ser Gly Lys Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln Tyr
385                 390                 395                 400
```

Glu Ile Leu

<210> 53
<211> 393
<212> PRT
<213> Artificial Sequence

<220>
<223> SLO(deltaC101) fragment W535A w/o leader, with G overhang from cleavage

<400> 53

Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
1 5 10 15

Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
20 25 30

Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
35 40 45

Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
50 55 60

Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn
65 70 75 80

Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
85 90 95

Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys
100 105 110

Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
115 120 125

Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
130 135 140

Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
145 150 155 160

Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
165 170 175

Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
180 185 190

Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
195 200 205

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe

```
    210                 215                 220

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
225                 230                 235                 240

Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
                245                 250                 255

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
            260                 265                 270

Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe
        275                 280                 285

Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser
    290                 295                 300

Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
305                 310                 315                 320

Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg
                325                 330                 335

Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
            340                 345                 350

Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
        355                 360                 365

Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
    370                 375                 380

Ser Gly Lys Ile Asn Leu Ser His Gln
385                 390


<210>  54
<211>  393
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  SLO(deltaC101) fragment W535A w/o leader, with G overhang from
       cleavage


<220>
<221>  MOD_RES
<222>  (21)..(21)
<223>  Xaa = nnAA (e.g pAMF)

<220>
<221>  MOD_RES
<222>  (35)..(35)
<223>  Xaa = nnAA (e.g pAMF)

<220>
<221>  MOD_RES
```

<222> (74)..(74)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (112)..(112)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (195)..(195)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (246)..(246)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (280)..(280)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (298)..(298)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (330)..(330)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (387)..(387)
<223> Xaa = nnAA (e.g pAMF)

<400> 54

Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
1   5   10   15

Lys Ser Glu Asp Xaa Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
            20   25   30

Asn Asp Xaa Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
        35   40   45

Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
    50   55   60

Lys Ala Asp Lys Phe Ile Val Ile Glu Xaa Lys Lys Lys Asn Ile Asn
65   70   75   80

Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
                85   90   95

Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Xaa
            100   105   110

Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp

```
        115                 120                 125

Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
    130                 135                 140

Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
145                 150                 155                 160

Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
                165                 170                 175

Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
            180                 185                 190

Asn Ser Xaa Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
        195                 200                 205

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
    210                 215                 220

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
225                 230                 235                 240

Lys Ser Val Thr Phe Xaa Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
                245                 250                 255

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
            260                 265                 270

Val Lys Leu Glu Thr Ser Ser Xaa Ser Asn Asp Val Glu Ala Ala Phe
        275                 280                 285

Ser Ala Ala Leu Lys Gly Thr Asp Val Xaa Thr Asn Gly Lys Tyr Ser
    290                 295                 300

Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
305                 310                 315                 320

Ala Ala Glu His Asn Lys Val Val Thr Xaa Asp Phe Asp Val Ile Arg
                325                 330                 335

Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
            340                 345                 350

Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
        355                 360                 365

Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
    370                 375                 380

Ser Gly Xaa Ile Asn Leu Ser His Gln
385                 390
```

<210> 55
<211> 393
<212> PRT
<213> Artificial Sequence

<220>
<223> SLO(deltaC101) fragment W535A - nnAA (e.g. pAMF) mutant 1 - w/o
       leader, with G overhang from cleavage

<220>
<221> MOD_RES
<222> (21)..(21)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (74)..(74)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (195)..(195)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (280)..(280)
<223> Xaa = nnAA (e.g pAMF)

<400> 55

Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
1   5   10   15

Lys Ser Glu Asp Xaa Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
            20   25   30

Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
        35   40   45

Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
    50   55   60

Lys Ala Asp Lys Phe Ile Val Ile Glu Xaa Lys Lys Lys Asn Ile Asn
65   70   75   80

Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
                85   90   95

Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys
            100   105   110

Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
        115   120   125

Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
    130   135   140

```
Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
145                 150                 155                 160

Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
                165                 170                 175

Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
            180                 185                 190

Asn Ser Xaa Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
        195                 200                 205

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
    210                 215                 220

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
225                 230                 235                 240

Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
                245                 250                 255

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
            260                 265                 270

Val Lys Leu Glu Thr Ser Ser Xaa Ser Asn Asp Val Glu Ala Ala Phe
        275                 280                 285

Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser
    290                 295                 300

Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
305                 310                 315                 320

Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg
                325                 330                 335

Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
            340                 345                 350

Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
        355                 360                 365

Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
    370                 375                 380

