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(54) Title: THERAPEUTIC NUCLEASE MOLECULES WITH ALTERED GLYCOSYLATION AND METHODS

(57) Abstract: The invention provides for polypeptides having one or more nuclease domains (e.g., an RNase and/or DNase domain) with altered glycosylation, preferably operably coupled to a pharmacokinetic moiety, which optionally has altered glycosylation. The invention also provides methods of treating or preventing a condition associated with an abnormal immune response.

THERAPEUTIC NUCLEASE MOLECULES WITH ALTERED GLYCOSYLATION AND METHODS

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Patent Application Serial No. 61/898,370, filed October 31, 2013, U.S. Provisional Patent Application Serial No. 61/898,384, filed October 31, 2013, and U.S. Provisional Patent Application Serial No. 61/898,393, filed October 31, 2013. The entire content of each of the above-referenced patent applications is incorporated herein by this reference.

BACKGROUND

Excessive release of (ribo)nucleoprotein particles from dead and dying cells can cause lupus pathology by two mechanisms: (i) Deposition or *in situ* formation of chromatin / anti-chromatin complexes causes nephritis and leads to loss of renal function; and (ii) nucleoproteins activate innate immunity through toll-like receptor (TLR) 3, 7, 8, and 9 as well as TLR-independent pathway(s). Release of nucleoproteins can serve as a potent antigen for autoantibodies in SLE, providing amplification of B cell and DC activation through co-engagement of antigen receptors and TLRs. Thus, there exists a need for a means to remove inciting antigens and/or attenuate immune stimulation, immune amplification, and immune complex mediated disease in subjects in need thereof, for example, with long-acting nuclease molecules that attack circulating immune complexes by digesting nucleic acids contained therein.

SUMMARY OF THE INVENTION

The invention relates, in part, to nuclease molecules with altered glycosylation, preferably operably coupled to a pharmacokinetic (PK) moiety, such as polyethylene glycol (PEG), albumin, Fc, and transferrin, or variants or fragments thereof, wherein the nuclease molecules exhibit enhanced pharmacokinetics relative to the corresponding glycosylated nuclease molecules. When coupled with a PK moiety, such nuclease molecules are herein referred to as "hybrid nuclease-PK molecules."

In some embodiments, the hybrid nuclease-PK molecule is a polypeptide comprising an amino acid sequence of a first nuclease domain operably coupled to an amino acid sequence of a PK moiety (or variant or fragment thereof). In some embodiments, the molecule includes a first linker domain, and the first nuclease domain is operably coupled to a PK moiety, or a variant or fragment thereof, via the first linker domain.

In some embodiments, the first nuclease domain is an RNase or DNase (e.g., human RNase1 or human DNase1), and the nuclease domain is aglycosylated, underglycosylated, or deglycosylated. Preferably, the nuclease domain is aglycosylated.

In some embodiments, the hybrid nuclease-PK molecule further includes a second nuclease domain (e.g., an RNase or DNase domain), which is operably coupled to the first nuclease domain or the N- or C-terminus of a PK moiety, or a variant or fragment thereof, optionally via a linker. In some embodiments, the first and second nuclease domains are the same, e.g., RNase and RNase, or DNase and DNase. In other embodiments, the first and second nuclease domains are different, e.g., RNase and DNase.

In some embodiments, the RNase domain is a wild-type RNase, such as wild-type human RNase1 (SEQ ID NO: 1). In other embodiments, the RNase domain is a mutant RNase, such as an aglycosylated, underglycosylated, or deglycosylated RNase1. In one embodiment, the human RNase1 includes an alteration (e.g., a substitution) at one or more sites of N-linked glycosylation, such as N34, N76 and N88 as set forth in SEQ ID NO: 2. In another embodiment, the human RNase1 includes an alteration (e.g., a substitution) at one of the following: N34; N76; N88; N34 and N76; N34 and N88; N76 and N88; and N34, N76, and N88. In yet another embodiment, the human RNase1 includes an alteration at one or more of N34, N76, and N88, and combinations thereof, such as a substitution, e.g., N34S/N76S/N88S (SEQ ID NO: 2).

In some embodiments, the RNase containing hybrid nuclease-PK molecule degrades circulating RNA and RNA in immune complexes, or inhibits interferon-alpha production, or both. In yet other embodiments, the activity of the RNase is not less than about 10-

fold less, such as 9-fold less, 8-fold less, 7-fold less, 6-fold less, 5-fold less, 4-fold less, 3-fold less, or 2-fold less than the activity of a control RNase molecule. In yet other embodiments, the activity of the RNase is about equal to the activity of a control RNase molecule.

In some embodiments, the DNase domain is wild type DNase, such as wild type, human DNase1 (SEQ ID NO: 3). In other embodiments, the DNase domain is a mutant DNase domain, such as mutant, human DNase1 A114F (SEQ ID NO: 4) or an aglycosylated, underglycosylated, or deglycosylated human DNase. In one embodiment, the human DNase1 includes an alteration (e.g., a substitution) at one or more sites of N-linked glycosylation, such as N18 and N106 as set forth in SEQ IN NO: 4. In another embodiment, the human DNase1 includes an alteration (e.g., a substitution) at the following: N18; N106; and N18 and N106. In yet another embodiment, the human DNase1 includes an alteration at one or more of N18, N106, and A114, such as a substitution, e.g., N18S/N106S/A114F (SEQ ID NO: 5) and combinations thereof.

In some embodiments, the DNase domain is a mutant DNase domain, such as mutant, human DNase1 and an aglycosylated, underglycosylated, or deglycosylated DNase domain, such as an aglycosylated, underglycosylated, or deglycosylated human DNase1. In one embodiment, the human DNase1 includes an alteration (e.g., a substitution) at one or more sites of N-linked glycosylation, such as N18 and N106 and at least one additional mutation selected from A114, E13, N74, T205, and combinations thereof. In another embodiment, the human DNase1 includes an alteration (e.g., a substitution) at N18, N106, or both N18 and N106 and an additional alertation (e.g., a substitution) at A114, E13, N74, T205, and combinations thereof. In yet another embodiment, the human DNase1 includes an alteration at N18, N106, A114, E13, N74 and T205, such as a substitution, e.g., N18S/N106S/A114F/E13R/N74K/T205K (SEQ ID NO: 88).

In some embodiments, the DNase containing hybrid nuclease-PK molecule degrades circulating DNA and DNA in immune complexes, or inhibits interferon-alpha production, or both. In yet other embodiments, the activity of the DNase is not less than about 10-

fold less, such as 9-fold less, 8-fold less, 7-fold less, 6-fold less, 5-fold less, 4-fold less, 3-fold less, or 2-fold less than the activity of a control DNase molecule. In yet other embodiments, the activity of the DNase is about equal to the activity of a control DNase molecule.

In some embodiments, the hybrid nuclease-PK molecule has a gly-ser linker separating the first and second nuclease domains, and/or the nuclease domains from the PK moiety, or a variant or fragment thereof.

In some embodiments, the hybrid nuclease-PK molecule increases the serum half-life and/or activity of the molecule relative to a molecule that does not contain the PK moiety, or variant or fragment thereof.

In some embodiments, the PK moiety is wild type human serum albumin (HSA; SEQ ID NO: 6), or a variant or fragment thereof. In some embodiments, the PK moiety is wild type human serum transferrin (HST; SEQ ID NO: 7), or a variant or fragment thereof. In some embodiments, the PK moiety is a wild type human IgG1 Fc domain (SEQ ID NO: 10), or a variant or fragment thereof. In some embodiments, the PK moiety is a PEG or derivative thereof. In some embodiments, the PK moiety variants are more than 80%, such greater than 85%, greater than 90%, or greater than 95% identical to the amino acid sequence of the corresponding wild-type PK moieties. In some embodiments, the PK moiety is a fragment of the PK moiety or a variant thereof. In some embodiments, the fragment is at least 20 amino acids, such as at least 40 amino acids, at least 60 amino acids, at least 80 amino acids, at least 100 amino acids, at least 150 amino acids, at least 200 amino acids, at least 300 amino acids, at least 400 amino acids, or at least 500 amino acids in length.

In some aspects, the hybrid nuclease-PK molecule with altered glycosylation includes the mutant, human DNase1 A114F domain set forth in (SEQ ID NO: 4). In another embodiment, the hybrid nuclease-PK molecule with altered glycosylation includes the mutant, human DNase1 N18S/N106S/A114F domain set forth in SEQ ID NO: 5. In yet

another embodiment, the hybrid nuclease-PK molecule with altered glycosylation includes the mutant, human DNase1 N18S/N106S/A114F/E13R/N74K/T205K domain set forth in SEQ ID NO: 88.

In another embodiment, the hybrid nuclease-PK molecule with altered glycosylation includes the human, wild-type RNase1 domain set forth in SEQ ID NO: 1. In another embodiment, the hybrid nuclease-PK molecule with altered glycosylation includes the human, mutant RNase1 N34S/N76S/N88S domain set forth in SEQ ID NO: 2. In another embodiment, the hybrid nuclease-PK molecule with altered glycosylation includes the wild-type, mature HSA set forth in SEQ ID NO: 6.

In another embodiment, the hybrid nuclease-PK molecule with altered glycosylation includes the wild-type, human serum transferrin set forth in SEQ ID NO: 7. In another embodiment, the hybrid nuclease-PK molecule with altered glycosylation includes the mutant, human serum transferrin N413S/N611S set forth in SEQ ID NO: 8. In another embodiment, the hybrid nuclease-PK molecule with altered glycosylation includes the mutant, human serum transferrin S12A/N413S/N611S set forth in SEQ ID NO: 9 (S12 is a potential O-linked glycosylation site).

In another embodiment, the hybrid nuclease-PK molecule with altered glycosylation includes the wild-type, human IgG1 Fc region set forth in SEQ ID NO: 10. In another embodiment, the hybrid nuclease-PK molecule with altered glycosylation includes a mutant, human IgG1 Fc region N83S (i.e., N297S by Kabat numbering) set forth in SEQ ID NO: 11. In another embodiment, the hybrid nuclease-PK molecule with altered glycosylation includes a mutant human IgG1 Fc region having a cysteine substitution in the hinge region (SCC) set forth in SEQ ID NO: 40. In yet another embodiment, the hybrid nuclease-PK molecule with altered glycosylation includes a mutant human IgG1 Fc region having 3 cysteine substitutions in the hinge region (SSS) set forth in SEQ ID NO: 41.

In another embodiment, the hybrid nuclease-PK molecule with altered glycosylation includes a mutant, human IgG1 Fc region P238S, P331S set forth in SEQ ID NO: 65. In another embodiment, the hybrid nuclease-PK molecule with altered glycosylation includes a mutant human IgG1 Fc region P238S, P331S having a cysteine substitution in the hinge region (SCC) as set forth in SEQ ID NO: 66. In yet another embodiment, the hybrid nuclease-PK molecule with altered glycosylation includes a mutant human IgG1 Fc region P238S, P331S having 3 cysteine substitutions in the hinge region (SSS) set forth in SEQ ID NO: 67.

In another embodiment, the hybrid nuclease-PK molecule with altered glycosylation includes a mutant, human IgG1 Fc region P238S, P331S, N297S set forth in SEQ ID NO: 68. In another embodiment, the hybrid nuclease-PK molecule with altered glycosylation includes a mutant human IgG1 Fc region P238S, P331S, N297S having a cysteine substitution in the hinge region (SCC) as set forth in SEQ ID NO: 69. In yet another embodiment, the hybrid nuclease-PK molecule with altered glycosylation includes a mutant human IgG1 Fc region P238S, P331S, N297S having 3 cysteine substitutions in the hinge region (SSS) set forth in SEQ ID NO: 70.

In another embodiment, the hybrid nuclease-PK molecule with altered glycosylation includes a PEG. In another embodiment, the hybrid nuclease-PK molecule with altered glycosylation includes the $(Gly_4Ser)_3$ linker domain set forth in SEQ ID NO: 12. In another embodiment, the hybrid nuclease-PK molecule with altered glycosylation includes a VK3LP leader (SEQ ID NO: 13). These individual domains can be operably coupled to each other in any order to form a hybrid nuclease-PK molecule with altered glycosylation that is enzymatically active.

In some aspects, the invention provides a polypeptide with altered glycosylation having human, mutant RNase1 N34S/N76S/N88S, operably linked with or without a linker to a mutant human IgG1 Fc domain optionally having a mutant hinge region (e.g., a cysteine substitution, such as with serine, e.g., SCC), and one or more CH2 mutations to reduce Fcγ receptor binding (e.g., P238S, P331S or both P238S and P331S). In one embodiment,

the polypeptide with altered glycosylation has human, mutant RNase1 N34S/N76S/N88S, operably linked to a mutant human IgG1 Fc domain having SCC hinge, P238S and P331S mutations. In yet another embodiment, the Fc domain further includes a mutation at a site of N-linked glycosylation, such as a substitution at N297 (numbering by Kabat). In another embodiment, the polypeptide with altered glycosylation has human, mutant RNase1 N34S/N76S/N88S, operably linked to a mutant human IgG1 Fc domain having SCC hinge, P238S, P331S and N297S mutations. In another embodiment, the polypeptide comprises an amino acid sequence set forth in SEQ ID NOs: 71-74. In other aspects, the polypeptide has an amino acid sequence at least 90% identical or at least 95% identical to the amino acid sequences set forth in SEQ ID NOs: 71-74.

In some aspects, the invention provides hybrid nuclease-PK molecules comprising polypeptides with altered glycosylation having an amino acid sequence set forth in SEQ ID NOs: 14-21 and 47-54. In other aspects, the polypeptide has an amino acid sequence at least 90% identical or at least 95% identical to the amino acid sequences set forth in SEQ ID NOs: 14-21, 47-54, and 71-82.

In some aspects, the invention provides compositions including the hybrid nuclease-PK molecules with altered glycosylation and a carrier, such as a pharmaceutically acceptable carrier or diluent.

In some aspects, the invention provides nucleic acid molecules that encode the hybrid nuclease-PK molecules with altered glycosylation disclosed herein. In some embodiments, the invention provides a recombinant expression vector having a nucleic acid molecule that encodes the hybrid nuclease-PK molecules with altered glycosylation disclosed herein. In some embodiments, the invention provides host cells transformed with the recombinant expression vectors containing the nucleic acid sequences encoding the hybrid nuclease-PK molecules with altered glycosylation disclosed herein. Also disclosed herein is a method of making a hybrid nuclease-PK molecule with altered glycosylation disclosed herein involving providing a host cell comprising a nucleic acid sequence that encodes the hybrid nuclease-PK molecule with altered glycosylation; and

maintaining the host cell under conditions in which the hybrid nuclease-PK molecule with altered glycosylation is expressed.

Also disclosed herein is a method for treating or preventing a condition associated with an abnormal immune response by administering to a patient in need thereof an effective amount of an isolated hybrid nuclease-PK molecule with altered glycosylation disclosed herein. In some embodiments, the condition is an autoimmune disease. In some embodiments, the autoimmune disease is selected from the group consisting of insulin-dependent diabetes mellitus, multiple sclerosis, experimental autoimmune encephalomyelitis, rheumatoid arthritis, experimental autoimmune arthritis, myasthenia gravis, thyroiditis, an experimental form of uveoretinitis, Hashimoto's thyroiditis, primary myxoedema, thyrotoxicosis, pernicious anaemia, autoimmune atrophic gastritis, Addison's disease, premature menopause, male infertility, juvenile diabetes, Goodpasture's syndrome, pemphigus vulgaris, pemphigoid, sympathetic ophthalmia, phacogenic uveitis, autoimmune haemolytic anaemia, idiopathic leucopenia, primary biliary cirrhosis, active chronic hepatitis Hbs-ve, cryptogenic cirrhosis, ulcerative colitis, Sjogren's syndrome, scleroderma, Wegener's granulomatosis, polymyositis, dermatomyositis, discoid LE, systemic lupus erythematosus (SLE), and connective tissue disease. In some embodiments, the autoimmune disease is SLE or Sjogren's syndrome.

Also disclosed herein is a method of treating SLE or Sjogren's syndrome comprising administering to a subject a nuclease-containing composition in an amount effective to degrade immune complexes containing RNA, DNA or both RNA and DNA. In some aspects, the composition includes a pharmaceutically acceptable carrier and a hybrid nuclease-PK molecule with altered glycosylation as described herein. In other aspects, the composition includes a hybrid nuclease-PK molecule comprising a polypeptide with altered glycosylation having an amino acid sequence set forth in SEQ ID NO: 14-21, 47-54, and 71-82.

In another aspect, the invention relates to hybrid nuclease-PK molecules with altered glycosylation for use in treating diseases characterized by defective clearance or

processing of apoptotic cells and cell debris, such as SLE. In some embodiments, the hybrid nuclease-PK molecules comprise a polypeptide with altered glycosylation comprising an amino acid sequence set forth in SEQ ID NOs: 14-21, 47-54 and 71-82.

In another aspect, the invention relates to the use of the hybrid nuclease-PK molecules with altered glycosylation for manufacturing a medicament for treating diseases characterized by defective clearance or processing of apoptotic cells and cell debris, such as SLE. In some embodiments, the hybrid nuclease-PK molecules comprise a polypeptide with altered glycosylation having an amino acid sequence set forth in SEQ ID NOs: 14-21, 47-54 and 71-82.

In another aspect, the invention relates to the use of a hybrid nuclease-PK molecule that upon administration to a subject achieves increased overall exposure of the subject to nuclease activity. In certain embodiments, administration of aglycosylated RSLV-132 to a subject achieves increased overall exposure of the subject to nuclease activity as compared to RSLV-132. In certain embodiments, aglycosylated RSLV-132 is AG1-RSLV-132 or AG2-RSLV-132.

In another aspect, the invention relates to a composition comprising hybrid nuclease-PK molecules with increased homogeneity. In certain embodiments, a composition comprising aglycosylated RSLV-132 has increased homogeneity as compared to RSLV-132. In certain embodiments, aglycosylated RSLV-132 is AG1-RSLV-132 or AG2-RSLV-132.

In another aspect, the invention relates to a composition comprising hybrid nuclease-PK molecules with reduced heterogeneity. In certain embodiments, a composition comprising aglycosylated RSLV-132 has reduced heterogeneity as compared to RSLV-132. In certain embodiments, aglycosylated RSLV-132 is AG1-RSLV-132 or AG2-RSLV-132.

BRIEF DESCRIPTION OF THE DRAWINGS

These and other features, aspects, and advantages of the present invention will become better understood with regard to the following description, and accompanying drawing, where:

FIG 1A-B. is a schematic depicting the various configurations of the hybrid nuclease-PK molecules with altered glycosylation described herein.

FIG 2. is a schematic representation of the aglycosylated variants AG1-RSLV-132 and AG2-RSLV-132. The 3 N-linked glycosylation sites within human RNase1 domain are shown (N54S, N96S and N108S). Numbering corresponds to numbering in the RSLV-132 molecule which includes a 20 amino acid heterologous leader sequence. Numbering corresponds to N34, N76 and N88 in the mature human RNase (which lacks a leader sequence) and N62, N104 and N116 in human RNase (P07998) with the 28 amino acid native leader sequence. The single N-linked site within the Fc domain was eliminated by introducing an asparagine to serine mutation at N231S in AG2-RSLV-132 (numbering according to the RSLV-132 molecule. This site corresponds to the site of N-linked glycosylation in human IgG1 Fc at N297 according to Kabat numbering (or N83 as shown in SEQ ID NO:11).

FIG 3A. shows the results of SDS gel electrophoresis of AG1-RSLV-132, AG2-RSLV-132 compared to RSLV-132 under non-reducing and reducing conditions. Sample1: RSLV-132; Sample 2: AG1-RSLV-132; Sample 3: AG2-RSLV-132. The left lane of the gel contained MW standards. Following electrophoresis, the gel was stained with Coomassie blue.