Ser Gly Lys Ile Asn Leu Ser His Gln
385                 390


<210>  56
<211>  393
<212>  PRT
<213>  Artificial Sequence
```

<220>
<223> SLO(deltaC101) fragment W535A - nnAA (e.g. pAMF) mutant 2 - w/o
       leader, with G overhang from cleavage

<220>
<221> MOD_RES
<222> (35)..(35)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (112)..(112)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (246)..(246)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (298)..(298)
<223> Xaa = nnAA (e.g pAMF)

<400> 56

Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
1   5   10   15

Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
            20   25   30

Asn Asp Xaa Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
        35   40   45

Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
    50   55   60

Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn
65   70   75   80

Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
                85   90   95

Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Xaa
            100   105   110

Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
        115   120   125

Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
    130   135   140

Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
145   150   155   160

Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr

```
                165                 170                 175

Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
            180                 185                 190

Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
        195                 200                 205

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
    210                 215                 220

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
225                 230                 235                 240

Lys Ser Val Thr Phe Xaa Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
                245                 250                 255

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
            260                 265                 270

Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe
        275                 280                 285

Ser Ala Ala Leu Lys Gly Thr Asp Val Xaa Thr Asn Gly Lys Tyr Ser
    290                 295                 300

Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
305                 310                 315                 320

Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg
                325                 330                 335

Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
            340                 345                 350

Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
        355                 360                 365

Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
    370                 375                 380

Ser Gly Lys Ile Asn Leu Ser His Gln
385                 390
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<210> 57
<211> 393
<212> PRT
<213> Artificial Sequence

<220>
<223> SLO(deltaC101) fragment W535A - nnAA (e.g. pAMF) mutant 3 - w/o leader, with G overhang from cleavage

<220>
<221> MOD_RES
<222> (74)..(74)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (195)..(195)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (280)..(280)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (330)..(330)
<223> Xaa = nnAA (e.g pAMF)

<400> 57

```
Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
1               5                   10                  15


Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
            20                  25                  30


Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
        35                  40                  45


Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
    50                  55                  60


Lys Ala Asp Lys Phe Ile Val Ile Glu Xaa Lys Lys Lys Asn Ile Asn
65                  70                  75                  80


Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
                85                  90                  95


Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys
            100                 105                 110


Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
        115                 120                 125


Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
    130                 135                 140


Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
145                 150                 155                 160


Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
                165                 170                 175


Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
            180                 185                 190
```

```
Asn Ser Xaa Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
        195                 200                 205


Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
    210                 215                 220


Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
225                 230                 235                 240


Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
                245                 250                 255


Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
            260                 265                 270


Val Lys Leu Glu Thr Ser Ser Xaa Ser Asn Asp Val Glu Ala Ala Phe
        275                 280                 285


Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser
    290                 295                 300


Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
305                 310                 315                 320


Ala Ala Glu His Asn Lys Val Val Thr Xaa Asp Phe Asp Val Ile Arg
                325                 330                 335


Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
            340                 345                 350


Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
        355                 360                 365


Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
    370                 375                 380


Ser Gly Lys Ile Asn Leu Ser His Gln
385                 390


<210>  58
<211>  393
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  SLO(deltaC101) fragment W535A - nnAA (e.g. pAMF) mutant 4 -  w/o
       leader, with G overhang from cleavage


<220>
<221>  MOD_RES
<222>  (74)..(74)
<223>  Xaa = nnAA (e.g pAMF)

<220>
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<221> MOD_RES
<222> (195)..(195)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (298)..(298)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (387)..(387)
<223> Xaa = nnAA (e.g pAMF)

<400> 58

```
Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
1               5                   10                  15


Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
            20                  25                  30


Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
        35                  40                  45


Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
    50                  55                  60


Lys Ala Asp Lys Phe Ile Val Ile Glu Xaa Lys Lys Lys Asn Ile Asn
65                  70                  75                  80


Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
                85                  90                  95


Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys
            100                 105                 110


Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
        115                 120                 125


Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
    130                 135                 140


Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
145                 150                 155                 160


Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
                165                 170                 175


Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
            180                 185                 190


Asn Ser Xaa Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
        195                 200                 205


Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
```

```
    210                 215                 220

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
225                 230                 235                 240

Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
                245                 250                 255

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
            260                 265                 270

Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe
        275                 280                 285

Ser Ala Ala Leu Lys Gly Thr Asp Val Xaa Thr Asn Gly Lys Tyr Ser
    290                 295                 300

Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
305                 310                 315                 320

Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg
                325                 330                 335

Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
            340                 345                 350

Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
        355                 360                 365

Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
    370                 375                 380

Ser Gly Xaa Ile Asn Leu Ser His Gln
385                 390


<210>  59
<211>  393
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  SLO(deltaC101) fragment W535A - nnAA (e.g. pAMF) mutant 5 -  w/o
       leader, with G overhang from cleavage


<220>
<221>  MOD_RES
<222>  (35)..(35)
<223>  Xaa = nnAA (e.g pAMF)

<220>
<221>  MOD_RES
<222>  (195)..(195)
<223>  Xaa = nnAA (e.g pAMF)

<220>
<221>  MOD_RES
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<222> (280)..(280)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (387)..(387)
<223> Xaa = nnAA (e.g pAMF)

<400> 59

Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
1   5   10   15

Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
            20   25   30

Asn Asp Xaa Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
        35   40   45

Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
    50   55   60

Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn
65   70   75   80

Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
                85   90   95

Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys
            100   105   110

Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
        115   120   125

Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
    130   135   140

Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
145   150   155   160

Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
                165   170   175

Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
            180   185   190

Asn Ser Xaa Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
        195   200   205

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
    210   215   220

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
225   230   235   240

Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
245 250 255

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
260 265 270

Val Lys Leu Glu Thr Ser Ser Xaa Ser Asn Asp Val Glu Ala Ala Phe
275 280 285

Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser
290 295 300

Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
305 310 315 320

Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg
325 330 335

Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
340 345 350

Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
355 360 365

Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
370 375 380

Ser Gly Xaa Ile Asn Leu Ser His Gln
385 390

<210> 60
<211> 393
<212> PRT
<213> Artificial Sequence

<220>
<223> SLO(deltaC101) fragment W535A - nnAA (e.g. pAMF) mutant 6 - w/o leader, with G overhang from cleavage

<220>
<221> MOD_RES
<222> (21)..(21)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (112)..(112)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (280)..(280)
<223> Xaa = nnAA (e.g pAMF)

<400> 60

Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys

```
1               5                   10                  15

Lys Ser Glu Asp Xaa Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
            20                  25                  30

Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
        35                  40                  45

Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
    50                  55                  60

Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn
65                  70                  75                  80

Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
                85                  90                  95

Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Xaa
            100                 105                 110

Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
        115                 120                 125

Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
    130                 135                 140

Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
145                 150                 155                 160

Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
                165                 170                 175

Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
            180                 185                 190

Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
        195                 200                 205

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
    210                 215                 220

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
225                 230                 235                 240

Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
                245                 250                 255

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
            260                 265                 270

Val Lys Leu Glu Thr Ser Ser Xaa Ser Asn Asp Val Glu Ala Ala Phe
        275                 280                 285
```

Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser
290 295 300

Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
305 310 315 320

Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg
325 330 335

Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
340 345 350

Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
355 360 365

Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
370 375 380

Ser Gly Lys Ile Asn Leu Ser His Gln
385 390

<210> 61
<211> 393
<212> PRT
<213> Artificial Sequence

<220>
<223> SLO(deltaC101) fragment W535A - nnAA (e.g. pAMF) mutant 7 - w/o leader, with G overhang from cleavage

<220>
<221> MOD_RES
<222> (21)..(21)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (35)..(35)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (112)..(112)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (246)..(246)
<223> Xaa = nnAA (e.g pAMF)

<400> 61

Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
1 5 10 15

Lys Ser Glu Asp Xaa Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
20 25 30

```
Asn Asp Xaa Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
        35                  40                  45

Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
    50                  55                  60

Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn
65                  70                  75                  80

Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
                85                  90                  95

Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Xaa
            100                 105                 110

Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
        115                 120                 125

Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
    130                 135                 140

Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
145                 150                 155                 160

Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
                165                 170                 175

Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
            180                 185                 190

Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
        195                 200                 205

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
    210                 215                 220

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
225                 230                 235                 240

Lys Ser Val Thr Phe Xaa Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
                245                 250                 255

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
            260                 265                 270

Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe
        275                 280                 285

Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser
    290                 295                 300
```

```
Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
305                 310                 315                 320


Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg
                325                 330                 335


Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
            340                 345                 350


Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
        355                 360                 365


Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
    370                 375                 380


Ser Gly Lys Ile Asn Leu Ser His Gln
385                 390


<210>  62
<211>  393
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  SLO(deltaC101) fragment W535A - nnAA (e.g. pAMF) mutant 8 -  w/o
       leader, with G overhang from cleavage


<220>
<221>  MOD_RES
<222>  (21)..(21)
<223>  Xaa = nnAA (e.g pAMF)

<220>
<221>  MOD_RES
<222>  (74)..(74)
<223>  Xaa = nnAA (e.g pAMF)

<220>
<221>  MOD_RES
<222>  (195)..(195)
<223>  Xaa = nnAA (e.g pAMF)

<400>  62

Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
1               5                   10                  15


Lys Ser Glu Asp Xaa Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
            20                  25                  30


Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
        35                  40                  45


Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
    50                  55                  60


Lys Ala Asp Lys Phe Ile Val Ile Glu Xaa Lys Lys Lys Asn Ile Asn
65                  70                  75                  80
```

```
Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
                85                  90                  95

Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys
            100                 105                 110

Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
        115                 120                 125

Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
    130                 135                 140

Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
145                 150                 155                 160

Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
                165                 170                 175

Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
            180                 185                 190

Asn Ser Xaa Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
        195                 200                 205

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
    210                 215                 220

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
225                 230                 235                 240

Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
                245                 250                 255

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
            260                 265                 270

Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe
        275                 280                 285

Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser
    290                 295                 300

Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
305                 310                 315                 320

Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg
                325                 330                 335

Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
            340                 345                 350
```

Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
        355   360   365

Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
    370   375   380

Ser Gly Lys Ile Asn Leu Ser His Gln
385   390

<210> 63
<211> 393
<212> PRT
<213> Artificial Sequence

<220>
<223> SLO(deltaC101) fragment W535A - nnAA (e.g. pAMF) mutant 9 - w/o
       leader, with G overhang from cleavage

<220>
<221> MOD_RES
<222> (35)..(35)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (195)..(195)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (298)..(298)
<223> Xaa = nnAA (e.g pAMF)

<400> 63

Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
1   5   10   15

Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
            20   25   30

Asn Asp Xaa Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
        35   40   45

Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
    50   55   60

Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn
65   70   75   80

Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
                85   90   95

Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys
            100   105   110

Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp

```
        115                 120                 125

Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
    130                 135                 140

Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
145                 150                 155                 160

Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
                165                 170                 175

Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
            180                 185                 190

Asn Ser Xaa Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
        195                 200                 205

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
    210                 215                 220

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
225                 230                 235                 240

Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
                245                 250                 255

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
            260                 265                 270

Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe
        275                 280                 285

Ser Ala Ala Leu Lys Gly Thr Asp Val Xaa Thr Asn Gly Lys Tyr Ser
    290                 295                 300

Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
305                 310                 315                 320

Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg
                325                 330                 335

Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
            340                 345                 350

Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
        355                 360                 365

Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
    370                 375                 380

Ser Gly Lys Ile Asn Leu Ser His Gln
385                 390
```

<210> 64
<211> 393
<212> PRT
<213> Artificial Sequence

<220>
<223> SLO(deltaC101) fragment W535A - nnAA (e.g. pAMF) mutant 10 - w/o
       leader, with G overhang from cleavage

<220>
<221> MOD_RES
<222> (35)..(35)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (246)..(246)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (330)..(330)
<223> Xaa = nnAA (e.g pAMF)

<400> 64

Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
1   5   10   15

Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
            20   25   30

Asn Asp Xaa Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
        35   40   45

Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
    50   55   60

Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn
65   70   75   80

Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
                85   90   95

Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys
            100   105   110

Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
        115   120   125

Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
    130   135   140

Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
145   150   155   160

```
Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
                165                 170                 175

Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
            180                 185                 190

Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
        195                 200                 205

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
    210                 215                 220

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
225                 230                 235                 240

Lys Ser Val Thr Phe Xaa Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
                245                 250                 255

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
            260                 265                 270

Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe
        275                 280                 285

Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser
    290                 295                 300

Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
305                 310                 315                 320

Ala Ala Glu His Asn Lys Val Val Thr Xaa Asp Phe Asp Val Ile Arg
                325                 330                 335

Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
            340                 345                 350

Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
        355                 360                 365

Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
    370                 375                 380

Ser Gly Lys Ile Asn Leu Ser His Gln
385                 390
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<210> 65
<211> 393
<212> PRT
<213> Artificial Sequence

<220>
<223> SLO(deltaC101) fragment W535A - nnAA (e.g. pAMF) mutant 10 - w/o leader, with G overhang from cleavage

<220>
<221> MOD_RES
<222> (21)..(21)
<223> Xaa = nnAA (e.g pAMF)

<220>
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<223> Xaa = nnAA (e.g pAMF)

<220>
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<223> Xaa = nnAA (e.g pAMF)

<220>
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<222> (280)..(280)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (330)..(330)
<223> Xaa = nnAA (e.g pAMF)

<400> 65

Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
1   5   10   15

Lys Ser Glu Asp Xaa Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
            20   25   30

Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
        35   40   45

Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
    50   55   60

Lys Ala Asp Lys Phe Ile Val Ile Glu Xaa Lys Lys Lys Asn Ile Asn
65   70   75   80

Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
                85   90   95

Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys
            100   105   110

Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
        115   120   125

Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
    130   135   140

Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
145   150   155   160

Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr

```
                165                 170                 175

Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
            180                 185                 190

Asn Ser Xaa Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
        195                 200                 205

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
    210                 215                 220

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
225                 230                 235                 240

Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
                245                 250                 255

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
            260                 265                 270

Val Lys Leu Glu Thr Ser Ser Xaa Ser Asn Asp Val Glu Ala Ala Phe
        275                 280                 285

Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser
    290                 295                 300

Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
305                 310                 315                 320

Ala Ala Glu His Asn Lys Val Val Thr Xaa Asp Phe Asp Val Ile Arg
                325                 330                 335

Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
            340                 345                 350

Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
        355                 360                 365

Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
    370                 375                 380