FIG 3B. shows RNase activity of the three preparations using the RNaseAlert® format. Dilutions of the three preparations were subjected to kinetic analysis. Initial rates of hydrolysis were calculated and compared to a standard curve generated with RSLV-132.

FIG 4. graphically depicts the specific activity of three AG1-RSLV-132 (AG1) preparations relative to RSLV-132 in the Version 3 activity format. The mean specific activity (U/mg) was calculated and shown as a function of the preparation.

FIG 5. graphically depicts a dose response curve for AG1-RSLV-132 (AG1) and RSLV-132 with respect to inhibition of poly(I:C). Where indicated, RSLV-132 or AG1-RSLV-132 was added to the cells in the absence of poyl(I:C). The amount of SEAP activity detected is indicated (A_{620}) as a function of fusion construct concentration.

FIG 6A-B. 6A, Top Panel: shows the elution profile of AG1-RSLV-132 (y-axis corresponding to absorbance at 280nM) versus elution time. The main A_{280} peak eluted at 8.62 min. and represented >97% of the total recovered A_{280} absorbance. 6A, Bottom Panel: shows the elution profile obtained with a set of calibration standards. The 8.167 min and 9.701 min peaks correspond to 158 kDa and 44 kDa, respectively, RSLV-132 typically elutes at 8.2 to 8.3 min. 6B: depicts the comparison of the SEC elution profiles of RSLV-132 (Pilot 2 lot #P3094253 and AG1-RSLV-1 32(AG1)) at comparable loads.

FIG 7. graphically depicts the mean serum concentration of RSLV-132 or AG1-RSLV-132 (RSLV-132_AG1) observed (n=3/group) over the 12 day time period following a single 30 mg/kg IV bolus dose to male Cynomolgus monkeys.

FIG 8. graphically depicts individual animal RSLV-132 and AG1-RSLV-132 (RSLV-132_AG1) concentration versus time profiles following a single 30 mg/kg IV bolus dose to male Cynomolgus monkeys.

DETAILED DESCRIPTION

Overview

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease characterized by the presence of high titer autoantibodies directed against self nucleoprotein particles. There is strong evidence that defective clearance or processing of dead and dying cells in SLE leads to disease, predominantly through exposure of ribo- and deoxy-ribonucleoprotein particles (abbreviated nucleoproteins). The nucleoproteins cause damage through three mechanisms: i) serve as antigens to generate circulating high affinity autoantibodies; ii) activation of the innate immune system to produce inflammatory cytokines as a result of immune complex formation; and iii) mediate organ dysfunction as a result of the deposition of immune complexes at local sites such as the kidney.

The present invention provides methods for treating diseases characterized by defective clearance or processing of apoptotic cells and cell debris, such as SLE and Sjogren's syndrome, by administering an effective amount of a nuclease activity to degrade extracellular RNA and DNA containing immune complexes. Such treatment can inhibit the production of Type I interferons (IFNs), which are prominent cytokines in SLE and are strongly correlated with disease activity and nephritis.

The present invention relates, in part, to the provision of such enzymatically active nucleases. In particular, the invention relates to a polypeptide comprising one or more nuclease domains with altered glycosylation, preferably operably coupled to a PK moiety (which can also have altered glycosylation) which extends serum half-life of the polypeptide, such as PEG, albumin, Fc, or transferrin, or variants or fragments thereof. When coupled to a PK moiety, such polypeptides are herein referred to as "hybrid nuclease-PK molecules."

Accordingly, in one embodiment, a subject with a disease characterized by defective clearance or processing of apoptotic cells and cell debris is treated by administering a

hybrid nuclease-PK molecule with altered glycosylation, wherein the hybrid nuclease-PK molecule has increased bioavailability and/or serum half-life relative to the corresponding glycosylated hybrid nuclease-PK molecule (i.e., a hybrid nuclease-PK molecule, wherein the glycosylation sites in the various domains are retained).

In another aspect, a method of treating SLE or Sjogren's syndrome is provided in which a sufficient or effective amount of a composition containing a hybrid nuclease-PK molecule with altered glycosylation is administered to a subject. In one aspect, treatment results in degradation of immune complexes containing RNA, DNA or both RNA and DNA. In another aspect, treatment results in inhibition of Type I interferons, such as interferon-α in a subject. In one aspect, a method of treating a subject comprises administering an effective amount of a composition of a hybrid nuclease-PK molecule comprising a polypeptide with altered glycosylation comprising an amino acid sequence set forth in SEQ ID NOs: 14-21, 47-54 and 71-82.

In another aspect, the invention relates to hybrid nuclease-PK molecules with altered glycosylation for use in treating diseases characterized by defective clearance or processing of apoptotic cells and cell debris, such as SLE. In some embodiments, the hybrid nuclease-PK molecule comprises a polypeptide with altered glycosylation comprising an amino acid sequence set forth in SEQ ID NOs: 14-21, 47-54 and 71-82.

In another aspect, the invention relates to the use of the hybrid nuclease-PK molecules with altered glycosylation for manufacturing a medicament for treating diseases characterized by defective clearance or processing of apoptotic cells and cell debris, such as SLE. In some embodiments, the hybrid nuclease-PK molecule comprise a polypeptide with altered glycosylation comprising an amino acid sequence set forth in SEQ ID NOs: 14-21, 47-54 and 71-82.

Definitions

Terms used in the claims and specification are defined as set forth below unless otherwise specified. In the case of direct conflict with a term used in a parent provisional patent application, the term used in the instant specification shall control.

"Amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g. , hydroxyproline, γ-carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., an a carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refer to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that function in a manner similar to a naturally occurring amino acid.

Amino acids can be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, can be referred to by their commonly accepted single-letter codes.

An "amino acid substitution" refers to the replacement of at least one existing amino acid residue in a predetermined amino acid sequence (an amino acid sequence of a starting polypeptide) with a second, different "replacement" amino acid residue. An "amino acid insertion" refers to the incorporation of at least one additional amino acid into a predetermined amino acid sequence. While the insertion will usually consist of the insertion of one or two amino acid residues, larger "peptide insertions" can be made, e.g. insertion of about three to about five or even up to about ten, fifteen, or twenty amino acid residues. The inserted residue(s) may be naturally occurring or non-naturally

occurring as disclosed above. An "amino acid deletion" refers to the removal of at least one amino acid residue from a predetermined amino acid sequence.

"Polypeptide," "peptide," and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer.

"Nucleic acid" refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions) and complementary sequences, as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions can be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., *Nucleic Acid Res* 1991;19:5081; Ohtsuka et al., *JBC* 1985;260:2605-8); Rossolini et al., *Mol Cell Probes* 1994;8:91-8). For arginine and leucine, modifications at the second base can also be conservative. The term nucleic acid is used interchangeably with gene, cDNA, and mRNA encoded by a gene.

Polynucleotides of the present invention can be composed of any polyribonucleotide or polydeoxribonucleotide, which can be unmodified RNA or DNA or modified RNA or DNA. For example, polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that can be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the polynucleotide can be composed of triple-stranded regions comprising RNA or DNA or

both RNA and DNA. A polynucleotide can also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

As used herein, the term "operably linked" or "operably coupled" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner.

As used herein, the term "glycosylation" or "glycosylated" refers to a process or result of adding sugar moieties to a molecule (e.g., a hybrid nuclease-PK molecule).

As used herein, the term "altered glycosylation" refers to a molecule that is aglycosylated, deglycosylated, or underglycosylated.

As used herein, "glycosylation site(s)" refers to both sites that potentially could accept a carbohydrate moiety, as well as sites within the protein on which a carbohydrate moiety has actually been attached and includes any amino acid sequence that could act as an acceptor for an oligosaccharide and/or carbohydrate.

As used herein, the term "aglycosylation" or "aglycosylated" refers to the production of a molecule (e.g., a hybrid nuclease-PK molecule) in an unglycosylated form (e.g., by engineering a hybrid nuclease-PK molecule to lack amino acid residues that serve as acceptors of glycosylation). Alternatively, the hybrid nuclease-PK molecule can be expressed in, e.g., *E. coli*, to produce an aglycosylated hybrid nuclease-PK molecule.

As used herein, the term "deglycosylation" or "deglycosylated" refers to the process or result of enzymatic removal of sugar moieties on a molecule.

As used herein, the term "underglycosylation" or "underglycosylated" refers to a molecule in which one or more carbohydrate structures that would normally be present if produced in a mammalian cell has been omitted, removed, modified, or masked.

The term "pharmacokinetic moiety" or "PK moiety" refers to a moiety (e.g., a protein, polypeptide, or compound) that affects the pharmacokinetic properties of a biologically active molecule when operably coupled to the molecule. PK encompasses properties of a compound, such as distribution, metabolism, absorption, and elimination by a subject. Exemplary PK moieties include albumin, Fc, transferrin, and polyethylene glycol (PEG). When a polypeptide having one or more nuclease domains is operably coupled to a PK moiety, it is referred to herein as a "hybrid nuclease-PK molecule."

The term "albumin" refers to a protein having the same, or very similar three dimensional structure as human albumin (SEQ ID NO: 22) and having a long serum half-life. Exemplary albumin proteins include human serum albumin (HSA; SEQ ID NO: 6), chimpanzee serum albumin, gorilla serum albumin, macaque serum albumin, hamster serum albumin, guinea pig serum albumin, mouse serum albumin, rat serum albumin, cow serum albumin, horse serum albumin, donkey serum albumin, rabbit serum albumin, goat serum albumin, sheep serum albumin, dog serum albumin, chicken serum albumin, and pig serum albumin. When a polypeptide comprising one or more nucleases is operably coupled to an albumin, or variant or fragment thereof, the resulting polypeptide is referred to as a "hybrid nuclease-albumin molecule." SEQ ID NOs: 14-21 and 47-54 are examples of hybrid nuclease-albumin molecules with altered glycosylation.

The term "transferrin" refers to a vertebrate glycoprotein that functions to bind and transport iron. Human transferrin is a glycosylated 698 amino acid protein (SEQ ID NO: 23). In a preferred embodiment, the transferrin is human serum transferrin ("HST"; SEQ ID NO: 7), which lacks the 19 amino acid leader found in human transferrin.

The term "polyethylene glycol" or "PEG" as used herein refers to an oligomer or polymer of ethylene oxide. PEG typically has the following structure: $HO\text{-}(CH_2\text{-}CH_2\text{-}O)_n\text{-}H$, wherein n is the average number of repeating oxyethylene units. PEG compounds are

often referred to by their average molecular weight, e.g., “PEG400” would signify PEG having an average molecular weight of 400 daltons. PEG is also known as polyethylene oxide (PEO) or polyoxyethylene (POE) depending on its molecular weight. PEG is commercially available from, e.g., Union Carbide, Dow, and Texaco Chemical. PEG typically has an average molecular weight of between 200 and 6000.

As used herein, the term "Fc region" is defined as the portion of a native immunoglobulin formed by the respective Fc domains (or Fc moieties) of its two heavy chains.

As used herein, the term "Fc domain" refers to a portion of a single immunoglobulin (Ig) heavy chain. An Fc domain can also be referred to as "Ig" or "IgG." The wild type human IgG1 Fc domain has the amino acid sequence set forth in SEQ ID NO: 10.

In some embodiments, an Fc domain begins in the hinge region just upstream of the papain cleavage site and ending at the C-terminus of the antibody. Accordingly, a complete Fc domain comprises at least a hinge domain, a CH2 domain, and a CH3 domain. In certain embodiments, an Fc domain comprises at least one of: a hinge (e.g., upper, middle, and/or lower hinge region) domain, a CH2 domain, a CH3 domain, a CH4 domain, or a variant, portion, or fragment thereof. In other embodiments, an Fc domain comprises a complete Fc domain (i.e., a hinge domain, a CH2 domain, and a CH3 domain). In one embodiment, an Fc domain comprises a hinge domain (or portion thereof) fused to a CH3 domain (or portion thereof). In another embodiment, an Fc domain comprises a CH2 domain (or portion thereof) fused to a CH3 domain (or portion thereof). In another embodiment, an Fc domain consists of a CH3 domain or portion thereof. In another embodiment, an Fc domain consists of a hinge domain (or portion thereof) and a CH3 domain (or portion thereof). In another embodiment, an Fc domain consists of a CH2 domain (or portion thereof) and a CH3 domain. In another embodiment, an Fc domain consists of a hinge domain (or portion thereof) and a CH2 domain (or portion thereof). In one embodiment, an Fc domain lacks at least a portion of a CH2 domain (e.g., all or part of a CH2 domain). In one embodiment, an Fc domain of the invention comprises at least the portion of an Fc molecule known in the art to be required for FcRn binding. In one embodiment, an Fc domain of the invention comprises

at least the portion of an Fc molecule known in the art to be required for Protein A binding. In one embodiment, an Fc domain of the invention comprises at least the portion of an Fc molecule known in the art to be required for protein G binding. An Fc domain herein generally refers to a polypeptide comprising all or part of the Fc domain of an immunoglobulin heavy-chain. This includes, but is not limited to, polypeptides comprising the entire CHI, hinge, CH2, and/or CH3 domains as well as fragments of such peptides comprising only, e.g., the hinge, CH2, and CH3 domain. The Fc domain may be derived from an immunoglobulin of any species and/or any subtype, including, but not limited to, a human IgGl, IgG2, IgG3, IgG4, IgD, IgA, IgE, or IgM antibody. The Fc domain encompasses native Fc and Fc variant molecules. As with Fc variants and native Fc's, the term Fc domain includes molecules in monomeric or multimeric form, whether digested from whole antibody or produced by other means.

As set forth herein, it will be understood by one of ordinary skill in the art that any Fc domain may be modified such that it varies in amino acid sequence from the native Fc domain of a naturally occurring immunoglobulin molecule.

The Fc domains of a polypeptide of the invention may be derived from different immunoglobulin molecules. For example, an Fc domain of a polypeptide may comprise a CH2 and/or CH3 domain derived from an IgGl molecule and a hinge region derived from an IgG3 molecule. In another example, an Fc domain can comprise a chimeric hinge region derived, in part, from an IgGl molecule and, in part, from an IgG3 molecule. In another example, an Fc domain can comprise a chimeric hinge derived, in part, from an IgGl molecule and, in part, from an IgG4 molecule.

The term "activity," when used in the context of a PK moiety, refers to the ability of the PK moiety, or a variant or fragment thereof, to prolong the serum half-life of a polypeptide comprising one or more nuclease domains compared to a nuclease domain not fused to the PK moiety. In the context of, e.g., albumin, "albumin activity" may also refer to the ability of an albumin, or a variant or fragment thereof, to bind to the neonatal Fc receptor (FcRn) receptor, e.g., human FcRn.

The term "wild-type" (WT) PK moiety means a PK moiety (such as albumin, Fc, or transferrin) having the same amino acid sequence as naturally found in an animal or in a human being. In one embodiment, the wild type albumin is a HSA with the amino acid sequence set forth in SEQ ID NO: 6. In another embodiment, the wild type transferrin is HST, which has the amino acid sequence set forth in SEQ ID NO: 7. In another embodiment, the wild type Fc is a human IgG1 Fc domain with the amino acid sequence set forth in SEQ ID NO: 10.

The term "variant," when used in the context of a PK moiety, refers to a polypeptide derived from a wild-type PK moiety and differs from the wild-type PK moiety by one or more alteration(s), i.e., a substitution, insertion, and/or deletion, at one or more positions. A substitution means a replacement of an amino acid occupying a position with a different amino acid. A deletion means removal of an amino acid occupying a position. An insertion means adding 1 or more, such as 1-3 amino acids, immediately adjacent to an amino acid occupying a position. Variant PK moieties necessarily have less than 100% sequence identity or similarity with the wild-type PK moiety. In a preferred embodiment, the variant PK moiety will have an amino acid sequence from about 75% to less than 100% amino acid sequence identity or similarity with the amino acid sequence of the wild-type PK moiety, more preferably from about 80% to less than 100%, more preferably from about 85% to less than 100%, more preferably from about 90% to less than 100% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%o, 99%) and most preferably from about 95% to less than 100%, e.g., over the length of the variant molecule.

The term "fragment," when used in the context of a PK moiety, refers to any fragment of the full-length PK moiety or a variant thereof that retains the ability to extend the serum half-life of one or more nuclease domains to which it is fused or conjugated to relative to the corresponding non-fused nuclease domain.

As used herein, the term "serum half-life" refers to the time required for the *in vivo* serum hybrid nuclease-PK molecule with altered glycosylation to decline by 50%. The shorter the serum half-life of the molecule, the shorter time it will have to exert a therapeutic

effect, although in some embodiments as discussed *infra*, a shorter serum half-life of the polypeptide. A "longer serum half-life" and similar expressions are understood to be in relationship to the corresponding glycosylated hybrid nuclease-PK molecule (e.g., a hybrid nuclease-PK molecule which retains residues that potentially undergo N-glycoyslation). Thus, a hybrid nuclease-PK molecule with altered glycosylation with longer serum half-life means that the molecule has a longer serum half-life than the corresponding glycosylated hybrid nuclease-PK molecule.

In certain aspects, the hybrid nuclease-PK molecules with altered glycosylation of the invention can employ one or more "linker domains," such as polypeptide linkers. As used herein, the term "linker domain" refers to a sequence which connects two or more domains in a linear sequence. As used herein, the term "polypeptide linker" refers to a peptide or polypeptide sequence (e.g., a synthetic peptide or polypeptide sequence) which connects two or more domains in a linear amino acid sequence of a polypeptide chain. For example, polypeptide linkers may be used to connect a nuclease domain to a PK moiety, e.g., an albumin, or a variant or fragment thereof. Preferably, such polypeptide linkers can provide flexibility to the hybrid nuclease-PK molecule with altered glycosylation. In certain embodiments the polypeptide linker is used to connect (e.g., genetically fuse) a PK moiety, or a variant or fragment thereof, with one or more nuclease domains. A hybrid nuclease-PK molecule with altered glycosylation of the invention may comprise more than one linker domain or peptide linker. Various peptide linkers are known in the art.

As used herein, the term "gly-ser polypeptide linker" refers to a peptide that consists of glycine and serine residues. An exemplary gly/ser polypeptide linker comprises the amino acid sequence $(Gly_4Ser)_n$. In some embodiments, n is 1 or more, such as 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, or 10 or more (e.g., $(Gly_4Ser)_{10}$). Another exemplary gly/ser polypeptide linker comprises the amino acid sequence $Ser(Gly_4Ser)_n$. In some embodiments, n is 1 or more, such as 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, or 10 or more (e.g., $Ser(Gly_4Ser)_{10}$).

As used herein, the terms “coupled,” “linked,” “fused,” or “fusion,” are used interchangeably. These terms refer to the joining together of two more elements or components or domains, by whatever means including chemical conjugation or recombinant means. Methods of chemical conjugation (e.g., using heterobifunctional crosslinking agents) are known in the art.

A polypeptide or amino acid sequence "derived from" a designated polypeptide or protein refers to the origin of the polypeptide. Preferably, the polypeptide or amino acid sequence which is derived from a particular sequence has an amino acid sequence that is essentially identical to that sequence or a portion thereof, wherein the portion consists of at least 10-20 amino acids, preferably at least 20-30 amino acids, more preferably at least 30-50 amino acids, or which is otherwise identifiable to one of ordinary skill in the art as having its origin in the sequence. Polypeptides derived from another peptide may have one or more mutations relative to the starting polypeptide, e.g., one or more amino acid residues which have been substituted with another amino acid residue or which has one or more amino acid residue insertions or deletions.

In one embodiment, there is one amino acid difference between a starting polypeptide sequence and the sequence derived therefrom. Identity or similarity with respect to this sequence is defined herein as the percentage of amino acid residues in the candidate sequence that are identical (i.e., same residue) with the starting amino acid residues, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity.