Ser Gly Lys Ile Asn Leu Ser His Gln
385                 390


<210>  66
<211>  393
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  SLO(deltaC101) fragment W535A - nnAA (e.g. pAMF) mutant 10 -  w/o
       leader, with G overhang from cleavage
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<220>
<221> MOD_RES
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<223> Xaa = nnAA (e.g pAMF)

<220>
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<222> (112)..(112)
<223> Xaa = nnAA (e.g pAMF)

<220>
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<222> (246)..(246)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (298)..(298)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (387)..(387)
<223> Xaa = nnAA (e.g pAMF)

<400> 66

Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
1   5   10   15

Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
            20   25   30

Asn Asp Xaa Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
        35   40   45

Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
    50   55   60

Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn
65   70   75   80

Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
                85   90   95

Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Xaa
            100   105   110

Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
        115   120   125

Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
    130   135   140

Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
145   150   155   160

Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
                165   170   175

```
Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
            180                 185                 190

Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
        195                 200                 205

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
    210                 215                 220

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
225                 230                 235                 240

Lys Ser Val Thr Phe Xaa Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
                245                 250                 255

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
            260                 265                 270

Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe
        275                 280                 285

Ser Ala Ala Leu Lys Gly Thr Asp Val Xaa Thr Asn Gly Lys Tyr Ser
    290                 295                 300

Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
305                 310                 315                 320

Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg
                325                 330                 335

Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
            340                 345                 350

Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
        355                 360                 365

Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
    370                 375                 380

Ser Gly Xaa Ile Asn Leu Ser His Gln
385                 390
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<210> 67
<211> 393
<212> PRT
<213> Artificial Sequence

<220>
<223> SLO(deltaC101) fragment W535A - nnAA (e.g. pAMF) mutant 10 - w/o leader, with G overhang from cleavage

<220>

<221> MOD_RES
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<223> Xaa = nnAA (e.g pAMF)

<220>
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<222> (74)..(74)
<223> Xaa = nnAA (e.g pAMF)

<220>
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<222> (195)..(195)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (280)..(280)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (330)..(330)
<223> Xaa = nnAA (e.g pAMF)

<400> 67

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Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
1               5                   10                  15


Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
            20                  25                  30


Asn Asp Xaa Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
        35                  40                  45


Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
    50                  55                  60


Lys Ala Asp Lys Phe Ile Val Ile Glu Xaa Lys Lys Lys Asn Ile Asn
65                  70                  75                  80


Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
                85                  90                  95


Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys
            100                 105                 110


Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
        115                 120                 125


Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
    130                 135                 140


Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
145                 150                 155                 160


Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
                165                 170                 175
```

```
Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
            180                 185                 190

Asn Ser Xaa Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
        195                 200                 205

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
    210                 215                 220

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
225                 230                 235                 240

Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
                245                 250                 255

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
            260                 265                 270

Val Lys Leu Glu Thr Ser Ser Xaa Ser Asn Asp Val Glu Ala Ala Phe
        275                 280                 285

Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser
    290                 295                 300

Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
305                 310                 315                 320

Ala Ala Glu His Asn Lys Val Val Thr Xaa Asp Phe Asp Val Ile Arg
                325                 330                 335

Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
            340                 345                 350

Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
        355                 360                 365

Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
    370                 375                 380

Ser Gly Lys Ile Asn Leu Ser His Gln
385                 390
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<210> 68
<211> 393
<212> PRT
<213> Artificial Sequence

<220>
<223> SLO(deltaC101) fragment W535A - nnAA (e.g. pAMF) mutant 10 - w/o leader, with G overhang from cleavage

<220>
<221> MOD_RES

<222> (21)..(21)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
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<223> Xaa = nnAA (e.g pAMF)

<220>
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<222> (195)..(195)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (280)..(280)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (330)..(330)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (387)..(387)
<223> Xaa = nnAA (e.g pAMF)

<400> 68

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Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
1               5                   10                  15


Lys Ser Glu Asp Xaa Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
            20                  25                  30


Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
        35                  40                  45


Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
    50                  55                  60


Lys Ala Asp Lys Phe Ile Val Ile Glu Xaa Lys Lys Lys Asn Ile Asn
65                  70                  75                  80


Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
                85                  90                  95


Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys
            100                 105                 110


Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
        115                 120                 125


Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
    130                 135                 140


Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
145                 150                 155                 160
```

```
Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
                165                 170                 175

Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
            180                 185                 190

Asn Ser Xaa Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
        195                 200                 205

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
    210                 215                 220

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
225                 230                 235                 240

Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
                245                 250                 255

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
            260                 265                 270

Val Lys Leu Glu Thr Ser Ser Xaa Ser Asn Asp Val Glu Ala Ala Phe
        275                 280                 285

Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser
    290                 295                 300

Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
305                 310                 315                 320

Ala Ala Glu His Asn Lys Val Val Thr Xaa Asp Phe Asp Val Ile Arg
                325                 330                 335

Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
            340                 345                 350

Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
        355                 360                 365

Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
    370                 375                 380

Ser Gly Xaa Ile Asn Leu Ser His Gln
385                 390
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<210> 69
<211> 393
<212> PRT
<213> Artificial Sequence

<220>
<223> SLO(deltaC101) fragment W535A - nnAA (e.g. pAMF) mutant 10 - w/o leader, with G overhang from cleavage

<220>
<221> MOD_RES
<222> (35)..(35)
<223> Xaa = nnAA (e.g pAMF)

<220>
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<222> (74)..(74)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (112)..(112)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (246)..(246)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (298)..(298)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (387)..(387)
<223> Xaa = nnAA (e.g pAMF)

<400> 69

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Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
1               5                   10                  15


Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
            20                  25                  30


Asn Asp Xaa Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
        35                  40                  45


Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
    50                  55                  60


Lys Ala Asp Lys Phe Ile Val Ile Glu Xaa Lys Lys Lys Asn Ile Asn
65                  70                  75                  80


Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
                85                  90                  95


Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Xaa
            100                 105                 110


Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
        115                 120                 125


Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
    130                 135                 140
```

```
Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
145                 150                 155                 160

Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
                165                 170                 175

Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
            180                 185                 190

Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
        195                 200                 205

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
    210                 215                 220

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
225                 230                 235                 240

Lys Ser Val Thr Phe Xaa Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
                245                 250                 255

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
            260                 265                 270

Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe
        275                 280                 285

Ser Ala Ala Leu Lys Gly Thr Asp Val Xaa Thr Asn Gly Lys Tyr Ser
    290                 295                 300

Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
305                 310                 315                 320

Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg
                325                 330                 335

Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
            340                 345                 350

Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
        355                 360                 365

Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
    370                 375                 380

Ser Gly Xaa Ile Asn Leu Ser His Gln
385                 390
```