In one embodiment, a polypeptide of the invention consists of, consists essentially of, or comprises an amino acid sequence selected from Table 1 and functionally active variants thereof. In an embodiment, a polypeptide includes an amino acid sequence at least 80%, such as at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to an amino acid sequence set forth in Table 1. In some embodiments, a polypeptide includes a contiguous amino acid sequence at least 80%, such as at least 81%,

at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a contiguous amino acid sequence set forth in Table 1. In some embodiments, a polypeptide includes an amino acid sequence having at least 10, such as at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 100, at least 200, at least 300, at least 400, or at least 500 (or any integer within these numbers) contiguous amino acids of an amino acid sequence set forth in Table 1.

In some embodiments, the polypeptides of the invention are encoded by a nucleotide sequence. Nucleotide sequences of the invention can be useful for a number of applications, including: cloning, gene therapy, protein expression and purification, mutation introduction, DNA vaccination of a host in need thereof, antibody generation for, e.g., passive immunization, PCR, primer and probe generation, siRNA design and generation (see, e.g., the Dharmacon siDesign website), and the like. In some embodiments, the nucleotide sequence of the invention comprises, consists of, or consists essentially of, a nucleotide sequence that encodes the amino acid sequence of the polypeptides selected from Table 1. In some embodiments, a nucleotide sequence includes a nucleotide sequence at least 80%, such as at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a nucleotide sequence encoding the amino acid sequence of the polypeptides in Table 1. In some embodiments, a nucleotide sequence includes a contiguous nucleotide sequence at least 80%, such as at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a contiguous nucleotide sequence encoding the amino acid sequences set forth in Table 1. In some embodiments, a nucleotide sequence includes a nucleotide sequence having at least 10, such as at least 15, such as at least 20, at least 25, at least 30, at least 35, at least

40, at least 45, at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 100, at least 200, at least 300, at least 400, or at least 500 (or any integer within these numbers) contiguous nucleotides of a nucleotide sequence encoding the amino acid sequences set forth in Table 1.

In some embodiments, polypeptide sequences of the invention are not immunogenic and/or have reduced immunogenicity.

It will also be understood by one of ordinary skill in the art that the hybrid nuclease-PK molecules with altered glycosylation of the invention may be altered such that they vary in sequence from the naturally occurring or native sequences from which their components (e.g., nuclease domains, linker domains, and PK moieties) are derived, while retaining the desirable activity of the native sequences. For example, nucleotide or amino acid substitutions leading to conservative substitutions or changes at "non-essential" amino acid residues may be made. An isolated nucleic acid molecule encoding a non-natural variant of a PK moiety derived from a wild type PK moiety can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of the PK moiety such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations may be introduced by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis.

The hybrid nuclease-PK molecules with altered glycosylation of the invention may comprise conservative amino acid substitutions at one or more amino acid residues, e.g., at essential or non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine),

and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, a nonessential amino acid residue in a hybrid nuclease-PK molecule with altered glycosylation is preferably replaced with another amino acid residue from the same side chain family. In another embodiment, a string of amino acids can be replaced with a structurally similar string that differs in order and/or composition of side chain family members. Alternatively, in another embodiment, mutations may be introduced randomly along all or part of a coding sequence, such as by saturation mutagenesis, and the resultant mutants can be incorporated into the hybrid nuclease-PK molecules with altered glycosylation of the invention and screened for their ability to bind to the desired target.

The term "ameliorating" refers to any therapeutically beneficial result in the treatment of a disease state, e.g., an autoimmune disease state (e.g., SLE, Sjogren's syndrome), including prophylaxis, lessening in the severity or progression, remission, or cure thereof.

The term "in situ" refers to processes that occur in a living cell growing separate from a living organism, e.g., growing in tissue culture.

The term "*in vivo*" refers to processes that occur in a living organism.

The term "mammal" or "subject" or "patient" as used herein includes both humans and non-humans and include but is not limited to humans, non-human primates, canines, felines, murines, bovines, equines, and porcines.

The term percent "identity," in the context of two or more nucleic acid or polypeptide sequences, refer to two or more sequences or subsequences that have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned for maximum correspondence, as measured using one of the sequence comparison algorithms described below (e.g., BLASTP and BLASTN or other algorithms available to persons of skill) or by visual inspection. Depending on the application, the percent "identity" can exist over a region of the sequence being compared, e.g., over a functional domain, or, alternatively, exist over the full length of the two sequences to be compared.

For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, *Adv Appl Math* 1981;2:482, by the homology alignment algorithm of Needleman & Wunsch, *J Mol Biol* 1970;48:443, by the search for similarity method of Pearson & Lipman, *PNAS* 1988;85:2444, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by visual inspection (see generally Ausubel et al, *infra*).

One example of an algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul et al., *J Mol Biol* 1990;215:403-10. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information website.

The term "sufficient amount" means an amount sufficient to produce a desired effect, e.g., an amount sufficient to modulate protein aggregation in a cell.

The term "therapeutically effective amount" is an amount that is effective to ameliorate a symptom of a disease. A therapeutically effective amount can be a "prophylactically effective amount" as prophylaxis can be considered therapy.

The term “about” will be understood by persons of ordinary skill and will vary to some extent depending on the context in which it is used. If there are uses of the term which are not clear to persons of ordinary skill given the context in which it is used, “about” will mean up to plus or minus 10% of the particular value.

It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise.

Hybrid nuclease-PK molecules with altered glycosylation

Glycosylation status (e.g., O-linked or N-linked glycosylation) can impact the serum half-life of molecules by, e.g., minimizing their removal from circulation by mannose and asialoglycoprotein receptors and other lectin-like receptors. Accordingly, the present invention, in general, relates to nucleases with altered glycosylation, such as aglycosylated nucleases, deglycosylated nucleases, and underglycosylated nucleases. Preferably, the nuclease is aglycosylated.

In some aspects, the invention relates to a polypeptide comprising one or more nuclease domains (e.g., RNase, DNase), wherein the one or more nuclease domains have altered glycosylation, and preferably include a PK moiety (e.g., an albumin, Fc, transferrin, or PEG, which alters the serum half-life of the polypeptide) which optionally has altered glycosylation. When operably coupled to a PK moiety, the polypeptide is referred to herein as a "hybrid nuclease-PK molecule." In preferred embodiments, the nuclease domain(s) of the hybrid nuclease-PK molecule are aglycosylated. In other embodiments, the entire hybrid nuclease-PK molecule is aglycosylated.

In some embodiments, all potential N-linked glycosylation sites in a polypeptide comprising one or more nuclease domains, or a hybrid nuclease-PK molecule, are eliminated to yield an aglycosylated polypeptide or hybrid nuclease-PK molecule. For example, N-linked glycosylation sites can be eliminated by introducing mutations in the nucleic acid sequence of the polypeptide or hybrid nuclease-PK molecule such that some or all asparagines residues conforming to the Asn-X-Ser/Thr (X can be any other naturally occurring amino acid except for P) consensus for N-linked glycosylation are mutated to residues that do not serve as acceptors of N-linked glycosylation (e.g., serine, glutamine), thereby reducing or eliminating glycosylation. Accordingly, in some embodiments, the polypeptide comprising one or more nuclease domains or hybrid

nuclease-PK molecule is produced in a mammalian cell. In one embodiment, the mammalian cell is a CHO cell. In a specific embodiment, a polypeptide comprising one or more nuclease domains or hybrid nuclease-PK molecule is engineered to eliminate all potential N-glycosylation sites and is expressed in a CHO cell.

In some embodiments, the nuclease domain is operably coupled to the PK moiety, or a variant or fragment thereof, directly. In other embodiments, the nuclease domain is operably coupled to the PK moiety, or a variant or fragment thereof, via a linker domain. In some embodiments, the linker domain is a linker peptide. In some embodiments, the linker domain is a linker nucleotide.

In some embodiments, the hybrid nuclease-PK molecule with altered glycosylation includes a leader molecule, e.g., a leader peptide. In some embodiments, the leader molecule is a leader peptide positioned at the N-terminus of the nuclease domain. In some embodiments, a hybrid nuclease-PK molecule with altered glycosylation of the invention comprises a leader peptide at the N-terminus of the molecule, wherein the leader peptide is later cleaved from the hybrid nuclease-PK molecule with altered glycosylation. Methods for generating nucleic acid sequences encoding a leader peptide fused to a recombinant protein are well known in the art. In some embodiments, any of the hybrid nuclease-PK molecules with altered glycosylation of the present invention can be expressed either with or without a leader fused to their N-terminus. The protein sequence of a hybrid nuclease-PK molecule with altered glycosylation following cleavage of a fused leader peptide can be predicted and/or deduced by one of skill in the art.

Examples of hybrid nuclease-PK molecules with altered glycosylation of the present invention additionally including a VK3 leader peptide (VK3LP), wherein the leader peptide is fused to the N-terminus of the hybrid nuclease-PK molecule with altered glycosylation, are set forth in SEQ ID NOs: 14-21 (RSLV-315, RSLV-316 RSLV-317, RSLV-318, RSLV-319, RSLV-320, RSLV-321, and RSLV-322, respectively). Such leader sequences can improve the level of synthesis and secretion of the hybrid nuclease-

PK molecules with altered glycosylation in mammalian cells. In some embodiments, the leader is cleaved, yielding hybrid nuclease-PK molecules with altered glycosylation having the sequences set forth in SEQ ID NOs: 47-54. In some embodiments, a hybrid nuclease-PK molecule with altered glycosylation is expressed without a leader peptide fused to its N-terminus, and the resulting hybrid nuclease-PK molecule with altered glycosylation has an N-terminal methionine.

In some embodiments, the hybrid nuclease-PK molecule with altered glycosylation will include a stop codon. In some embodiments, the stop codon will be at the C-terminus of the PK moiety, or a variant or fragment thereof. In other embodiments, the stop codon will be at the C-terminus of the nuclease domain (e.g., RNase and/or DNase domain). Appropriate positioning of a stop codon will differ depending on the configuration of components within the hybrid nuclease-PK molecule with altered glycosylation, and will be evident to the skilled artisan.

In some embodiments, the hybrid nuclease-PK molecule with altered glycosylation further includes a second nuclease domain. In some embodiments, the second nuclease domain is operably coupled to the PK moiety, or variant or fragment thereof, via a second linker domain. In some embodiments, the second linker domain will be at the C-terminus of the PK moiety, or a variant or fragment thereof.

Figure 1 displays exemplary configurations of the hybrid nuclease-PK molecules with altered glycosylation.

In some embodiments, a hybrid nuclease-PK molecule with altered glycosylation is an RNase domain or DNase domain or a multi-nuclease domain (e.g., both RNase and DNase or two RNA or DNA nucleases with different specificity for substrate) fused to a PK moiety, or a variant or fragment thereof that specifically binds to extracellular immune complexes. In some embodiments, the PK moiety (e.g., albumin), or a variant or fragment thereof, shows increased binding to the FcRn receptor, thereby increasing the serum half-life and bioavailability of the hybrid nuclease-PK molecule in circulation. In

other embodiments, the hybrid nuclease-PK molecule with altered glycosylation has activity against single and/or double-stranded RNA substrates.

In one embodiment, the nuclease domain is operably coupled (e.g., chemically conjugated or genetically fused (e.g., either directly or via a polypeptide linker)) to the N-terminus of a PK moiety, or a variant or fragment thereof. In another embodiment, the nuclease domain is operably coupled (e.g., chemically conjugated or genetically fused (e.g., either directly or via a polypeptide linker)) to the C-terminus of a PK moiety, or a variant or fragment thereof. In other embodiments, a nuclease domain is operably coupled (e.g., chemically conjugated or genetically fused (e.g., either directly or via a polypeptide linker)) via an amino acid side chain of a PK moiety, or a variant or fragment thereof.

In certain embodiments, the hybrid nuclease-PK molecules with altered glycosylation comprise two or more nuclease domains and at least one PK moiety, or a variant or fragment thereof. For example, nuclease domains may be operably coupled to both the N-terminus and C-terminus of a PK moiety, or a variant or fragment thereof, with optional linkers between the nuclease domains and PK moiety, or variant or fragment thereof. In some embodiments, the nuclease domains are identical, e.g., RNase and RNase, or DNase1 and DNase1. In other embodiments, the nuclease domains are different, e.g., DNase and RNase.

In other embodiments, two or more nuclease domains are operably coupled to each other (e.g., via a polypeptide linker) in series, and the tandem array of nuclease domains is operably coupled (e.g., chemically conjugated or genetically fused (e.g., either directly or via a polypeptide linker)) to either the C-terminus or the N-terminus of a PK moiety, or a variant or fragment thereof. In other embodiments, the tandem array of nuclease domains is operably coupled to both the C-terminus and the N-terminus of a PK moiety, or a variant or fragment thereof.

In other embodiments, one or more nuclease domains may be inserted between two PK moieties, or variants or fragments thereof. For example, one or more nuclease domains

may form all or part of a polypeptide linker of a hybrid nuclease-PK molecule with altered glycosylation.

Preferred hybrid nuclease-PK molecules with altered glycosylation of the invention comprise at least one nuclease domain (e.g., RNase or DNase), at least one linker domain, and at least one PK moiety, or a variant or fragment thereof.

Accordingly, in some embodiments, the hybrid nuclease-PK molecules with altered glycosylation of the invention include a PK moiety, or a variant or fragment thereof, as described *supra*, thereby increasing serum half-life and bioavailability of the hybrid nuclease-PK molecules with altered glycosylation. In some embodiments, a hybrid nuclease-PK molecule with altered glycosylation is as shown in any of SEQ ID NOs: 14-21, 47-54 and 71-82.

It will be understood by the skilled artisan that other configurations of the nuclease domains and PK moiety are possible, with the inclusion of optional linkers between the nuclease domains and/or between the nuclease domains and PK moiety. It will also be understood that domain orientation can be altered, so long as the nuclease domains are active in the particular configuration tested.

In certain embodiments, the hybrid nuclease-PK molecules with altered glycosylation of the invention have at least one nuclease domain specific for a target molecule which mediates a biological effect. In another embodiment, binding of the hybrid nuclease-PK molecules with altered glycosylation of the invention to a target molecule (e.g. DNA or RNA) results in the reduction or elimination of the target molecule, e.g., from a cell, a tissue, or from circulation.

In other embodiments, the hybrid nuclease-PK molecules with altered glycosylation may be assembled together or with other polypeptides to form binding proteins having two or more polypeptides ("multimers"), wherein at least one polypeptide of the multimer is a hybrid nuclease-PK molecule with altered glycosylation. Exemplary multimeric forms include dimeric, trimeric, tetrameric, and hexameric altered binding proteins and the like.

In one embodiment, the polypeptides of the multimer are the same (i.e., homomeric altered binding proteins, e.g., homodimers, homotetramers). In another embodiment, the polypeptides of the multimer are different (e.g., heteromeric).

In some embodiments, the hybrid nuclease-PK molecules with altered glycosylation include a PK moiety, or a variant or fragment thereof, that, e.g., allows binding to the FcRn receptor and thereby increases serum half-life and bioavailability. In some embodiments, the hybrid nuclease-PK molecules can be active towards extracellular immune complexes containing DNA and/or RNA, e.g., either in soluble form or deposited as insoluble complexes.

In some embodiments, the activity of a hybrid nuclease-PK molecule with altered glycosylation is detectable *in vitro* and/or *in vivo*.

In another aspect, a multifunctional RNase or DNase molecule is provided that is attached to another enzyme or antibody having binding specificity, such as an scFv targeted to RNA or DNA or a second nuclease domain with the same or different specificities as the first domain.

In some embodiments, linker domains include (Gly_4Ser) 3, 4 or 5 variants that alter the length of the linker by 5 amino acid progressions. In another embodiment, a linker domain is approximately 18 amino acids in length and includes an N-linked glycosylation site, which can be sensitive to protease cleavage *in vivo*. In some embodiments, an N-linked glycosylation site can protect the hybrid nuclease-PK molecule with altered glycosylation from cleavage in the linker domain. In some embodiments, an N-linked glycosylation site can assist in separating the folding of independent functional domains separated by the linker domain.

In some embodiments, the hybrid nuclease-PK molecule with altered glycosylation includes substantially all or at least an enzymatically active fragment of a DNase. In some embodiments, the DNase is a Type I secreted DNase, preferably a human DNase such as mature human pancreatic DNase 1 (UnitProtKB entry P24855, SEQ ID NO: 3;

human DNase1 precursor, SEQ ID NO: 24). In some embodiments, a naturally occurring variant allele, A114F (SEQ ID NO: 4), which shows reduced sensitivity to actin is included in a DNase1 hybrid nuclease-PK molecule (see Pan et al., *JBC* 1998;273:18374-81; Zhen et al., *BBRC* 1997;231:499-504; Rodriguez et al., *Genomics* 1997;42:507-13). In other embodiments, the DNase1 is a naturally occurring variant allele, G105R (SEQ ID NO: 25), which exhibits high DNase activity relative to wild type DNase1, is included in a DNase1 hybrid nuclease-PK molecule (see Yasuda et al., *Int J Biochem Cell Biol* 2010;42:1216-25). In some embodiments, this mutation is introduced into a hybrid nuclease-PK molecule to generate a more stable derivative of human DNase1. In some embodiments, the DNase is wild type, human DNase1 or mutant, human DNase1 A114F mutated to remove some or all potential N-linked glycosylation sites. In some embodiments, 1 or both sites of N-linked glycosylation are altered, e.g., by substitution with another amino acid residue, e.g., serine. In some embodiments, the asparagine residues at positions 18 and 106 of human DNase1 domain (SEQ ID NO: 3) are altered, e.g., by substitution (i.e., human DNase1 N18S/N106S/A114F, SEQ ID NO: 5), which correspond to asparagine residues at positions 40 and 128, respectively, of full length pancreatic DNase1 with the native leader (SEQ ID NO: 24). In other embodiments, either or both of the asparagine residues at positions 18 and 106 are altered, e.g., by substitution with, e.g., serine, to yield a DNase1 N18S/A114F domain (SEQ ID NO: 26) or DNase1 N106S/A114F domain (SEQ ID NO: 27).

In some embodiments, the DNase is a human DNase1 comprising one or more basic (*i.e.*, positively charged) amino acid substitutions to increase DNase functionality and chromatin cleavage. In some embodiments, basic amino acids are introduced into human DNase1 at the DNA binding interface to enhance binding with negatively charged phosphates on DNA substrates (see e.g., US 7407785 and US 6391607). This hyperactive DNase1 may be referred to as "chromatin cutter."

In some embodiments, 1, 2, 3, 4, 5 or 6 basic amino acid substitutions are introduced into human DNase1. For example, one or more of the following residues is mutated to enhance DNA binding: Gln9, Glu13, Thr14, His44, Asn74, Asn110, Thr205. In some

embodiments one or more of the foregoing amino acids are substituted with basic amino acids such as, arginine, lysine and/or histidine. In one embodiment, a mutant human DNase1 includes one or more of the following substitutions : Q9R, E13R, T14K, H44K, N74K, N110R, T205K. In some embodiments, the mutant human DNase1 also includes an A114F substitution, which reduces sensitivity to actin (see e.g., US 6348343). In one embodiment, the mutant human DNase1 includes the following substitutions: E13R, N74K, A114F and T205K.

In some embodiments, the mutant human DNase1 further includes mutations to remove potential glycosylation sites, *e.g.,* asparagine residues at positions 18 and 106 of the human DNase1 domain set forth in SEQ ID NO:66, which correspond to asparagines residues at positions 40 and 128, respectively of full length pancreatic DNase1 with the native leader (SEQ ID NO:67). In one embodiment, the mutant human DNase1 includes the following substitutions: E13R/N74K/A114F/T205K/N18S/N106S.

In some embodiments, the DNase is DNase 1-like (DNaseL) enzyme, 1-3 (UniProtKB entry Q13609; SEQ ID NO: 28). In some embodiments, the DNase is three prime repair exonuclease 1 (TREX1; UniProtKB entry Q9NSU2; SEQ ID NO: 29). In some embodiments, the DNase is DNase2. In some embodiments, the DNase2 is DNAse2 alpha (i.e., DNase2; UnitProtKB entry O00115SEQ ID NO: 30) or DNase2 beta (i.e., DNase2-like acid DNase; UnitProtKB entry Q8WZ79; SEQ ID NO: 31). In some embodiments, the N-linked glycosylation sites of DNase 1L3, TREX1, DNase2 alpha, or DNase2 beta are mutated such as to remove potential N-linked glycosylation sites.

In some embodiments, a DNase-linker-PK molecule containing a 20 or 25 aa linker domain is made. In some embodiments, hybrid nuclease-PK molecules with altered glycosylation include RNase-PK-linker-DNase1, wherein the DNase1 domain is located at the COOH side of the PK moiety. In other embodiments, hybrid nuclease-PK molecules with altered glycosylation include DNase1-PK-linker-RNase, wherein the DNase1 domain is located at the NH2 side of the PK moiety. In some embodiments, hybrid nuclease-PK molecules with altered glycosylation are made that incorporate DNase1 and include: DNase1-PK, DNase1-linker-PK, PK-DNase1, PK-linker-DNase1,

DNase1-PK-RNase, RNase-PK-DNase1, DNase1-linker-PK-linker-RNase, RNase-linker-PK-linker-DNase1, DNase1-linker-RNase-PK, RNase-linker-DNase1-PK, PK-DNase1-linker-RNase, and PK-RNase-linker DNase1, wherein the RNase domain and/or DNase domain lack potential N-glycosylation sites. In some embodiments, the PK moiety also lacks potential N-glycosylation sites. In these embodiments, RNase can be, for example, human RNase1.

In some embodiments, the DNase enzyme exhibits comparable functional activity regardless of its position in the hybrid nuclease-PK molecule with altered glycosylation. In some embodiments, other hybrid nuclease-PK molecules with altered glycosylation can be designed to test whether a particular configuration demonstrates improved expression and/or function of the hybrid nuclease-PK molecule components.

In some embodiments, a hybrid nuclease-PK molecule with altered glycosylation includes TREX1 (SEQ ID NO: 29). In some embodiments, a TREX1 hybrid nuclease-PK molecule with altered glycosylation can digest chromatin. In some embodiments, a TREX1 hybrid nuclease-PK molecule with altered glycosylation is expressed by a cell. In some embodiments, the expressed hybrid nuclease-PK molecule with altered glycosylation includes murine TREX-1 and a PK moiety, or a variant or fragment thereof. In some embodiments, a hydrophobic region of approximately 72 aa can be removed from the COOH end of TREX-1 prior to fusion to a PK moiety, or a variant or fragment thereof, via the linker domain. In some embodiments, a 20 amino acid linker domain version of the hybrid nuclease-PK molecule with altered glycosylation exhibits high expression levels compared to controls and/or other hybrid nuclease-PK molecules with altered glycosylation. In some embodiments, kinetic enzyme assays are used to compare the enzyme activity of hybrid nuclease-PK molecules with altered glycosylation and controls in a quantitative manner.

In some embodiments, further optimization of the fusion junction chosen for truncation of a TREX1 enzyme can be used to improve expression of the hybrid nuclease-PK molecules with altered glycosylation.

In some embodiments, the hybrid nuclease-PK molecule with altered glycosylation includes a human TREX1-linker-PK domain with 20 and/or 25 aa linker domains. In some embodiments, the linker domain(s) are variants of a $(Gly_4Ser)_4$ or $(Gly_4Ser)_5$ cassette with one or more restriction sites attached for incorporation into the hybrid nuclease-PK molecule construct. In some embodiments, because of the head-to-tail dimerization useful for TREX1 enzyme activity; a flexible, longer linker domain can be used to facilitate proper folding.

In some embodiments, the hybrid nuclease-PK molecule with altered glycosylation is a TREX1-tandem hybrid nuclease-PK molecule. In some embodiments, an alternative method for facilitating head-to-tail folding of TREX1 is to generate a TREX1-TREX1-PK molecule that incorporates two TREX1 domains in tandem, followed by a linker domain and a PK moiety. In some embodiments, positioning of TREX1 cassettes in a head-to-tail manner can be corrected for head-to tail folding on either arm of the immunoenzyme and introduce a single TREX1 functional domain into each arm of the molecule. In some embodiments, each immunoenzyme of a hybrid nuclease-PK molecule with altered glycosylation has two functional TREX1 enzymes attached to a single PK molecule, or a variant or fragment thereof.

In some embodiments, the hybrid nuclease-PK molecule with altered glycosylation includes TREX1-linker1-PK-linker2-RNase. In some embodiments, the hybrid nuclease-PK molecule with altered glycosylation includes RNase-PK-linker-TREX1. In some embodiments, cassettes are derived for both amino and carboxyl fusion of each enzyme for incorporation into hybrid nuclease-PK molecules with altered glycosylation where the enzyme configuration is reversed. In some embodiments, the RNase enzyme exhibits comparable functional activity regardless of its position in the hybrid nuclease-PK molecules with altered glycosylation. In some embodiments, other hybrid nuclease-PK molecules with altered glycosylation can be designed to test whether a particular configuration demonstrates improved expression and/or function of the hybrid nuclease-PK molecule components.

In some embodiments, the hybrid nuclease-PK molecule with altered glycosylation includes DNase 1L3-PK. In some embodiments, the DNase 1L3 is constructed from a human (SEQ ID NO: 28) and murine (SEQ ID NO: 32) sequence and expressed. In some embodiments, a human DNase-1L3-PK-RNase hybrid nuclease-PK molecule with altered glycosylation is constructed and expressed. In some embodiments, the molecule includes human DNase 1L3-PK, human DNase L3-PK-RNase, and/or human RNase-PK-DNase-1L3.

In some embodiments, the hybrid nuclease-PK molecule with altered glycosylation includes DNase2 alpha (SEQ ID NO: 30) or DNase2 beta (SEQ ID NO: 31). In some embodiments, a human DNase2 alpha-PK-RNase or human DNase2 beta-PK-RNase hybrid nuclease-PK molecule with altered glycosylation is constructed and expressed. In some embodiments, the molecule includes human DNase2 alpha-PK, human DNase2 alpha-PK-RNase, and/or human RNase-PK-DNase2 alpha. In other embodiments, the molecule includes human DNase2 beta-PK, human DNase2 beta-PK-RNase, and/or human RNase-PK-DNase2 beta. In preferred embodiments, the DNase2 alpha and/or DNase2 beta in the hybrid nuclease-PK molecules are aglycosylated.

In some embodiments, a hybrid nuclease-PK molecule with altered glycosylation includes an RNase1, preferably human pancreatic RNase1 (UniProtKB entry P07998; SEQ ID NO: 1) of the RNase A family. In some embodiments, the human RNase1 is mutated to remove some or all potential N-linked glycosylation sites, i.e., asparagine residues at positions 34, 76, and 88 of the RNase1 domain set forth in SEQ ID NO: 1 (e.g., RNase1 N34S/N76S/N88S domain; SEQ ID NO: 2), which correspond to asparagine residues at positions 62, 104, and 116, respectively, of full length pancreatic RNase1 with the native leader (SEQ ID NO: 33). In other embodiments, 1, 2 or 3 sites of N-linked glycosylation are altered, e.g., by substitution with another amino acid residue, e.g., serine. Accordingly, in some embodiments, the RNase1 domain is an RNase1 N34S domain (SEQ ID NO: 34), an RNase1 N76S domain (SEQ ID NO: 35), an RNase1 N88S domain (SEQ ID NO: 36), an RNase1 N34S/N76S domain (SEQ ID NO: 37), an RNase1

N34S/N88S domain (SEQ ID NO: 38), or an RNase1 N76S/N88S domain (SEQ ID NO: 39).

In some aspects, the invention provides a polypeptide with altered glycosylation having a human, mutant RNase1 N34S/N76S/N88S, operably linked with or without a linker to a mutant human IgG1 Fc domain optionally having a mutant hinge region (e.g., a cysteine substitution, such as with serine, e.g., SCC), and one or more CH2 mutations to reduce Fcγ receptor binding (e.g., P238S, P331S or both P238S and P331S). In one embodiment, the polypeptide with altered glycosylation has human, mutant RNase1 N34S/N76S/N88S, operably linked to a mutant human IgG1 Fc domain having SCC hinge, P238S and P331S mutations. In yet another embodiment, the Fc domain further includes a mutation at a site of N-linked glycosylation, such as a substitution at N297 (numbering by Kabat). In another embodiment, the polypeptide with altered glycosylation has human, mutant RNase1 N34S/N76S/N88S, operably linked to a mutant human IgG1 Fc domain having SCC hinge, P238S, P331S and N297S mutations. In another embodiment, the polypeptide comprises an amino acid sequence set forth in SEQ ID NOs: 71-74. In other aspects, the polypeptide has an amino acid sequence at least 90% identical or at least 95% identical to the amino acid sequences set forth in SEQ ID NOs: 71-74.

In some embodiments, a RNase1-linker-PK molecule with altered glycosylation includes a 20 or 25 aa linker domain. In some embodiments, the hybrid nuclease-PK molecule with altered glycosylation includes DNase-PK-linker-RNase1, wherein the RNase1 domain is located at the COOH side of the PK moiety. In other embodiments, the hybrid nuclease-PK molecule with altered glycosylation includes RNase1-PK-linker-DNase, wherein the RNase1 domain is located at the NH2 side of the PK moiety. In some embodiments, hybrid nuclease-PK molecules with altered glycosylation are made that incorporate RNase1 and include: RNase1-PK, RNase1-linker-PK, PK-RNase1, PK-linker-RNase1, RNase1-PK-DNase, DNase-PK-RNase1, RNase1-linker-PK-linker-DNase, DNase-linker-PK-linker-RNase1, RNase1-linker-DNase-PK, DNase-linker-RNase1-PK, PK-RNase1-linker-DNase, and PK-DNase-linker-RNase1, wherein the RNase domain and/or DNase domain lack potential N-glycosylation sites. In some

embodiments, the PK moiety also lacks potential N-glycosylation sites. In these embodiments, DNase can be, for example, human DNase1.

In some embodiments, the RNase enzyme exhibits comparable functional activity regardless of its position in the hybrid nuclease-PK molecule with altered glycosylation. In some embodiments, other hybrid nuclease-PK molecules with altered glycosylation can be designed to test whether a particular configuration demonstrates improved expression and/or function of the hybrid nuclease-PK molecule components.

In some embodiments, fusion junctions between enzyme domains and the other domains of the hybrid nuclease-PK molecule with altered glycosylation is optimized.

In some embodiments, the targets of the RNase enzyme activity of a hybrid nuclease-PK molecule with altered glycosylation are primarily extracellular, consisting of, e.g., RNA contained in immune complexes with anti-RNP autoantibody and RNA expressed on the surface of cells undergoing apoptosis. In some embodiments, the hybrid nuclease-PK molecule with altered glycosylation is active in the acidic environment of the endocytic vesicles. In some embodiments, the hybrid nuclease-PK molecule with altered glycosylation is adapted to be active both extracellularly and in the endocytic environment. In some aspects, this allows a hybrid nuclease-PK molecule with altered glycosylation to stop TLR7 signaling through previously engulfed immune complexes or by RNAs that activate TLR7 after viral infection. In some embodiments, the RNase of a hybrid nuclease-PK molecule with altered glycosylation is not resistant to inhibition by an RNase cytoplasmic inhibitor. In some embodiments, the hybrid nuclease-PK molecule with altered glycosylation is not active in the cytoplasm of a cell.

In some embodiments, hybrid nuclease-PK molecules with altered glycosylation include both DNase and RNase. In some embodiments, these hybrid nuclease-PK molecules with altered glycosylation can improve the therapy of SLE because they can, e.g., digest immune complexes containing RNA, DNA, or a combination of both RNA and DNA; and are active both extracellularly and in the endocytic compartment where TLR7 and TLR9 can be located.

In some embodiments, a hybrid nuclease-PK molecule with altered glycosylation retains at least 50%, such as at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.7%, at least 99.9%, or 100% of the nuclease activity (e.g., RNase activity, DNase activity, or both RNase and DNase activity) of the corresponding glycosylated hybrid nuclease-PK molecule (e.g., a hybrid nuclease-PK molecule which retains all potential glycosylation sites).

In some embodiments, a hybrid nuclease-PK molecule with altered glycosylation has a serum half-life that is increased at least about 1.5-fold, such as at least 3-fold, at least 5-fold, at least 10-fold, at least about 20-fold, at least about 50-fold, at least about 100-fold, at least about 200-fold, at least about 300-fold, at least about 400-fold, at least about 500-fold, at least about 600-fold, at least about 700-fold, at least about 800-fold, at least about 900-fold, at least about 1000-fold, or 1000-fold or greater relative to the corresponding glycosylated hybrid nuclease molecules (e.g., a hybrid nuclease-PK molecule which retains all potential glycosylation sites). Routine art-recognized methods can be used to determine the serum half-life of hybrid nuclease-PK molecules with altered glycosylation.

In some embodiments, a hybrid nuclease-PK molecule with altered glycosylation retains at least 50%, such as at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% of the activity of the corresponding glycosylated hybrid nuclease-PK molecule (e.g., a hybrid nuclease-PK molecule which retains all potential glycosylation sites).

In some embodiments, altering the glycosylation of the hybrid nuclease-PK molecules may increase nuclease activity, either by directly increasing enzymatic activity, or by increasing bioavailability (e.g., serum half-life). Accordingly, in some embodiments, the nuclease activity of a hybrid nuclease-PK molecule with altered glycosylation is increased by at least 1.3-fold, such as at least 1.5-fold, at least 2-fold, at least 2.5-fold, at

least 3-fold, at least 3.5-fold, at least 4-fold, at least 4.5-fold, at least 5-fold, at least 5.5-fold, at least 6-fold, at least 6.5-fold, at least 7-fold, at least 7.5-fold, at least 8-fold, at least 8.5-fold, at least 9-fold, at least 9.5 fold, or 10-fold or greater, relative to the corresponding glycosylated hybrid nuclease-PK molecule (e.g., a hybrid nuclease-PK molecule which retains all potential glycosylation sites).

In some embodiments, the activity of the RNase in the hybrid nuclease-PK molecule with altered glycosylation is not less than about 10-fold less, such as 9-fold less, 8-fold less, 7-fold less, 6-fold less, 5-fold less, 4-fold less, 3-fold less, or 2-fold less than the activity of a control RNase molecule. In some embodiments, the activity of the RNase in the hybrid nuclease-PK molecule with altered glycosylation is about equal to the activity of a control RNase molecule.

In some embodiments, the activity of the DNase in the hybrid nuclease-PK molecule with altered glycosylation is not less than about 10-fold less, such as 9-fold less, 8-fold less, 7-fold less, 6-fold less, 5-fold less, 4-fold less, 3-fold less, or 2-fold less than the activity of a control DNase molecule. In some embodiments, the activity of the DNase in the hybrid nuclease-PK molecule with altered glycosylation is about equal to the activity of a control DNase molecule.

The skilled artisan can readily determine the glycosylation status of hybrid nuclease-PK molecules using art-recognized methods. In a preferred embodiment, the glycosylation status is determined using mass spectrometry. In other embodiments, interactions with Concanavalin A (Con A) can be assessed to determine whether a hybrid nuclease-PK molecule has altered glycosylation. An hybrid nuclease-PK molecule with reduced glycosylation is expected to exhibit reduced binding to Con A-Sepharose when compared to the corresponding glycosylated hybrid nuclease-PK molecule (e.g., a hybrid nuclease-PK molecule which retains all potential glycosylation sites). SDS-PAGE analysis can also be used to compare the mobility of an underglycosylated protein and corresponding glycosylated protein. The underglycosylated protein is expected to have a greater mobility in SDS-PAGE compared to the glycosylated protein. Other suitable art-

recognized methods for analyzing protein glycosylation status are disclosed in, e.g., Roth et al., *International Journal of Carbohydrate Chemistry* 2012;2012:1-10.

Pharmacokinetics, such as serum half-life, of hybrid nuclease-PK molecules with altered glycosylation can be assayed using routine methods, e.g., by introducing the hybrid nuclease-PK molecules with altered glycosylation into mice, e.g., intravenously, taking blood samples at pre-determined time points, and assaying and comparing levels and/or enzymatic activity of the hybrid nuclease-PK molecules with altered glycosylation in the samples.

RSLV-132 and RSLV-133

RSLV-132 and RSLV-133 are nuclease fusion constructs engineered on the Fc scaffold of human IgG1, described in WO 2012/149440, herein incorporated by reference. The Fc portion extends the circulatory half-life of the constructs *in vivo*.

RSLV-132 contains human RNase linked (without a linker) to the N-terminus of a mutant human IgG1 Fc region P238S, P331S having a cysteine substitution in the hinge region (SCC).

RSLV-133 contains RNase1 fused to the N-terminus the IgG1 Fc scaffold and DNase1 fused to the C-terminus (with an 18 amino acid linker inserted between the DNase and Fc domains). In RSLV-133 the DNase domain is positioned at the C-terminus of the Fc domain via an eighteen amino acid linker sequence composed of: VDGASSPVNVSSPSVQDI. This linker contains a potential site of N-linked glycosytalion (NVS sequon).

Examples of hybrid nuclease molecules of the present invention additionally including a VK3 leader peptide (VK3LP), wherein the leader peptide is fused to the N-terminus of the hybrid nuclease molecule, are set forth in SEQ ID NOS: 99 (RSLV-132) and 101 (RSLV-133). The corresponding nucleotide sequences are set forth in SEQ ID NOS: 100 and 102, respectively. In certain embodiments, following cleavage of the VK3 leader, these hybrid nuclease molecules have the sequences as set forth in SEQ ID NOS: 103

(RSLV-132) and 105 (RSLV-133), respectively. The corresponding nucleotide sequences are set forth in SEQ ID NOS: 104 and 106, respectively. In some embodiments, a hybrid nuclease molecule of the present invention is expressed without a leader peptide fused to its N-terminus, and the resulting hybrid nuclease molecule has an N-terminal methionine.

RSLV-132 molecules with altered glycosylation

In some aspects, the invention provides an aglycosylated RSLV-132 molecule. RSLV-132 is a fusion construct composed of human RNase1 operably linked to a mutant human IgG1 Fc domain. Human RNase1 contains 3 sites of N-linked glycosylation. These sites are conserved in RSLV-132 and a fourth N-linked site resides within the Fc region. The four potential sites for N-linked glycosylation (mature protein) correspond to: Asn^{34}, Asn^{76}, and Asn^{88} within the RNase domain and Asn^{211} within the Fc domain. Based on structural studies conducted on the RSLV-132, all four potential sites of N-linked glycosylation are utilized. However, the efficiency at which the individual sites are glycosylated varies; the occupancy rates being 28%, 26%, 55%, and 97% for Asn^{34}, Asn^{76}, Asn^{88}, and Asn^{211}, respectively. Moreover, the N-linked oligosaccharides are processed to a wide array of structures, with G2F and G1F species predominating, and terminally sialylated to various degrees. As a result, the RSLV-132 drug substance is heterogeneous in nature; this is readily visualized following SDS gel electrophoresis (reducing conditions)(Figure 3A). The extent to which the N-linked oligosaccharides affect human RNase1 activity is unknown. Bovine pancreatic RNase A shares 72% identity with human RNase1 but is aglycosylated; despite the absence of N-linked oligosaccharides, bovine RNase demonstrates high catalytic activity. Bovine pancreatic RNase is also produced in a number of glycosylated isoforms (B, C, and D) possessing the same amino acid sequence as RNase A. When the pharmacokinetic (PK) properties of the glycosylated variants were analyzed in rats, differences in the behavior were noted. Therefore, aglycosylated variants of RSLV-132 were generated to determine whether the absence of N-linked oligosaccharides altered the RNase activity and/or PK properties of the Fc fusion constructs.

PK moieties

In a preferred embodiment, the polypeptide comprising one or more nuclease domains is operably coupled to a PK moiety, which serves as a scaffold as well as a means to increase the serum half-life of the polypeptide, wherein the one or more nuclease domains and/or the PK moiety is aglycosylated, deglycosylated, or underglycosylated.