<210> 70
<211> 393
<212> PRT
<213> Artificial Sequence

<220>
<223> SLO(deltaC101) fragment W535A - nnAA (e.g. pAMF) mutant 10 - w/o
       leader, with G overhang from cleavage

<220>
<221> MOD_RES
<222> (21)..(21)
<223> Xaa = nnAA (e.g pAMF)

<220>
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<222> (35)..(35)
<223> Xaa = nnAA (e.g pAMF)

<220>
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<222> (112)..(112)
<223> Xaa = nnAA (e.g pAMF)

<220>
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<222> (246)..(246)
<223> Xaa = nnAA (e.g pAMF)

<220>
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<222> (298)..(298)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (387)..(387)
<223> Xaa = nnAA (e.g pAMF)

<400> 70

Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
1   5   10   15

Lys Ser Glu Asp Xaa Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
            20   25   30

Asn Asp Xaa Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
        35   40   45

Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
    50   55   60

Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn
65   70   75   80

Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
                85   90   95

Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Xaa
            100   105   110

Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
        115   120   125

```
Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
    130                 135                 140

Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
145                 150                 155                 160

Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
                165                 170                 175

Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
            180                 185                 190

Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
        195                 200                 205

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
    210                 215                 220

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
225                 230                 235                 240

Lys Ser Val Thr Phe Xaa Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
                245                 250                 255

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
            260                 265                 270

Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe
        275                 280                 285

Ser Ala Ala Leu Lys Gly Thr Asp Val Xaa Thr Asn Gly Lys Tyr Ser
    290                 295                 300

Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
305                 310                 315                 320

Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg
                325                 330                 335

Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
            340                 345                 350

Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
        355                 360                 365

Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
    370                 375                 380

Ser Gly Xaa Ile Asn Leu Ser His Gln
385                 390
```

<210> 71
<211> 393
<212> PRT
<213> Artificial Sequence

<220>
<223> SLO(deltaC101) fragment W535A - nnAA (e.g. pAMF) mutant 10 - w/o
       leader, with G overhang from cleavage

<220>
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<223> Xaa = nnAA (e.g pAMF)

<220>
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<223> Xaa = nnAA (e.g pAMF)

<220>
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<222> (112)..(112)
<223> Xaa = nnAA (e.g pAMF)

<220>
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<222> (195)..(195)
<223> Xaa = nnAA (e.g pAMF)

<220>
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<222> (280)..(280)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (330)..(330)
<223> Xaa = nnAA (e.g pAMF)

<220>
<221> MOD_RES
<222> (387)..(387)
<223> Xaa = nnAA (e.g pAMF)

<400> 71

Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
1   5   10   15

Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
            20   25   30

Asn Asp Xaa Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
        35   40   45

Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
    50   55   60

Lys Ala Asp Lys Phe Ile Val Ile Glu Xaa Lys Lys Lys Asn Ile Asn
65   70   75   80

Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
                85   90   95

```
Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Xaa
            100                 105                 110

Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
        115                 120                 125

Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
    130                 135                 140

Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
145                 150                 155                 160

Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
                165                 170                 175

Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
            180                 185                 190

Asn Ser Xaa Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
        195                 200                 205

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
    210                 215                 220

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
225                 230                 235                 240

Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
                245                 250                 255

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
            260                 265                 270

Val Lys Leu Glu Thr Ser Ser Xaa Ser Asn Asp Val Glu Ala Ala Phe
        275                 280                 285

Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser
    290                 295                 300

Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
305                 310                 315                 320

Ala Ala Glu His Asn Lys Val Val Thr Xaa Asp Phe Asp Val Ile Arg
                325                 330                 335

Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
            340                 345                 350

Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
        355                 360                 365
```

Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
    370   375   380

Ser Gly Xaa Ile Asn Leu Ser His Gln
385   390

<210> 72
<211> 393
<212> PRT
<213> Artificial Sequence

<220>
<223> SLO(deltaC101) fragment W535A - nnAA (e.g. pAMF) mutant 10 - w/o
       leader, with G overhang from cleavage

<220>
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<223> Xaa = nnAA (e.g pAMF)

<220>
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<223> Xaa = nnAA (e.g pAMF)

<220>
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<223> Xaa = nnAA (e.g pAMF)

<400> 72

Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
1   5   10   15

Lys Ser Glu Asp Xaa Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
            20   25   30

Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
        35   40   45

Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys

```
    50                  55                  60

Lys Ala Asp Lys Phe Ile Val Ile Glu Xaa Lys Lys Lys Asn Ile Asn
65                  70                  75                  80

Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
                85                  90                  95

Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Xaa
            100                 105                 110

Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
        115                 120                 125

Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
    130                 135                 140

Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
145                 150                 155                 160

Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
                165                 170                 175

Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
            180                 185                 190

Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
        195                 200                 205

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
    210                 215                 220

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
225                 230                 235                 240

Lys Ser Val Thr Phe Xaa Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
                245                 250                 255

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
            260                 265                 270

Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe
        275                 280                 285

Ser Ala Ala Leu Lys Gly Thr Asp Val Xaa Thr Asn Gly Lys Tyr Ser
    290                 295                 300

Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
305                 310                 315                 320

Ala Ala Glu His Asn Lys Val Val Thr Xaa Asp Phe Asp Val Ile Arg
                325                 330                 335
```

Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
            340   345   350

Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
        355   360   365

Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
    370   375   380

Ser Gly Xaa Ile Asn Leu Ser His Gln
385   390

<210> 73
<211> 393
<212> PRT
<213> Artificial Sequence

<220>
<223> SLO(deltaC101) fragment W535A - nnAA (e.g. pAMF) mutant 10 - w/o
       leader, with G overhang from cleavage

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<223> Xaa can be any naturally occurring amino acid

<220>
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<223> Xaa = nnAA (e.g pAMF)

<220>
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<223> Xaa = nnAA (e.g pAMF)

<400> 73

Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
1   5   10   15

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Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
            20                  25                  30

Asn Asp Xaa Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
        35                  40                  45

Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
    50                  55                  60

Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn
65                  70                  75                  80

Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
                85                  90                  95

Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Xaa
            100                 105                 110

Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
        115                 120                 125

Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
    130                 135                 140

Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
145                 150                 155                 160

Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
                165                 170                 175

Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
            180                 185                 190

Asn Ser Xaa Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
        195                 200                 205

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
    210                 215                 220

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
225                 230                 235                 240

Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
                245                 250                 255

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
            260                 265                 270

Val Lys Leu Glu Thr Ser Ser Xaa Ser Asn Asp Val Glu Ala Ala Phe
        275                 280                 285

Ser Ala Ala Leu Lys Gly Thr Asp Val Xaa Thr Asn Gly Lys Tyr Ser
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    290                 295                 300

Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
305                 310                 315                 320

Ala Ala Glu His Asn Lys Val Val Thr Xaa Asp Phe Asp Val Ile Arg
                325                 330                 335

Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
            340                 345                 350

Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
        355                 360                 365

Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
    370                 375                 380

Ser Gly Xaa Ile Asn Leu Ser His Gln
385                 390


<210>  74
<211>  393
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  SLO(deltaC101) fragment W535A - nnAA (e.g. pAMF) mutant 10 -  w/o
       leader, with G overhang from cleavage


<220>
<221>  MOD_RES
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<223>  Xaa = nnAA (e.g pAMF)

<220>
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<223>  Xaa = nnAA (e.g pAMF)

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<223>  Xaa = nnAA (e.g pAMF)

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<222>  (246)..(246)
<223>  Xaa = nnAA (e.g pAMF)