Suitable PK moieties are well-known in the art and include, but are not limited to, albumin, transferrin, Fc, and their variants, and polyethylene glycol (PEG) and its derivatives. Suitable PK moieties include, but are not limited to, HSA, or variants or fragments thereof, such as those disclosed in US 5,876,969, WO 2011/124718, and WO 2011/0514789; Fc and Fc variants, such as those disclosed in WO2011/053982, WO 02/060955, WO 02/096948, WO05/047327, WO05/018572, and US 2007/0111281; transferrin, or variants or fragments thereof, as disclosed in US 7,176,278 and US 8,158,579; and PEG or derivatives, such as those disclosed in Zalipsky et al. ("Use of Functionalized Poly(Ethylene Glycols) for Modification of Polypeptides" in Polyethylene Glycol Chemistry: Biotechnical and Biomedical Applications, J. M. Harris, Plenus Press, New York (1992)), and in Zalipsky et al. *Advanced Drug Reviews* 1995:16: 157-182), and US Pat. Nos. 4,640,835, 4,496,689, 4,301,144, 4,670,417, 4,791,192, 4,179,337, and 5,932,462 (the contents of the foregoing are incorporated herein by reference). It is within the abilities of the skilled artisan to use routine methods to introduce PK moieties (e.g., cloning, conjugation) into the hybrid nuclease-PK molecules with altered glycosylation of the invention.

In some embodiments, the PK moiety is HSA (SEQ ID NO: 6), which is naturally aglycosylated.

In some embodiments, the PK moiety is a wild type Fc (SEQ ID NO: 10).

In certain embodiments, an Fc domain is altered or modified, e.g., by amino acid mutation (e.g., addition, deletion, or substitution). As used herein, the term "Fc domain variant" refers to an Fc domain having at least one amino acid modification, such as an amino acid substitution, as compared to the wild-type Fc from which the Fc domain is

derived. For example, wherein the Fc domain is derived from a human IgG1 antibody, a variant comprises at least one amino acid mutation (e.g., substitution) as compared to a wild type amino acid at the corresponding position of the human IgG1 Fc region.

The amino acid substitution(s) of an Fc variant may be located at a position within the Fc domain referred to as corresponding to the portion number that that residue would be given in an Fc region in an antibody.

In one embodiment, the Fc variant comprises a substitution at an amino acid position located in a hinge domain or portion thereof. In another embodiment, the Fc variant comprises a substitution at an amino acid position located in a CH2 domain or portion thereof. In another embodiment, the Fc variant comprises a substitution at an amino acid position located in a CH3 domain or portion thereof. In another embodiment, the Fc variant comprises a substitution at an amino acid position located in a CH4 domain or portion thereof.

In some embodiments, the PK moiety is an Fc region with a mutation at N83 (i.e., N297 by Kabat numbering), yielding an aglycosylated Fc region (e.g., Fc N83S; SEQ ID NO: 11). In some embodiments, the Fc domain includes mutations in one or more of the three hinge cysteines. In some embodiments, one or more of the three hinge cysteines in the Fc domain (SEQ ID NO: 10) can be mutated to SCC (SEQ ID NO: 40) or SSS (SEQ ID NO: 41), where in "S" represents an amino acid substitution of cysteine with serine. Accordingly "SCC" indicates an amino acid substitution to serine of only the first cysteine of the three hinge region cysteines, whereas "SSS" indicates that all three cysteines in the hinge region are substituted with serine.

In some aspects, the PK moiety is a mutant human IgG1 Fc domain. In some aspects, a mutant Fc domain comprises one or more mutations in the hinge, CH2, and/or CH3 domains. In some aspects, a mutant Fc domain includes a P238S mutation. In some aspects, a mutant Fc domain includes a P331S mutation. In some aspects, a mutant Fc domain includes a P238S mutation and a P331S mutation. In some aspects, a mutant Fc domain comprises P238S and/or P331S, and may include mutations in one or more of the

three hinge cysteines. In some aspects, a mutant Fc domain comprises P238S and/or P331S, and/or one or more mutations in the three hinge cysteines. In some aspects, a mutant Fc domain comprises P238S and/or P331S, and/or mutations in a hinge cysteine to SCC or in the three hinge cysteines to SSS. In some aspects, a mutant Fc domain comprises P238S and P331S and mutations in at least one of the three hinge cysteines. In some aspects, a mutant Fc domain comprises P238S and P331S and SCC. In some aspects, a mutant Fc domain comprises P238S and P331S and SSS. In some aspects, a mutant Fc domain includes P238S and SCC or SSS. In some aspects, a mutant Fc domain includes P331S and SCC or SSS.

In some aspects, a mutant Fc domain includes a mutation at a site of N-linked glycosylation, such as N297, e.g., a substitution of asparagine for another amino acid such as serine, e.g., N297S. In some aspects, a mutant Fc domain includes a mutation at a site of N-linked glycosylation, such as N297, e.g., a substitution of asparagine for another amino acid such as serine, e.g., N297S and a mutation in one or more of the three hinge cysteines. In some aspects, a mutant Fc domain includes a mutation at a site of N-linked glycosylation, such as N297, e.g., a substitution of asparagine for another amino acid such as serine, e.g., N297S and mutations in one of the three hinge cysteines to SCC or all three cysteines to SSS. In some aspects, a mutant Fc domain includes a mutation at a site of N-linked glycosylation, such as N297, e.g., a substitution of asparagine for another amino acid such as serine, e.g., N297 and one or more mutations in the CH2 domain which decrease FcγR binding and/or complement activation, such as mutations at P238 or P331 or both, e.g., P238S or P331S or both P238S and P331S. In some aspects, such mutant Fc domains can further include a mutation in the hinge region, e.g., SCC or SSS. In some aspects, mutant Fc domain is as shown in SEQ ID NOs: 40, 41 and 65-70.

The molecules of the invention may employ art-recognized Fc variants which are known to impart an alteration in effector function and/or FcR binding. For example, a change (e.g., a substitution) at one or more of the amino acid positions disclosed in International PCT Publications WO88/07089A1, WO96/14339A1, WO98/05787A1, WO98/23289A1, WO99/51642A1, WO99/58572A1, WO00/09560A2, WO00/32767A1, WO00/42072A2, WO02/44215A2, WO02/060919A2, WO03/074569A2, WO04/016750A2,

WO04/029207A2, WO04/035752A2, WO04/063351 A2, WO04/074455A2, WO04/099249A2, WO05/040217A2, WO04/044859, WO05/070963A1, WO05/077981A2, WO05/092925A2, WO05/123780A2, WO06/019447A1, WO06/047350A2, and WO06/085967A2; US Patent Publication Nos. US2007/0231329, US2007/0231329, US2007/0237765, US2007/0237766, US2007/0237767, US2007/0243188, US20070248603, US20070286859, US20080057056; or U.S. Pat. Nos. 5,648,260; 5,739,277; 5,834,250; 5,869,046; 6,096,871; 6,121,022; 6,194,551; 6,242,195; 6,277,375; 6,528,624; 6,538,124; 6,737,056; 6,821,505; 6,998,253; 7,083,784; and 7,317,091, each of which is incorporated by reference herein. In one embodiment, the specific change (e.g., the specific substitution of one or more amino acids disclosed in the art) may be made at one or more of the disclosed amino acid positions. In another embodiment, a different change at one or more of the disclosed amino acid positions (e.g., the different substitution of one or more amino acid position disclosed in the art) may be made.

Other amino acid mutations in the Fc domain are contemplated to reduce binding to the Fc gamma receptor and Fc gamma receptor subtypes. The assignment of amino acids residue numbers to an Fc domain is in accordance with the definitions of Kabat. *See, e.g., Sequences of Proteins of Immunological Interest* (Table of Contents, Introduction and Constant Region Sequences sections), 5th edition, Bethesda, MD:NIH vol. 1:647-723 (1991); Kabat et al., "Introduction" *Sequences of Proteins of Immunological Interest*, US Dept of Health and Human Services, NIH, 5th edition, Bethesda, MD vol. 1:xiii-xcvi (1991); Chothia & Lesk, *J. Mol. Biol.* 196:901-917 (1987); Chothia et al., *Nature* 342:878-883 (1989), each of which is herein incorporated by reference for all purposes." For example, mutations at positions 238, 239, 248, 249, 252, 254, 255, 256, 258, 265, 267, 268, 269, 270, 272, 279, 280, 283, 285, 298, 289, 290, 292, 293, 294, 295, 296, 298, 301, 303, 305, 307, 312, 315, 322, 324, 327, 329, 330, 331, 333, 334, 335, 337, 338, 340, 356, 360, 373, 376, 378, 379, 382, 388, 389, 398, 414, 416, 419, 430, 434, 435, 437, 438 or 439 of the Fc region can alter binding as described in U.S. Pat. No. 6,737,056, issued May 18, 2004, incorporated herein by reference in its entirety. This patent reported that changing Pro331 in IgG3 to Ser resulted in six fold lower affinity as compared to

unmutated IgG3, indicating the involvement of Pro331 in Fc gamma RI binding. In addition, amino acid modifications at positions 234, 235, 236, and 237, 297, 318, 320 and 322 are disclosed as potentially altering receptor binding affinity in U.S. 5,624,821, issued April 29, 1997 and incorporated herein by reference in its entirety.

Further mutations contemplated for use include, e.g., those described in U.S. Pat. App. Pub. No. 2006/0235208, published October 19, 2006 and incorporated herein by reference in its entirety. This publications describe Fc variants that exhibit reduced binding to Fc gamma receptors, reduced antibody dependent cell-mediated cytotoxicity, or reduced complement dependent cytotoxicity, that comprise at least one amino acid modification in the Fc region, including 232G, 234G, 234H, 235D, 235G, 235H, 236I, 236N, 236P, 236R, 237K, 237L, 237N, 237P, 238K, 239R, 265G, 267R, 269R, 270H, 297S, 299A, 299I, 299V, 325A, 325L, 327R, 328R, 329K, 330I, 330L, 330N, 330P, 330R, and 331L (numbering is according to the EU index), as well as double mutants 236R/237K, 236R/325L, 236R/328R, 237K/325L, 237K/328R, 325L/328R, 235G/236R, 267R/269R, 234G/235G, 236R/237K/325L, 236R/325L/328R, 235G/236R/237K, and 237K/325L/328R. Other mutations contemplated for use as described in this publication include 227G, 234D, 234E, 234G, 234I, 234Y, 235D, 235I, 235S, 236S, 239D, 246H, 255Y, 258H, 260H, 2641, 267D, 267E, 268D, 268E, 272H, 272I, 272R, 281D, 282G, 283H, 284E, 293R, 295E, 304T, 324G, 324I, 327D, 327A, 328A, 328D, 328E, 328F, 328I, 328M, 328N, 328Q, 328T, 328V, 328Y, 330I, 330L, 330Y, 332D, 332E, 335D, an insertion of G between positions 235 and 236, an insertion of A between positions 235 and 236, an insertion of S between positions 235 and 236, an insertion of T between positions 235 and 236, an insertion of N between positions 235 and 236, an insertion of D between positions 235 and 236, an insertion of V between positions 235 and 236, an insertion of L between positions 235 and 236, an insertion of G between positions 235 and 236, an insertion of A between positions 235 and 236, an insertion of S between positions 235 and 236, an insertion of T between positions 235 and 236, an insertion of N between positions 235 and 236, an insertion of D between positions 235 and 236, an insertion of V between positions 235 and 236, an insertion of L between positions 235 and 236, an insertion of G between positions 297 and 298, an insertion of A between

positions 297 and 298, an insertion of S between positions 297 and 298, an insertion of D between positions 297 and 298, an insertion of G between positions 326 and 327, an insertion of A between positions 326 and 327, an insertion of T between positions 326 and 327, an insertion of D between positions 326 and 327, and an insertion of E between positions 326 and 327 (numbering is according to the EU index). Additionally, mutations described in U.S. Pat. App. Pub. No. 2006/0235208 include 227G/332E, 234D/332E, 234E/332E, 234Y/332E, 234I/332E, 234G/332E, 235I/332E, 235S/332E, 235D/332E, 235E/332E, 236S/332E, 236A/332E, 236S/332D, 236A/332D, 239D/268E, 246H/332E, 255Y/332E, 258H/332E, 260H/332E, 264I/332E, 267E/332E, 267D/332E, 268D/332D, 268E/332D, 268E/332E, 268D/332E, 268E/330Y, 268D/330Y, 272R/332E, 272H/332E, 283H/332E, 284E/332E, 293R/332E, 295E/332E, 304T/332E, 324I/332E, 324G/332E, 324I/332D, 324G/332D, 327D/332E, 328A/332E, 328T/332E, 328V/332E, 328I/332E, 328F/332E, 328Y/332E, 328M/332E, 328D/332E, 328E/332E, 328N/332E, 328Q/332E, 328A/332D, 328T/332D, 328V/332D, 328I/332D, 328F/332D, 328Y/332D, 328M/332D, 328D/332D, 328E/332D, 328N/332D, 328Q/332D, 330L/332E, 330Y/332E, 330I/332E, 332D/330Y, 335D/332E, 239D/332E, 239D/332E/330Y, 239D/332E/330L, 239D/332E/330I, 239D/332E/268E, 239D/332E/268D, 239D/332E/327D, 239D/332E/284E, 239D/268E/330Y, 239D/332E/268E/330Y, 239D/332E/327A, 239D/332E/268E/327A, 239D/332E/330Y/327A, 332E/330Y/268 E/327A, 239D/332E/268E/330Y/327A, Insert G>297-298/332E, Insert A>297-298/332E, Insert S>297-298/332E, Insert D>297-298/332E, Insert G>326-327/332E, Insert A>326-327/332E, Insert T>326-327/332E, Insert D>326-327/332E, Insert E>326-327/332E, Insert G>235-236/332E, Insert A>235-236/332E, Insert S>235-236/332E, Insert T>235-236/332E, Insert N>235-236/332E, Insert D>235-236/332E, Insert V>235-236/332E, Insert L>235-236/332E, Insert G>235-236/332D, Insert A>235-236/332D, Insert S>235-236/332D, Insert T>235-236/332D, Insert N>235-236/332D, Insert D>235-236/332D, Insert V>235-236/332D, and Insert L>235-236/332D (numbering according to the EU index) are contemplated for use. The mutant L234A/L235A is described, e.g., in U.S. Pat. App. Pub. No. 2003/0108548, published June 12, 2003 and incorporated herein by reference in its entirety. In embodiments, the described modifications are included either individually or in combination.

In some embodiments, the PK moiety is a wild type HST (SEQ ID NO: 7). In other embodiments, the PK moiety is a HST with a mutations at N413 and/or N611 and/or S12 (S12 is a potential O-linked glycosylation site), yielding a HST with altered glycosylation (i.e., HST N413S, SEQ ID NO: 42; HST N611S, SEQ ID NO: 43; HST N413S/N611S, SEQ ID NO: 8); and HST S12A/N413S/N611S, SEQ ID NO: 9).

Linker Domains

In some embodiments, a hybrid nuclease-PK molecule with altered glycosylation includes a linker domain. In some embodiments, a hybrid nuclease-PK molecule with altered glycosylation includes a plurality of linker domains. In some embodiments, the linker domain is a polypeptide linker. In certain aspects, it is desirable to employ a polypeptide linker to fuse the PK moiety, or a variant or fragment thereof, with one or more nuclease domains to form a hybrid nuclease-PK molecule with altered glycosylation.

In one embodiment, the polypeptide linker is synthetic. As used herein, the term "synthetic" with respect to a polypeptide linker includes peptides (or polypeptides) which comprise an amino acid sequence (which may or may not be naturally occurring) that is linked in a linear sequence of amino acids to a sequence (which may or may not be naturally occurring) to which it is not naturally linked in nature. For example, the polypeptide linker may comprise non-naturally occurring polypeptides which are modified forms of naturally occurring polypeptides (e.g., comprising a mutation such as an addition, substitution or deletion) or which comprise a first amino acid sequence (which may or may not be naturally occurring). The polypeptide linkers of the invention may be employed, for instance, to ensure that the PK moiety, or a variant or fragment thereof, is juxtaposed to ensure proper folding and formation of a functional PK moiety, or a variant or fragment thereof. Preferably, a polypeptide linker compatible with the instant invention will be relatively non-immunogenic and not inhibit any non-covalent association among monomer subunits of a binding protein.

In certain embodiments, the hybrid nuclease-PK molecules with altered glycosylation employ a polypeptide linker to join any two or more domains in frame in a single polypeptide chain. In one embodiment, the two or more domains may be independently selected from any of the PK moieties, or variants or fragments thereof, or nuclease domains discussed herein. For example, in certain embodiments, a polypeptide linker can be used to fuse identical PK moiety fragments, thereby forming a homomeric PK moiety region. In other embodiments, a polypeptide linker can be used to fuse different PK moiety fragments, thereby forming a heteromeric PK moiety region.

In one embodiment, a polypeptide linker comprises a portion of a PK moiety, or a variant or fragment thereof. For example, in one embodiment, a polypeptide linker can comprise an albumin fragment (e.g., domain I, II, or III), or a different portion of an albumin or different PK moiety or variant thereof.

In another embodiment, a polypeptide linker comprises or consists of a gly-ser linker. As used herein, the term "gly-ser linker" refers to a peptide that consists of glycine and serine residues. An exemplary gly/ser linker comprises an amino acid sequence of the formula $(Gly_4Ser)n$, wherein n is a positive integer (e.g., 1, 2, 3, 4, or 5). In certain embodiments the gly/ser linker is $(Gly_4Ser)_1$. In certain embodiments the gly/ser linker is $(Gly_4Ser)_2$. In certain embodiments the gly/ser linker is $(Gly_4Ser)_3$. In certain embodiments the gly/ser linker is $(Gly_4Ser)_4$. In certain embodiments the gly/ser linker is $(Gly_4Ser)_5$. In certain embodiments, the gly-ser linker may be inserted between two other sequences of the polypeptide linker (e.g., any of the polypeptide linker sequences described herein). In other embodiments, a gly-ser linker is attached at one or both ends of another sequence of the polypeptide linker (e.g., any of the polypeptide linker sequences described herein). In yet other embodiments, two or more gly-ser linker are incorporated in series in a polypeptide linker.

In other embodiments, a polypeptide linker of the invention comprises a biologically relevant peptide sequence or a sequence portion thereof. For example, a biologically relevant peptide sequence may include, but is not limited to, sequences derived from an

anti-rejection or anti-inflammatory peptide. Said anti-rejection or anti-inflammatory peptides may be selected from the group consisting of a cytokine inhibitory peptide, a cell adhesion inhibitory peptide, a thrombin inhibitory peptide, and a platelet inhibitory peptide. In a preferred embodiment, a polypeptide linker comprises a peptide sequence selected from the group consisting of an IL-1 inhibitory or antagonist peptide sequence, an erythropoietin (EPO)-mimetic peptide sequence, a thrombopoietin (TPO)-mimetic peptide sequence, G-CSF mimetic peptide sequence, a TNF-antagonist peptide sequence, an integrin-binding peptide sequence, a selectin antagonist peptide sequence, an anti-pathogenic peptide sequence, a vasoactive intestinal peptide (VIP) mimetic peptide sequence, a calmodulin antagonist peptide sequence, a mast cell antagonist, a SH3 antagonist peptide sequence, an urokinase receptor (UKR) antagonist peptide sequence, a somatostatin or cortistatin mimetic peptide sequence, and a macrophage and/or T-cell inhibiting peptide sequence. Exemplary peptide sequences, any one of which may be employed as a polypeptide linker, are disclosed in U.S. Pat. No. 6,660,843, which is incorporated by reference herein.

Other linkers that are suitable for use in the hybrid nuclease-PK molecules with altered glycosylation are known in the art, for example, the serine-rich linkers disclosed in US 5,525,491, the helix forming peptide linkers (e.g., A(EAAAK)nA (n=2-5)) disclosed in Arai et al. (*Protein Eng* 2001;14:529-32), and the stable linkers disclosed in Chen et al. (*Mol Pharm* 2011;8:457-65), i.e., the dipeptide linker LE, a thrombin-sensitive disulfide cyclopeptide linker, and the alpha-helix forming linker $LEA(EAAAK)_4ALEA(EAAAK)_4ALE$ (SEQ ID NO: 44).

Other exemplary linkers include GS linkers (i.e., (GS)n), GGSG (SEQ ID NO: 45) linkers (i.e., (GGSG)n), GSAT (SEQ ID NO: 46) linkers, SEG linkers, and GGS linkers (i.e., (GGSGGS)n), wherein n is a positive integer (e.g., 1, 2, 3, 4, or 5). Other suitable linkers for use in the hybrid nuclease-PK molecule with altered glycosylation can be found using publicly available databases, such as the Linker Database (ibi.vu.nl/programs/linkerdbwww). The Linker Database is a database of inter-domain

linkers in multi-functional enzymes which serve as potential linkers in novel fusion proteins (see, e.g., George et al., *Protein Engineering* 2002;15:871-9).

It will be understood that variant forms of these exemplary polypeptide linkers can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence encoding a polypeptide linker such that one or more amino acid substitutions, additions or deletions are introduced into the polypeptide linker. Mutations may be introduced by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis.

Polypeptide linkers of the invention are at least one amino acid in length and can be of varying lengths. In one embodiment, a polypeptide linker of the invention is from about 1 to about 50 amino acids in length. As used in this context, the term "about" indicates +/- two amino acid residues. Since linker length must be a positive integer, the length of from about 1 to about 50 amino acids in length, means a length of from 1 to 48-52 amino acids in length. In another embodiment, a polypeptide linker of the invention is from about 10-20 amino acids in length. In another embodiment, a polypeptide linker of the invention is from about 15 to about 50 amino acids in length.

In another embodiment, a polypeptide linker of the invention is from about 20 to about 45 amino acids in length. In another embodiment, a polypeptide linker of the invention is from about 15 to about 25 amino acids in length. In another embodiment, a polypeptide linker of the invention is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 ,57, 58, 59, 60, or 61 or more amino acids in length.

Polypeptide linkers can be introduced into polypeptide sequences using techniques known in the art. Modifications can be confirmed by DNA sequence analysis. Plasmid DNA can be used to transform host cells for stable production of the polypeptides produced.

Exemplary hybrid nuclease-PK molecules with altered glycosylation

The hybrid nuclease-PK molecules with altered glycosylation of the invention are modular, and can be configured to incorporate various individual domains, which can be glycosylated or aglycosylated. Exemplary molecules of the invention are shown schematically in Figure 1. For example, in one embodiment, the hybrid nuclease-PK molecule with altered glycosylation may include the mutant, human DNase1 A114F domain set forth in SEQ ID NO: 4. In another embodiment, the hybrid nuclease-PK molecule with altered glycosylation may include the mutant, human DNase1 N18S/N106S/A114F domain set forth in SEQ ID NO: 5. In another embodiment, the hybrid nuclease-PK molecule with altered glycosylation may include the wild-type, human RNase1 domain set forth in SEQ ID NO: 1. In another embodiment, the hybrid nuclease-PK molecule with altered glycosylation may include the mutant, human RNase1 N34S/N76S/N88S domain set forth in SEQ ID NO: 2.

In some aspects, the invention provides a polypeptide with altered glycosylation having human, mutant RNase1 N34S/N76S/N88S, operably linked with or without a linker to a mutant human IgG1 Fc domain optionally having a mutant hinge region (e.g., a cysteine substitution, such as with serine, e.g., SCC), and one or more CH2 mutations to reduce Fcγ receptor binding (e.g., P238S, P331S or both P238S and P331S). In one embodiment, the polypeptide with altered glycosylation has human, mutant RNase1 N34S/N76S/N88S, operably linked to a mutant human IgG1 Fc domain having SCC hinge, P238S and P331S mutations. In yet another embodiment, the Fc domain further includes a mutation at a site of N-linked glycosylation, such as a substitution at N297 (numbering by Kabat). In another embodiment, the polypeptide with altered glycosylation has human, mutant RNase1 N34S/N76S/N88S, operably linked to a mutant human IgG1 Fc domain having SCC hinge, P238S, P331S and N297S mutations. In another embodiment, the polypeptide comprises an amino acid sequence set forth in SEQ ID NOs: 71-74. In other aspects, the polypeptide has an amino acid sequence at least 90% identical or at least 95% identical to the amino acid sequences set forth in SEQ ID NOs: 71-74.

In another embodiment, the hybrid nuclease-PK molecule with altered glycosylation may include the wild-type, HSA set forth in SEQ ID NO: 6. In another embodiment, the hybrid nuclease-PK molecule with altered glycosylation may include the wild-type, HST set forth in SEQ ID NO: 7. In another embodiment, the hybrid nuclease-PK molecule with altered glycosylation may include the mutant, HST N413S/N611S set forth in SEQ ID NO: 8. In another embodiment, the hybrid nuclease-PK molecule with altered glycosylation may include the mutant, HST S12A/N413S/N611S set forth in SEQ ID NO: 9. In another embodiment, the hybrid nuclease-PK molecule with altered glycosylation may include the wild-type, human IgG1 Fc region set forth in SEQ ID NO: 10. In another embodiment, the hybrid nuclease-PK molecule with altered glycosylation may include the mutant, human IgG1 Fc region N83S (i.e., N297S by Kabat numbering) set forth in SEQ ID NO: 11. In another embodiment, the hybrid nuclease-PK molecule with altered glycosylation may include a PEG. In another embodiment, the hybrid nuclease-PK molecule with altered glycosylation may include the $(Gly_4Ser)3$ linker domain set forth in SEQ ID NO: 12. It will be understood to the skilled artisan that these individual domains can be operably coupled to each other in any order to form an enzymatically active hybrid nuclease-PK molecule with altered glycosylation. For example, as detailed in the specific examples below, RNase1 N34S/N76S/N88S can be operably coupled to mature HSA. In another example, RNase1 N34S/N76S/N88S can be operably coupled to mature HSA via a $(Gly_4Ser)_3$ linker domain. In yet another example, DNase1 N18S/N106S/A114F can be operably coupled to mature HSA. In yet another example, DNase1 N18S/N106S/A114F can be operably coupled to mature HSA via a $(Gly_4Ser)_3$ linker domain. Various other configurations are possible, with non-limiting exemplary configurations detailed below and in Figure 1.

In some embodiments, the hybrid nuclease-PK molecule is aglycosylated and comprises a mutant, human RNase1 domain operably coupled via a $(Gly_4Ser)_3$ linker to a wild-type PK moiety, or mutant or fragment thereof. In one embodiment, the aglycosylated hybrid nuclease-PK molecule comprises a VK3LP leader, followed by a mutant, human RNase1 N34S/N76S/N88S domain operably coupled via a $(Gly_4Ser)_3$ linker to the N-terminus of a wild-type HSA, or variant or fragment thereof (i.e., an RNase1 N34S/N76S/N88S-

linker-HSA molecule; RSLV-315 (SEQ ID NO: 14). In one embodiment, the RNase1 N34S/N76S/N88S-linker-HSA molecule lacks the VK3LP leader (SEQ ID NO: 47).

In another embodiment, the aglycosylated hybrid nuclease-PK molecule comprises a VK3LP leader, followed by a mutant, human RNase1 N34S/N76S/N88S domain operably coupled via a $(Gly_4Ser)_3$ linker to the C-terminus of a wild-type HSA, or variant or fragment thereof (i.e., an HSA-linker-RNase1 N34S/N76S/N88S molecule; RSLV-316 (SEQ ID NO: 15). In one embodiment, the HSA-linker-RNase1 N34S/N76S/N88S molecule lacks the VK3LP leader (SEQ ID NO: 48).

In some embodiments, an aglycosylated hybrid nuclease-PK molecule comprises a mutant, human DNase1 domain operably coupled via a $(Gly_4Ser)_3$ linker to a wild-type PK moiety, or mutant or fragment thereof. In one embodiment, the aglycosylated hybrid nuclease-PK molecule comprises a VK3LP leader, followed by mutant, human DNase1 N18S/N106S/A114F domain operably coupled via a $(Gly_4Ser)_3$ linker to the N-terminus of a wild-type HSA, or variant or fragment thereof (i.e., an DNase1 N18S/N106S/A114F-linker-HSA molecule; RSLV-317 (SEQ ID NO: 16). In one embodiment, the DNase1 N18S/N106S/A114F-linker-HSA molecule lacks the VK3LP leader (SEQ ID NO: 49).

In another embodiment, the aglycosylated hybrid nuclease-PK molecule comprises a VK3LP leader, followed by mutant, human DNase1 N18S/N106S/A114F domain operably coupled via a $(Gly_4Ser)_3$ linker to the C-terminus of a wild-type HSA, or variant or fragment thereof (i.e., an HSA-linker-DNase1 N18S/N106S/A114F molecule; RSLV-318 (SEQ ID NO: 17). In one embodiment, the HSA-linker-DNase1 N18S/N106S/A114F molecule lacks the VK3LP leader (SEQ ID NO: 50).

In some embodiments, the hybrid nuclease-PK molecule has altered glycosylation and comprises a mutant, human RNase1 domain operably coupled via a $(Gly_4Ser)_3$ linker to a wild-type PK moiety, or mutant or fragment thereof, which is operably coupled via a $(Gly_4Ser)_3$ linker to a mutant, human DNase1 domain. In one embodiment, the hybrid nuclease-PK molecule with altered glycosylation comprises a VK3LP leader, followed by

a mutant, human RNase1 N34S/N76S/N88S domain operably coupled via a $(Gly_4Ser)_3$ linker to the N-terminus of a wild-type HSA, or variant or fragment thereof, and a mutant, DNase A114F domain operably coupled via a $(Gly_4Ser)_3$ linker to the C-terminus of the wild-type HSA, or variant or fragment thereof (i.e., an RNase1 N34S/N76S/N88S-linker-HSA-linker-DNase1 A114F molecule; RSLV-319 (SEQ ID NO: 18). In some embodiments, the RNase1 N34S/N76S/N88S-linker-HSA-linker-DNase1 A114F molecule lacks the VK3LP leader (SEQ ID NO: 51).

In another embodiment, the hybrid nuclease-PK molecule with altered glycosylation comprises a VK3LP leader, followed by a mutant, DNase A114F domain operably coupled via a $(Gly_4Ser)_3$ linker to the N-terminus of a wild-type HSA, or variant or fragment thereof, and a mutant, human RNase1 N34S/N76S/N88S domain operably coupled via a $(Gly_4Ser)_3$ linker to the C-terminus of the wild-type HSA, or variant or fragment thereof (i.e., a DNase1 A114F-linker-HSA-linker-RNase1 N34S/N76S/N88S molecule; RSLV-320 (SEQ ID NO: 19). In some embodiments, the DNase1 A114F-linker-HSA-linker-RNase1 N34S/N76S/N88S molecule lacks the VK3LP leader (SEQ ID NO: 52).

In another embodiment, the aglycosylated hybrid nuclease-PK molecule comprises a VK3LP leader, followed by mutant, human RNase1 N34S/N76S/N88S domain operably coupled via a $(Gly_4Ser)_3$ linker to the N-terminus of a wild-type HSA, or variant or fragment thereof, and a mutant, DNase N18S/N106S/A114F domain operably coupled via a $(Gly_4Ser)_3$ linker to the C-terminus of the wild-type HSA, or variant or fragment thereof (i.e., an RNase1 N34S/N76S/N88S-linker-HSA-linker-DNase1 N18S/N106S/A114F molecule; RSLV-321 (SEQ ID NO: 20). In some embodiments, the RNase1 N34S/N76S/N88S-linker-HSA-linker-DNase1 N18S/N106S/A114F molecule lacks the VK3LP leader (SEQ ID NO: 53).

In another embodiment, the aglycosylated hybrid nuclease-PK molecule comprises a VK3LP leader, followed by a mutant, DNase N18S/N106S/A114F domain operably coupled via a $(Gly_4Ser)_3$ linker to the N-terminus of a wild-type HSA, or variant or

fragment thereof, and a mutant, human RNase1 N34S/N76S/N88S domain operably coupled via a $(Gly_4Ser)_3$ linker to the C-terminus of the wild-type HSA, or variant or fragment thereof (i.e., a DNase1 N18S/N106S/A114F-linker-HSA-linker-RNase1 N34S/N76S/N88S molecule; RSLV-322 (SEQ ID NO: 21). In some embodiments, the DNase1 N18S/N106S/A114F-linker-HSA-linker-RNase1 N34S/N76S/N88S molecule lacks the VK3LP leader (SEQ ID NO: 54).

In some embodiments, the aglycosylated hybrid nuclease-PK molecule comprises a VK3LP leader, followed by a wild-type HSA, or a variant or fragment thereof operably coupled at its C-terminus via a $(Gly4Ser)_3$ linker to a mutant human DNase1 E13R/N74K/A114F/T205K/N18S/N106S domain (e.g., an HSA-linker-DNase1 E13R/N74K/A114F/T205K/N18S/N106S molecule; (SEQ ID NO: 75). In one embodiment, the HSA-linker-DNase1 E13R/N74K/A114F/T205K/N18S/N106S molecule lacks the VK3LP leader (SEQ ID NO: 79).

In another embodiment, the aglycosylated hybrid nuclease-PK molecule comprises a VK3LP leader, followed by mutant human DNase1 E13R/N74K/A114F/T205K/N18S/N106S domain operably coupled via a $(Gly_4Ser)3$ linker to the N-terminus of wild-type HSA, or a variant or fragment thereof (e.g., DNase1 E13R/N74K/A114F/T205K/N18S/N106S-linker-HSA molecule; RSLV-330 (SEQ ID NO:76). In one embodiment, the DNase1 E13R/N74K/A114F/T205K/N18S/N106S-linker-HSA molecule lacks the VK3LP leader (SEQ ID NO: 80).

In some embodiments, an aglycosylated hybrid nuclease-PK molecule comprises a wild-type, human RNase1 domain operably coupled via a $(Gly_4Ser)_3$ linker domain to a wild-type albumin, or mutant or fragment thereof, which is operably coupled via a $(Gly_4Ser)_3$ linker domain to a mutant, human DNase1 domain. In one embodiment, the aglycosylated hybrid nuclease-album molecule comprises a VK3LP leader, followed by a wild-type, human RNase1 domain operably coupled via a $(Gly_4Ser)_3$ linker domain to the N-terminus of wild-type HSA, or a variant or fragment thereof, and the mutant human DNase1 E13R/N74K/A114F/T205K/N18S/N106S domain is operably coupled via a

$(Gly4Ser)_3$ linker domain to the C-terminus of wild-type HSA, or a variant or fragment thereof. (e.g., an RNase1-linker-HSA-linker-DNase1 E13R/N74K/A114F/T205K/N18S/N106S molecule; RSLV-331 (SEQ ID NO:77). In one embodiment, the RNase1-linker-HSA-linker-DNase1 E13R/N74K/A114F/T205K/N18S/N106S molecule lacks the VK3LP leader (SEQ ID NO:81).

In another embodiment, the aglycosylated hybrid nuclease-PK molecule comprises a VK3LP leader, followed by mutant human DNase1 E13R/N74K/A114F/T205K/N18S/N106S domain operably coupled via a $(Gly_4Ser)_3$ linker to the N-terminus of wild-type HSA, or a variant or fragment thereof, and a wild-type, human RNase1 domain operably coupled via a $(Gly_4Ser)_3$ linker domain to the C-terminus of the wild-type HSA, or a variant or fragment thereof (e.g., DNase1 E13R/N74K/A114F/T205K/N18S/N106S-linker-HSA-linker-RNase1 molecule; RSLV-332 (SEQ ID NO:78). In one embodiment, the DNase1 E13R/N74K/A114F/T205K/N18S/N106S-linker-HSA-linker-RNase1 molecule lacks the VK3LP leader (SEQ ID NO:82).

In some embodiments, a hybrid nuclease-PK molecule with altered glycosylation has an amino acid sequence at least 80% identical, such as 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or at least 99.5% identical to an amino acid sequence of any one of SEQ ID NOs: 14-21, 47-54 and 71-82.

It will be understood by one of ordinary skill that the leader and linker sequences are optional and are not limited to those described in the embodiments above. For example, the RNase and/or DNase domains can be directly fused to the N- and/or C-terminus of HSA, or variant or fragment thereof; the leader domain can be any of those known in the art to be useful for its intended purpose, e.g., to increase protein expression and/or secretion (e.g., a *Gaussia* luciferase signal peptide (MGVKVLFALICIAVAEA; SEQ ID NO: 55)); the linker can be any linker known in the art, e.g., $(Gly_4Ser)_n$, NLG (SEQ ID NO: 57), LE, thrombin-sensitive disulphide cyclopeptide linker,

$LEA(EAAAK)_4ALEA(EAAAK)_4$ (SEQ ID NO: 56), or an in vivo cleavable disulphide linker, as described herein. It will also be understood that HSA in the specific embodiments can be exchanged with any of the other mutant PK moieties listed above (e.g., mutant, HST N413S/N611S, SEQ ID NO: 8; mutant, HST S12A/N413S/N611S, SEQ ID NO: 9; mutant, human IgG1 Fc region N83S, SEQ ID NO: 11). It is within the abilities of a skilled artisan to make the corresponding changes to the amino acid sequences of the hybrid nuclease-PK molecules using routine cloning and recombination methods. It will also be understood that the asparagine residues in the nuclease domains (i.e., N34, N76, and N88 in RNase1, and N18 and N106 in DNase1, as well as those in the PK moieties listed above) can be substituted with an amino acid other than serine (e.g., glutamine), as long as the amino acid does not serve as an acceptor for N-linked glycosylation.

Methods of Making Hybrid Nuclease-PK Molecules with altered glycosylation

The hybrid nuclease-PK molecules with altered glycosylation of this invention largely may be made in transformed or transfected host cells using recombinant DNA techniques. To do so, a recombinant DNA molecule coding for the peptide is prepared. Methods of preparing such DNA molecules are well known in the art. For instance, sequences coding for the peptides could be excised from DNA using suitable restriction enzymes. Alternatively, the DNA molecule could be synthesized using chemical synthesis techniques, such as the phosphoramidate method. Also, a combination of these techniques could be used.

The invention also includes a vector capable of expressing the peptides in an appropriate host. The vector comprises the DNA molecule that codes for the peptides operably coupled to appropriate expression control sequences. Methods of affecting this operative linking, either before or after the DNA molecule is inserted into the vector, are well known. Expression control sequences include promoters, activators, enhancers, operators, ribosomal nuclease domains, start signals, stop signals, cap signals, polyadenylation signals, and other signals involved with the control of transcription or translation.

The resulting vector having the DNA molecule thereon is used to transform or transfect an appropriate host. This transformation or transfection may be performed using methods well known in the art.

Any of a large number of available and well-known host cells may be used in the practice of this invention. The selection of a particular host is dependent upon a number of factors recognized by the art. These include, for example, compatibility with the chosen expression vector, toxicity of the peptides encoded by the DNA molecule, rate of transformation or transfection, ease of recovery of the peptides, expression characteristics, bio-safety and costs. A balance of these factors must be struck with the understanding that not all hosts may be equally effective for the expression of a particular DNA sequence. Within these general guidelines, useful microbial hosts include bacteria (such as E. coli), yeast (such as Saccharomyces) and other fungi, insects, plants, mammalian (including human) cells in culture, or other hosts known in the art. In a preferred embodiment, the hybrid nuclease-PK molecules with altered glycosylation are produced in CHO cells.

Next, the transformed or transfected host is cultured and purified. Host cells may be cultured under conventional fermentation or culture conditions so that the desired compounds are expressed. Such fermentation and culture conditions are well known in the art. Finally, the peptides are purified from culture by methods well known in the art.