<220>
<221>  MOD_RES
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<222> (280)..(280)
<223> Xaa = nnAA (e.g pAMF)

<220>
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<223> Xaa = nnAA (e.g pAMF)

<400> 74

Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
1   5   10   15

Lys Ser Glu Asp Xaa Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
            20   25   30

Asn Asp Xaa Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
        35   40   45

Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
    50   55   60

Lys Ala Asp Lys Phe Ile Val Ile Glu Xaa Lys Lys Lys Asn Ile Asn
65   70   75   80

Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
                85   90   95

Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Xaa
            100   105   110

Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
        115   120   125

Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
    130   135   140

Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
145   150   155   160

Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
                165   170   175

Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
            180   185   190

Asn Ser Xaa Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
        195   200   205

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
    210   215   220

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
225   230   235   240

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Lys Ser Val Thr Phe Xaa Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
                245                 250                 255


Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
            260                 265                 270


Val Lys Leu Glu Thr Ser Ser Xaa Ser Asn Asp Val Glu Ala Ala Phe
        275                 280                 285


Ser Ala Ala Leu Lys Gly Thr Asp Val Xaa Thr Asn Gly Lys Tyr Ser
    290                 295                 300


Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
305                 310                 315                 320


Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg
                325                 330                 335


Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
            340                 345                 350


Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
        355                 360                 365


Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
    370                 375                 380


Ser Gly Lys Ile Asn Leu Ser His Gln
385                 390


<210>  75
<211>  393
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  SLO(deltaC101) fragment W535A - nnAA (e.g. pAMF) mutant 10 -  w/o
       leader, with G overhang from cleavage


<220>
<221>  MOD_RES
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<223>  Xaa = nnAA (e.g pAMF)

<220>
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<220>
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<222>  (195)..(195)
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<223> Xaa = nnAA (e.g pAMF)

<220>
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<223> Xaa = nnAA (e.g pAMF)

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<220>
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<223> Xaa = nnAA (e.g pAMF)

<220>
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<223> Xaa = nnAA (e.g pAMF)

<400> 75

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Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
1               5                   10                  15


Lys Ser Glu Asp Xaa Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
            20                  25                  30


Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
        35                  40                  45


Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
    50                  55                  60


Lys Ala Asp Lys Phe Ile Val Ile Glu Xaa Lys Lys Lys Asn Ile Asn
65                  70                  75                  80


Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
                85                  90                  95


Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Xaa
            100                 105                 110


Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
        115                 120                 125


Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
    130                 135                 140


Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
145                 150                 155                 160


Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
                165                 170                 175


Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
```

```
            180                 185                 190

Asn Ser Xaa Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
        195                 200                 205

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
    210                 215                 220

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
225                 230                 235                 240

Lys Ser Val Thr Phe Xaa Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
                245                 250                 255

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
            260                 265                 270

Val Lys Leu Glu Thr Ser Ser Xaa Ser Asn Asp Val Glu Ala Ala Phe
        275                 280                 285

Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser
    290                 295                 300

Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
305                 310                 315                 320

Ala Ala Glu His Asn Lys Val Val Thr Xaa Asp Phe Asp Val Ile Arg
                325                 330                 335

Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
            340                 345                 350

Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
        355                 360                 365

Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
    370                 375                 380

Ser Gly Xaa Ile Asn Leu Ser His Gln
385                 390


<210>  76
<211>  393
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  SLO(deltaC101) fragment W535A - nnAA (e.g. pAMF) mutant 10 -  w/o
       leader, with G overhang from cleavage


<220>
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<220>
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<223> Xaa = nnAA (e.g pAMF)

<400> 76

Gly Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
1   5   10   15

Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
            20   25   30

Asn Asp Xaa Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
        35   40   45

Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
    50   55   60

Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn
65   70   75   80

Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
                85   90   95

Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Xaa
            100   105   110

Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
        115   120   125

```
Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
    130                 135                 140

Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
145                 150                 155                 160

Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
                165                 170                 175

Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
            180                 185                 190

Asn Ser Xaa Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
        195                 200                 205

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
    210                 215                 220

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
225                 230                 235                 240

Lys Ser Val Thr Phe Xaa Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
                245                 250                 255

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
            260                 265                 270

Val Lys Leu Glu Thr Ser Ser Xaa Ser Asn Asp Val Glu Ala Ala Phe
        275                 280                 285

Ser Ala Ala Leu Lys Gly Thr Asp Val Xaa Thr Asn Gly Lys Tyr Ser
    290                 295                 300

Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
305                 310                 315                 320

Ala Ala Glu His Asn Lys Val Val Thr Xaa Asp Phe Asp Val Ile Arg
                325                 330                 335

Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
            340                 345                 350

Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
        355                 360                 365

Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
    370                 375                 380

Ser Gly Xaa Ile Asn Leu Ser His Gln
385                 390
```