The compounds may also be made by synthetic methods. For example, solid phase synthesis techniques may be used. Suitable techniques are well known in the art, and include those described in Merrifield (1973), Chem. Polypeptides, pp. 335-61 (Katsoyannis and Panayotis eds.); Merrifield (1963), J. Am. Chem. Soc. 85: 2149; Davis et al., *Biochem Intl* 1985;10: 394-414; Stewart and Young (1969), Solid Phase Peptide Synthesis; U.S. Pat. No. 3,941,763; Finn et al. (1976), The Proteins (3rd ed.) 2: 105-253; and Erickson et al. (1976), The Proteins (3rd ed.) 2: 257-527. Solid phase synthesis is the preferred technique of making individual peptides since it is the most cost-effective method of making small peptides. Compounds that contain derivatized peptides or which

contain non-peptide groups may be synthesized by well-known organic chemistry techniques.

Other methods are of molecule expression/synthesis are generally known in the art to one of ordinary skill.

Other methods of making hybrid nuclease-PK molecules with altered glycosylation are described *supra*.

Alternative methods for altering glycosylation

In some embodiments, all potential glycosylation sites conforming to the Asn-X-Ser/Thr (X can be any naturally occurring amino acid except for P) in a hybrid nuclease-PK molecule are removed by mutation of the nucleic acid encoding the hybrid nuclease-PK molecule, thereby eliminating glycosylation of the hybrid nuclease-PK molecule when synthesized in a cell that glycosylates proteins (e.g., CHO cells).

In other embodiments, less than all potential glycosylation sites are removed by mutation of the nucleic acid encoding the hybrid nuclease-PK molecule, thereby reducing glycosylation (underglycosylation) of the hybrid nuclease-PK molecule when synthesized in a cell that glycosylates proteins. Some (underglycosylation) or all (aglycosylation) of the asparagine residues conforming to the Asn-X-Ser/Thr (X can be any naturally occurring amino acid except for P) consensus for N-linked glycosylation in a hybrid nuclease-PK molecule can be mutated to residues that do not serve as acceptors of N-linked glycosylation (e.g., serine, glutamine). When some, but not all, of the asparagine residues conforming to the N-linked glycosylation consensus sequence are mutated to, e.g., serine or glutamine, the optimal combinations of mutations can be readily determined by the skilled artisan and the resulting hybrid nuclease-PK molecule tested for, e.g., nuclease activity or serum half-life, using routine methods. Preferably, the particular mutation(s) introduced into the hybrid nuclease-PK molecule (e.g., the nuclease domain) does not substantially decrease the enzymatic activity of the nuclease domain.

In some embodiments, a hybrid nuclease-PK molecule is produced in a host that produces an aglycosylated protein, e.g., *E. coli*, mammalian cells engineered to lack one or more enzymes important for glycosylation, or mammalian cells treated with agents that prevent glycosylation, such as tunicamycin (an inhibitor of Dol-PP-GlcNAc formation). In other embodiments, a hybrid nuclease-PK molecule with intact glycosylation sites are produced in mammalian cells (e.g., CHO cells) and subsequently subjected to chemical or enzymatic deglycosylation, as described herein.

In other embodiments, the hybrid nuclease-PK molecule is produced in a host engineered to introduce complex N-glycans, rather than high-mannose-type sugars (e.g., according to the methods and engineered organisms disclosed in US2007/0105127).

In some embodiments, glycosylated hybrid nuclease-PK molecules (e.g., those produced in mammalian cells such as CHO cells) are treated chemically or enzymatically to remove one or more carbohydrate residues (e.g., one or more mannose, fucose, and/or N-acetylglucosamine residues) or to modify or mask one or more carbohydrate residues. Such modifications or masking may reduce binding of the hybrid nuclease-PK molecules to mannose receptors, and/or asialoglycoprotein receptors, and/or other lectin-like receptors. Chemical deglycosylation can be achieved by treating a hybrid nuclease-PK molecule with trifluoromethane sulfonic acid (TFMS), as disclosed in, e.g., Sojar et al., *JBC* 1989;264:2552-9 and Sojar et al., *Methods Enzymol* 1987;138:341-50, or by treating with hydrogen fluoride, as disclosed in Sojar et al. (1987, *supra*). Enzymatic removal of N-linked carbohydrates from hybrid nuclease-PK molecules can be achieved by treating a hybrid nuclease-PK molecule with protein N-glycosidase (PNGase) A or F, as disclosed in Thotakura et al. (*Methods Enzymol* 1987;138:350-9). Other art-recognized commercially available deglycosylating enzymes that are suitable for use include endo-alpha-N-acetyl-galactosaminidase, endoglycosidase F1, endoglycosidase F2, endoglycosidase F3, and endoglycosidase H. In some embodiments, one or more of these enzymes can be used to deglycosylate the hybrid nuclease-PK molecules of the invention. Alternative methods for deglycosylation are disclosed in, e.g., US 8,198,063.

In some embodiments, the hybrid nuclease-PK molecules are partially deglycosylated. Partial deglycosylation can be achieved by treating the hybrid nuclease-PK molecules with an endoglycosidase (e.g., endoglycosidase H), which cleaves N-linked high mannose carbohydrate but not complex type carbohydrates, leaving a single GlcNAc residue linked to the asparagine. Hybrid nuclease-PK molecules treated with endoglycosidase H will lack high mannose carbohydrates, resulting in a reduced interaction with the hepatic mannose receptor. Although this receptor recognizes terminal GlcNAc, the probability of a productive interaction with the single GlcNAc on the protein surface is not as great as with an intact high mannose structure.

In other embodiments, glycosylation of a hybrid nuclease-PK molecule is modified, e.g., by oxidation, reduction, dehydration, substitution, esterification, alkylation, sialylation, carbon-carbon bond cleavage, or the like, to reduce clearance of hybrid nuclease-PK molecules from blood. In some embodiments, the hybrid nuclease-PK molecules are treated with periodate and sodium borohydride to modify the carbohydrate structure. Periodate treatment oxidizes vicinal diols, cleaving the carbon-carbon bond and replacing the hydroxyl groups with aldehyde groups; borohydride reduces the aldehydes to hydroxyls. Many sugar residues include vicinal diols and, therefore, are cleaved by this treatment. Prolonged serum half-life with periodate and sodium borohydride is exemplified by the sequential treatment of the lysosomal enzyme β-glucuronidase with these agents (see, e.g., Houba et al. (1996) *Bioconjug Chem* 1996:7:606-11; Stahl et al. *PNAS* 1976;73:4045-9; Achord et al. *Pediat. Res* 1977;11:816-22; Achord et al. *Cell* 1978;15:269-78). A method for treatment with periodate and sodium borohydride is disclosed in Hickman et al., *BBRC* 1974;57:55-61. A method for treatment with periodate and cyanoborohydride, which increases the serum half-life and tissue distribution of ricin, is disclosed in Thorpe et al. *Eur J Biochem* 1985;147:197-206.

In one embodiment, the carbohydrate structures of a hybrid nuclease-PK molecule can be masked by addition of one or more additional moieties (e.g., carbohydrate groups, phosphate groups, alkyl groups, etc.) that interfere with recognition of the structure by a mannose or asialoglycoprotein receptor or other lectin-like receptors.

In some embodiments, it may be desirable to selectively aglycosylate the nuclease domain of the hybrid nuclease-PK molecules by mutating the potential N-linked glycosylation sites therein if, e.g., the resultant underglycosylated hybrid nuclease-PK molecule exhibits increased nuclease activity or increased serum half-life. In other embodiments, it may be desirable to aglycosylate portions of the hybrid nuclease-PK molecule such that regions other than the nuclease domain lack N-glycosylation if, for example, such a modification improves the serum half-life of the hybrid nuclease-PK molecule without affecting nuclease activity. Alternatively, other amino acids in the vicinity of glycosylation acceptors can be modified, disrupting a recognition motif for glycosylation enzymes without necessarily changing the amino acid that would normally be glycosylated.

In some embodiments, glycosylation of a hybrid nuclease-PK molecule can be altered by introducing glycosylation sites. For example, the amino acid sequence of the hybrid nuclease-PK molecule can be modified to introduce the consensus sequence for N-linked glycosylation of Asp-X-Ser/Thr (X is any amino acid other than proline). Additional N-linked glycosylation sites can be added anywhere throughout the amino acid sequence of the hybrid nuclease-PK molecule. Preferably, the glycosylation sites are introduced in position in the amino acid sequence that does not substantially reduce the nuclease (e.g., RNase and/or DNase) activity of the hybrid nuclease-PK molecule.

The addition of O-linked glycosylation sites has been reported to alter serum half-life of proteins, such as growth hormone, follicle-stimulating hormone, IGFBP-6, Factor IX, and many others (e.g., as disclosed in Okada et al., *Endocr Rev* 2011;32:2-342; Weenen et al., *J Clin Endocrinol Metab* 2004;89:5204-12; Marinaro et al., *European Journal of Endocrinology* 2000;142:512-6; US 2011/0154516). Accordingly, in some embodiments, O-linked glycosylation (on serine/threonine residues) of the hybrid nuclease-PK molecules is altered. Methods for altering O-linked glycosylation are routine in the art and can be achieved, e.g., by beta-elimination (see, e.g., Huang et al., *Rapid Communications in Mass Spectrometry* 2002;16:1199-204; Conrad, *Curr Protoc Mol Biol* 2001; Chapter 17:Unit17.15A; Fukuda, *Curr Protoc Mol Biol* 2001;Chapter

17;Unit 17.15B; Zachara et al., *Curr Protoc Mol Biol* 2011; Unit 17.6;); by using commercially available kits (e.g., GlycoProfile™ Beta-Elimination Kit, Sigma); or by subjecting the hybrid nuclease-PK molecule to treatment with a series of exoglycosidases such as, but not limited to, β1-4 galactosidase and β –N-acetylglucosaminidase, until only Gal β1-3GalNAc and/or GlcNAc β1-3GalNAc remains, followed by treatment with, e.g., endo-α-N-acetylgalactosaminidase (i.e., O-glycosidase). Such enzymes are commercially available from, e.g., New England Biolabs. In yet other embodiments, the hybrid nuclease-PK molecules are altered to introduce O-linked glycosylation in the hybrid nuclease-PK molecule as disclosed in, e.g., Okada et al. (*supra*), Weenen et al. (*supra*), US2008/0274958; and US2011/0171218. In some embodiments, one or more O-linked glycosylation consensus sites are introduced into the hybrid nuclease-PK molecule, such as CXXGG-T/S-C (SEQ ID NO: 58) (van den Steen et al., In *Critical Reviews in Biochemistry and Molecular Biology*, Michael Cox, ed., 1998;33:151-208), NST-E/D-A (SEQ ID NO: 59), NITQS (SEQ ID NO: 60), QSTQS (SEQ ID NO: 61), D/EFT-R/K-V (SEQ ID NO: 62), C-E/D-SN (SEQ ID NO: 63), and GGSC-K/R (SEQ ID NO: 64). Additional O-linked glycosylation sites can be added anywhere throughout the amino acid sequence of the hybrid nuclease-PK molecule. Preferably, the glycosylation sites are introduced in position in the amino acid sequence that does not substantially reduce the nuclease (e.g., RNase and/or DNase) activity of the hybrid nuclease-PK molecule. Alternatively, O-linked sugar moieties are introduced by chemically modifying an amino acid in the hybrid nuclease-PK molecule as described in, e.g., WO 87/05330 and Aplin et al., *CRC Crit Rev Biochem* 1981;259-306).

In some embodiments, both N-linked and O-linked glycosylation sites are introduced into the hybrid nuclease-PK molecules, preferably in position in the amino acid sequence that does not substantially reduce the nuclease (e.g., RNase and/or DNase) activity of the hybrid nuclease-PK molecule.

It is well within the abilities of the skilled artisan to introduce, reduce, or eliminate glycosylation (e.g., N-linked or O-linked glycosylation) in a hybrid nuclease-PK molecule and determine using routine methods in the art whether such modifications in

glycosylation status increases or decreases the nuclease activity or serum half-life of the hybrid nuclease-PK molecule.

In some embodiments, the hybrid nuclease molecule may comprise an altered glycoform (e.g., an underfucosylated or fucose-free glycan).

Pharmaceutical Compositions

In certain embodiments, a hybrid nuclease-PK molecule with altered glycosylation is administered alone. In certain embodiments, a hybrid nuclease-PK molecule with altered glycosylation is administered prior to the administration of at least one other therapeutic agent. In certain embodiments, a hybrid nuclease-PK molecule with altered glycosylation is administered concurrent with the administration of at least one other therapeutic agent. In certain embodiments, a hybrid nuclease-PK molecule with altered glycosylation is administered subsequent to the administration of at least one other therapeutic agent. In other embodiments, a hybrid nuclease-PK molecule with altered glycosylation is administered prior to the administration of at least one other therapeutic agent. As will be appreciated by one of skill in the art, in some embodiments, the hybrid nuclease-PK molecule with altered glycosylation is combined with the other agent/compound. In some embodiments, the hybrid nuclease-PK molecule with altered glycosylation and other agent are administered concurrently. In some embodiments, the hybrid nuclease-PK molecule with altered glycosylation and other agent are not administered simultaneously, with the hybrid nuclease-PK molecule being administered before or after the agent is administered. In some embodiments, the subject receives both the hybrid nuclease-PK molecule with altered glycosylation and the other agent during a same period of prevention, occurrence of a disorder, and/or period of treatment.

Pharmaceutical compositions of the invention can be administered in combination therapy, i.e., combined with other agents. In certain embodiments, the combination therapy comprises the hybrid nuclease-PK molecule with altered glycosylation, in combination with at least one other agent. Agents include, but are not limited to, *in vitro* synthetically prepared chemical compositions, antibodies, antigen binding regions, and

combinations and conjugates thereof. In certain embodiments, an agent can act as an agonist, antagonist, allosteric modulator, or toxin.

In certain embodiments, the invention provides for pharmaceutical compositions comprising a hybrid nuclease-PK molecule with altered glycosylation together with a pharmaceutically acceptable diluent, carrier, solubilizer, emulsifier, preservative and/or adjuvant.

In certain embodiments, the invention provides for pharmaceutical compositions comprising a hybrid nuclease-PK molecule with altered glycosylation and a therapeutically effective amount of at least one additional therapeutic agent, together with a pharmaceutically acceptable diluent, carrier, solubilizer, emulsifier, preservative and/or adjuvant.

In certain embodiments, acceptable formulation materials preferably are nontoxic to recipients at the dosages and concentrations employed. In some embodiments, the formulation material(s) are for s.c. and/or I.V. administration. In certain embodiments, the pharmaceutical composition can contain formulation materials for modifying, maintaining or preserving, for example, the pH, osmolality, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption or penetration of the composition. In certain embodiments, suitable formulation materials include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine or lysine); antimicrobials; antioxidants (such as ascorbic acid, sodium sulfite or sodium hydrogen-sulfite); buffers (such as borate, bicarbonate, Tris-HCl, citrates, phosphates or other organic acids); bulking agents (such as mannitol or glycine); chelating agents (such as ethylenediamine tetraacetic acid (EDTA)); complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin or hydroxypropyl-beta-cyclodextrin); fillers; monosaccharides; disaccharides; and other carbohydrates (such as glucose, mannose or dextrins); proteins (such as gelatin); coloring, flavoring and diluting agents; emulsifying agents; hydrophilic polymers (such as polyvinylpyrrolidone); low molecular weight polypeptides; salt-forming counterions (such as sodium); preservatives (such as benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol,

methylparaben, propylparaben, chlorhexidine, sorbic acid or hydrogen peroxide); solvents (such as glycerin, propylene glycol or polyethylene glycol); sugar alcohols (such as mannitol or sorbitol); suspending agents; surfactants or wetting agents (such as pluronics, PEG, sorbitan esters, polysorbates such as polysorbate 20, polysorbate 80, triton, tromethamine, lecithin, cholesterol, tyloxapal); stability enhancing agents (such as sucrose or sorbitol); tonicity enhancing agents (such as alkali metal halides, preferably sodium or potassium chloride, mannitol sorbitol); delivery vehicles; diluents; excipients and/or pharmaceutical adjuvants. (Remington's Pharmaceutical Sciences, 18th Edition, A. R. Gennaro, ed., Mack Publishing Company (1995). In some embodiments, the formulation comprises PBS; 20 mM NaOAC, pH 5.2, 50 mM NaCl; and/or 10 mM NAOAC, pH 5.2, 9% Sucrose.

In certain embodiments, a hybrid nuclease-PK molecule with altered glycosylation and/or a therapeutic molecule is linked to a half-life extending vehicle known in the art. Such vehicles include, but are not limited to, polyethylene glycol, glycogen (e.g., glycosylation of the hybrid nuclease-PK molecule), and dextran. Such vehicles are described, e.g., in U.S. application Ser. No. 09/428,082, now U.S. Pat. No. 6,660,843 and published PCT Application No. WO 99/25044.

In certain embodiments, the optimal pharmaceutical composition will be determined by one skilled in the art depending upon, for example, the intended route of administration, delivery format and desired dosage. See, for example, Remington's Pharmaceutical Sciences, *supra*. In certain embodiments, such compositions may influence the physical state, stability, rate of *in vivo* release and rate of *in vivo* clearance of the antibodies of the invention.

In certain embodiments, the primary vehicle or carrier in a pharmaceutical composition can be either aqueous or non-aqueous in nature. For example, in certain embodiments, a suitable vehicle or carrier can be water for injection, physiological saline solution or artificial cerebrospinal fluid, possibly supplemented with other materials common in compositions for parenteral administration. In some embodiments, the saline comprises isotonic phosphate-buffered saline. In certain embodiments, neutral buffered saline or

saline mixed with serum albumin are further exemplary vehicles. In certain embodiments, pharmaceutical compositions comprise Tris buffer of about pH 7.0-8.5, or acetate buffer of about H 4.0-5.5, which can further include sorbitol or a suitable substitute therefore. In certain embodiments, a composition comprising a hybrid nuclease-PK molecule with altered glycosylation, with or without at least one additional therapeutic agents, can be prepared for storage by mixing the selected composition having the desired degree of purity with optional formulation agents (Remington's Pharmaceutical Sciences, *supra*) in the form of a lyophilized cake or an aqueous solution. Further, in certain embodiments, a composition comprising a hybrid nuclease-PK molecule with altered glycosylation, with or without at least one additional therapeutic agent, can be formulated as a lyophilizate using appropriate excipients such as sucrose.

In certain embodiments, the pharmaceutical composition can be selected for parenteral delivery. In certain embodiments, the compositions can be selected for inhalation or for delivery through the digestive tract, such as orally. The preparation of such pharmaceutically acceptable compositions is within the ability of one skilled in the art.

In certain embodiments, the formulation components are present in concentrations that are acceptable to the site of administration. In certain embodiments, buffers are used to maintain the composition at physiological pH or at a slightly lower pH, typically within a pH range of from about 5 to about 8.

In certain embodiments, when parenteral administration is contemplated, a therapeutic composition can be in the form of a pyrogen-free, parenterally acceptable aqueous solution comprising a desired hybrid nuclease-PK molecule with altered glycosylation, with or without additional therapeutic agents, in a pharmaceutically acceptable vehicle. In certain embodiments, a vehicle for parenteral injection is sterile distilled water in which a hybrid nuclease-PK molecule with altered glycosylation, with or without at least one additional therapeutic agent, is formulated as a sterile, isotonic solution, properly preserved. In certain embodiments, the preparation can involve the formulation of the desired molecule with an agent, such as injectable microspheres, bio-erodible particles, polymeric compounds (such as polylactic acid or polyglycolic acid), beads or liposomes,

that can provide for the controlled or sustained release of the product which can then be delivered via a depot injection. In certain embodiments, hyaluronic acid can also be used, and can have the effect of promoting sustained duration in the circulation. In certain embodiments, implantable drug delivery devices can be used to introduce the desired molecule.

In certain embodiments, a pharmaceutical composition can be formulated for inhalation. In certain embodiments, a hybrid nuclease-PK molecule with altered glycosylation, with or without at least one additional therapeutic agent, can be formulated as a dry powder for inhalation. In certain embodiments, an inhalation solution comprising a hybrid nuclease-PK molecule with altered glycosylation, with or without at least one additional therapeutic agent, can be formulated with a propellant for aerosol delivery. In certain embodiments, solutions can be nebulized. Pulmonary administration is further described in PCT application no. PCT/US94/001875, which describes pulmonary delivery of chemically modified proteins.

In certain embodiments, it is contemplated that formulations can be administered orally. In certain embodiments, a hybrid nuclease-PK molecule with altered glycosylation, with or without at least one additional therapeutic agents, that is administered in this fashion can be formulated with or without those carriers customarily used in the compounding of solid dosage forms such as tablets and capsules. In certain embodiments, a capsule can be designed to release the active portion of the formulation at the point in the gastrointestinal tract when bioavailability is maximized and pre-systemic degradation is minimized. In certain embodiments, at least one additional agent can be included to facilitate absorption of a hybrid nuclease-PK molecule with altered glycosylation and/or any additional therapeutic agents. In certain embodiments, diluents, flavorings, low melting point waxes, vegetable oils, lubricants, suspending agents, tablet disintegrating agents, and binders can also be employed.

In certain embodiments, a pharmaceutical composition can involve an effective quantity of a hybrid nuclease-PK molecule with altered glycosylation, with or without at least one additional therapeutic agents, in a mixture with non-toxic excipients which are suitable

for the manufacture of tablets. In certain embodiments, by dissolving the tablets in sterile water, or another appropriate vehicle, solutions can be prepared in unit-dose form. In certain embodiments, suitable excipients include, but are not limited to, inert diluents, such as calcium carbonate, sodium carbonate or bicarbonate, lactose, or calcium phosphate; or binding agents, such as starch, gelatin, or acacia; or lubricating agents such as magnesium stearate, stearic acid, or talc.

Additional pharmaceutical compositions will be evident to those skilled in the art, including formulations involving a hybrid nuclease-PK molecule with altered glycosylation, with or without at least one additional therapeutic agent(s), in sustained- or controlled-delivery formulations. In certain embodiments, techniques for formulating a variety of other sustained- or controlled-delivery means, such as liposome carriers, bio-erodible microparticles or porous beads and depot injections, are also known to those skilled in the art. See for example, PCT Application No. PCT/US93/00829 which describes the controlled release of porous polymeric microparticles for the delivery of pharmaceutical compositions. In certain embodiments, sustained-release preparations can include semipermeable polymer matrices in the form of shaped articles, e.g. films, or microcapsules. Sustained release matrices can include polyesters, hydrogels, polylactides (U.S. Pat. No. 3,773,919 and EP 058,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman et al, *Biopolymers*, 22:547-556 (1983)), poly (2-hydroxyethyl-methacrylate) (Langer et al., *J Biomed Mater Res*, 15: 167-277 (1981) and Langer, *Chem Tech*, 12:98-105 (1982)), ethylene vinyl acetate (Langer et al, *supra*) or poly-D(-)-3-hydroxybutyric acid (EP 133,988). In certain embodiments, sustained release compositions can also include liposomes, which can be prepared by any of several methods known in the art. See, e.g., Eppstein et al, *PNAS*, 82:3688-3692 (1985); EP 036,676; EP 088,046 and EP 143,949.

The pharmaceutical composition to be used for *in vivo* administration typically is sterile. In certain embodiments, this can be accomplished by filtration through sterile filtration membranes. In certain embodiments, where the composition is lyophilized, sterilization using this method can be conducted either prior to or following lyophilization and reconstitution. In certain embodiments, the composition for parenteral administration can

be stored in lyophilized form or in a solution. In certain embodiments, parenteral compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

In certain embodiments, once the pharmaceutical composition has been formulated, it can be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or as a dehydrated or lyophilized powder. In certain embodiments, such formulations can be stored either in a ready-to-use form or in a form (e.g., lyophilized) that is reconstituted prior to administration.

In certain embodiments, kits are provided for producing a single-dose administration unit. In certain embodiments, the kit can contain both a first container having a dried protein and a second container having an aqueous formulation. In certain embodiments, kits containing single and multi-chambered pre-filled syringes (e.g., liquid syringes and lyosyringes) are included.

In certain embodiments, the effective amount of a pharmaceutical composition comprising a hybrid nuclease-PK molecule with altered glycosylation, with or without at least one additional therapeutic agent, to be employed therapeutically will depend, for example, upon the therapeutic context and objectives. One skilled in the art will appreciate that the appropriate dosage levels for treatment, according to certain embodiments, will thus vary depending, in part, upon the molecule delivered, the indication for which a hybrid nuclease-PK molecule with altered glycosylation, with or without at least one additional therapeutic agent, is being used, the route of administration, and the size (body weight, body surface or organ size) and/or condition (the age and general health) of the patient. In certain embodiments, the clinician can titer the dosage and modify the route of administration to obtain the optimal therapeutic effect. In certain embodiments, a typical dosage can range from about 0.1 μg/kg to up to about 100 mg/kg or more, depending on the factors mentioned above. In certain embodiments, the dosage can range from 0.1 μg/kg up to about 100 mg/kg; or 1 μg/kg up to about 100 mg/kg; or 5 μg/kg up to about 100 mg/kg.

In certain embodiments, the frequency of dosing will take into account the pharmacokinetic parameters of a hybrid nuclease-PK molecule with altered glycosylation and/or any additional therapeutic agents in the formulation used. In certain embodiments, a clinician will administer the composition until a dosage is reached that achieves the desired effect. In certain embodiments, the composition can therefore be administered as a single dose, or as two or more doses (which may or may not contain the same amount of the desired molecule) over time, or as a continuous infusion via an implantation device or catheter. Further refinement of the appropriate dosage is routinely made by those of ordinary skill in the art and is within the ambit of tasks routinely performed by them. In certain embodiments, appropriate dosages can be ascertained through use of appropriate dose-response data.

In certain embodiments, the route of administration of the pharmaceutical composition is in accord with known methods, e.g. orally, through injection by intravenous, intraperitoneal, intracerebral (intra-parenchymal), intracerebroventricular, intramuscular, subcutaneously, intra-ocular, intraarterial, intraportal, or intralesional routes; by sustained release systems or by implantation devices. In certain embodiments, the compositions can be administered by bolus injection or continuously by infusion, or by implantation device.

In certain embodiments, the composition can be administered locally via implantation of a membrane, sponge or another appropriate material onto which the desired molecule has been absorbed or encapsulated. In certain embodiments, where an implantation device is used, the device can be implanted into any suitable tissue or organ, and delivery of the desired molecule can be via diffusion, timed-release bolus, or continuous administration.

In certain embodiments, it can be desirable to use a pharmaceutical composition comprising a hybrid nuclease-PK molecule with altered glycosylation, with or without at least one additional therapeutic agent, in an *ex vivo* manner. In such instances, cells, tissues and/or organs that have been removed from the patient are exposed to a pharmaceutical composition comprising a hybrid nuclease-PK molecule with altered glycosylation, with or without at least one additional therapeutic agent, after which the cells, tissues and/or organs are subsequently implanted back into the patient.

In certain embodiments, a hybrid nuclease-PK molecule with altered glycosylation and/or any additional therapeutic agents can be delivered by implanting certain cells that have been genetically engineered, using methods such as those described herein, to express and secrete the polypeptides. In certain embodiments, such cells can be animal or human cells, and can be autologous, heterologous, or xenogeneic. In certain embodiments, the cells can be immortalized. In certain embodiments, in order to decrease the chance of an immunological response, the cells can be encapsulated to avoid infiltration of surrounding tissues. In certain embodiments, the encapsulation materials are typically biocompatible, semi-permeable polymeric enclosures or membranes that allow the release of the protein product(s) but prevent the destruction of the cells by the patient's immune system or by other detrimental factors from the surrounding tissues.

***In vitro* assays**

Various *in vitro* assays known in the art can be used to assess the efficacy of the hybrid nuclease-PK molecules with altered glycosylation of the invention, such as those disclosed in WO 2011/053982.

For example, cultured human PBMCs from normal or lupus patients are isolated, cultured, and treated with various stimuli (e.g., TLR ligands, costimulatory antibodies, immune complexes, and normal or autoimmune sera), in the presence or absence of the hybrid nuclease-PK molecules with altered glycosylation. Cytokine production by the stimulated cells can be measured using commercially available reagents, such as the antibody pair kits from Biolegend (San Diego, CA) for various cytokines (e.g., IL-6, IL-8, IL-10, IL-4, IFN-gamma, and TNF-alpha). Culture supernatants are harvested at various time points as appropriate for the assay (e.g., 24, 48 hours, or later time points) to determine the effects that the hybrid nuclease-PK molecules with altered glycosylation have on cytokine production. IFN-alpha production is measured by ELISA using, e.g., anti-human IFN-alpha antibodies and standard curve reagents available from PBL interferon source (Piscataway, NJ). Similar assays are performed using human lymphocyte subpopulations (isolated monocytes, B cells, pDCs, T cells, etc.); purified using, e.g., commercially available magnetic bead based isolation kits available from Miltenyi Biotech (Auburn, CA).

Multi-color flow cytometry can be used to assess the effects of the hybrid nuclease-PK molecules with altered glycosylation on immune cell activation following exposure to TLR ligands and/or immune complexes by measuring the expression of lymphocyte activation receptors such as CD5, CD23, CD69, CD80, CD86, and CD25 in PBMCs or isolated cell subpopulations at various time points after stimulation using routine art-recognized methods.

The efficacy of hybrid nuclease-PK molecules with altered glycosylation can also be tested by incubating SLE patient serum with normal human pDCs to activate IFN output, as described in, e.g., Ahlin et al., *Lupus* 2012:21:586-95; Mathsson et al., *Clin Expt Immunol* 2007;147:513-20; and Chiang et al., *J Immunol* 2011;186:1279-1288. Without being bound by theory, circulating nucleic acid-containing immune complexes in SLE patient sera facilitate nucleic acid antigen entry into pDC endosomes via Fc receptor-mediated endocytosis, followed by binding of nucleic acids to and activation of endosomal TLRs 7, 8, and 9. To assess the impact of the hybrid nuclease-PK molecules with altered glycosylation, SLE patient sera or plasma are pretreated with the hybrid nuclease-PK molecules, followed by addition cultures of pDC cells isolated from healthy volunteers. Levels of IFN-α produced are then determined at multiple time points. By degrading nucleic-acid containing immune complexes, effective hybrid nuclease-PK molecules with altered glycosylation are expected to reduce the quantity of IFN-α produced by the pDC cells.

The efficacy of hybrid nuclease-PK molecules can be tested by profiling their ability to degrade Toll-like receptor (TLR) ligands. HEK Blue cells (Invivogen) can be engineered to express human TLRs, including TLR3 and TLR9. When cultured in the presence of an appropriate ligand, HEK Blue cells secrete an alkaline phosphatase (SEAP) that is readily detected in the conditioned medium using a colorometric substrate. For example, TLR3 recognizes dsRNA ligands, such as poly(I:C), whereas TLR9 recognizes specific unmethylated CpG-ODN sequences. Incubation of the appropriate ligand with various RSLV constructs will cause a concentration-dependent inhibition in the output of SEAP. After treatment, the amount of SEAP produced is reduced to the levels generated by non-

TLR-stimulated cells. This will be consistent with the nuclease activity (RNase or DNase).

The effectiveness of hybrid nuclease-PK molecules with altered glycosylation is demonstrated by comparing the results of an assay from cells treated with a hybrid nuclease-PK molecules with altered glycosylation disclosed herein to the results of the assay from cells treated with control formulations. After treatment, the levels of the various markers (e.g., cytokines, cell-surface receptors, proliferation) described above are generally improved in an effective hybrid nuclease-PK molecule treated group relative to the marker levels existing prior to the treatment, or relative to the levels measured in a control group.

Methods of treatment

The hybrid nuclease-PK molecules with altered glycosylation of the invention are particularly effective in the treatment of autoimmune disorders or abnormal immune responses. In this regard, it will be appreciated that the hybrid nuclease-PK molecules with altered glycosylation may be used to control, suppress, modulate, treat, or eliminate unwanted immune responses to both external and autoantigens.

In another aspect, a hybrid nuclease-PK molecule with altered glycosylation is adapted for preventing (prophylactic) or treating (therapeutic) a disease or disorder, such as an autoimmune disease, in a mammal by administering a hybrid nuclease-PK molecule with altered glycosylation in a therapeutically effective amount or a sufficient amount to the mammal in need thereof, wherein the disease is prevented or treated. Any route of administration suitable for achieving the desired effect is contemplated by the invention (e.g., intravenous, intramuscular, subcutaneous). Treatment of the disease condition may result in a decrease in the symptoms associated with the condition, which may be long-term or short-term, or even a transient beneficial effect.

Numerous disease conditions are suitable for treatment with the hybrid nuclease-PK molecules with altered glycosylation of the invention. For example, in some aspects, the disease or disorder is an autoimmune disease or cancer. In some such aspects, the

autoimmune disease is insulin-dependent diabetes mellitus, multiple sclerosis, experimental autoimmune encephalomyelitis, rheumatoid arthritis, experimental autoimmune arthritis, myasthenia gravis, thyroiditis, an experimental form of uveoretinitis, Hashimoto's thyroiditis, primary myxoedema, thyrotoxicosis, pernicious anaemia, autoimmune atrophic gastritis, Addison's disease, premature menopause, male infertility, juvenile diabetes, Goodpasture's syndrome, pemphigus vulgaris, pemphigoid, sympathetic ophthalmia, phacogenic uveitis, autoimmune haemolytic anaemia, idiopathic leucopenia, primary biliary cirrhosis, active chronic hepatitis Hbs-ve, cryptogenic cirrhosis, ulcerative colitis, Sjogren's syndrome, scleroderma, Wegener's granulomatosis, polymyositis, dermatomyositis, discoid LE, SLE, or connective tissue disease.

In a specific embodiment, a hybrid nuclease-PK molecule with altered glycosylation is used to prevent or treat SLE or Sjogren's syndrome. The effectiveness of the hybrid nuclease-PK molecule is demonstrated by comparing the IFN-alpha levels, IFN-alpha response gene levels, autoantibody titers, kidney function and pathology, and/or circulating immune complex levels in mammals treated with the hybrid nuclease-PK molecule to mammals treated with control formulations.

For example, a human subject in need of treatment is selected or identified (e.g., a patient who fulfills the American College of Rheumatology criteria for SLE, or a patient who fulfills the American-European Consensus Sjogren's Classification Criteria). The subject can be in need of, e.g., reducing a cause or symptom of SLE or Sjogren's syndrome. The identification of the subject can occur in a clinical setting, or elsewhere, e.g., in the subject's home through the subject's own use of a self-testing kit.

At time zero, a suitable first dose of a hybrid nuclease-PK molecule with altered glycosylation is administered to the subject. The hybrid nuclease-PK molecule is formulated as described herein. After a period of time following the first dose, e.g., 7 days, 14 days, and 21 days, the subject's condition is evaluated, e.g., by measuring IFN-alpha levels, IFN-alpha response gene levels, autoantibody titers, kidney function and pathology, and/or circulating immune complex levels. Other relevant criteria can also be measured. The number and strength of doses are adjusted according to the subject's

needs. After treatment, the subject's IFN-alpha levels, IFN-alpha response gene levels, autoantibody titers, kidney function and pathology, and/or circulating immune complex levels are lowered and/or improved relative to the levels existing prior to the treatment, or relative to the levels measured in a similarly afflicted but untreated/control subject.

In another example, a rodent or monkey subject in need of treatment is selected or identified. The identification of the subject can occur in a laboratory setting or elsewhere. At time zero, a suitable first dose of a hybrid nuclease-PK molecule with altered glycosylation is administered to the subject. The hybrid nuclease-PK molecule is formulated as described herein. After a period of time following the first dose, e.g., 7 days, 14 days, and 21 days, the subject's condition is evaluated, e.g., by measuring IFN-alpha levels, IFN-alpha response gene levels, autoantibody titers, kidney function and pathology, and/or circulating immune complex levels. Other relevant criteria can also be measured. The number and strength of doses are adjusted according to the subject's needs.

After treatment, the subject's IFN-alpha levels, IFN-alpha response gene levels, autoantibody titers, kidney function and pathology, and/or circulating immune complex levels are lowered and/or improved relative to the levels existing prior to the treatment, or relative to the levels measured in a similarly afflicted but untreated/control subject.

Another aspect of the present invention is to use gene therapy methods for treating or preventing disorders, diseases, and conditions with one or more hybrid nuclease-PK molecules with altered glycosylation. The gene therapy methods relate to the introduction of a nucleic acid (DNA, RNA and antisense DNA or RNA) sequence encoding a hybrid nuclease-PK molecule with altered glycosylation into an animal in need thereof to achieve expression of the polypeptide or polypeptides of the present invention. This method can include introduction of one or more polynucleotides encoding a hybrid nuclease-PK molecule with altered glycosylation operably coupled to a promoter and any other genetic elements necessary for the expression of the polypeptide by the target tissue.

In gene therapy applications, hybrid nuclease-PK molecule (with altered glycosylation) genes are introduced into cells in order to achieve *in vivo* synthesis of a therapeutically effective genetic product. "Gene therapy" includes both conventional gene therapies where a lasting effect is achieved by a single treatment, and the administration of gene therapeutic agents, which involves the one time or repeated administration of a therapeutically effective DNA or mRNA. The oligonucleotides can be modified to enhance their uptake, *e.g.*, by substituting their negatively charged phosphodiester groups by uncharged groups.

EXAMPLES

Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

The practice of the present invention will employ, unless otherwise indicated, conventional methods of protein chemistry, biochemistry, recombinant DNA techniques and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. See, e.g., T.E. Creighton, Proteins: Structures and Molecular Properties (W.H. Freeman and Company, 1993); A.L. Lehninger, Biochemistry (Worth Publishers, Inc., current addition); Sambrook, et al, Molecular Cloning: A Laboratory Manual (2nd Edition, 1989); Methods In Enzymology (S. Colowick and N. Kaplan eds., Academic Press, Inc.); Remington's Pharmaceutical Sciences, 18th Edition (Easton, Pennsylvania: Mack Publishing Company, 1990); Carey and Sundberg Advanced Organic Chemistry 3rd Ed. (Plenum Press) Vols A and B (l 992).

EXAMPLE 1

Generating expression vectors for hybrid nuclease-PK molecules with altered glycosylation

Various embodiments of hybrid nuclease-PK molecules with altered glycosylation of the invention are shown in Figure 1, with amino acid sequences of each presented in Table 1. As exemplary hybrid nuclease-PK molecules with altered glycosylation, RSLV-319 and RSLV-320 were constructed (RSLV-319-320 are hybrid nuclease-albumin molecules). Specifically, starting from the amino acid sequence of these RSLV constructs, polynucleotides encoding them were directly synthesized using codon optimization by Genescript (Genescript, Piscatawy, N.J.) to allow for optimal expression in mammalian cells. The process of optimization involved, e.g., avoiding regions of very high (>80%) or very low (<30%) GC content when possible, and avoiding cis-acting sequence motifs, such as internal TATA-boxes, chi-sites and ribosomal entry sites, AT-rich or GC-rich sequence stretches, RNA instability motifs, repeat sequences and RNA secondary structures, and cryptic splice donor and acceptor sites in higher eukaryotes. DNAs encoding the hybrid nuclease-albumin molecules were cloned into the pcDNA3.1+ mammalian expression vector. The exemplary hybrid nuclease-albumin molecules referred to as RSLV-319 and RSLV-320 with altered glycosylation were generated. Hybrid nuclease-albumin molecules referred to as RSLV-315, RSLV-316, RSLV-317, RSLV-318, RSLV-321, and RSLV-322 can also be generated using the above protocol.

RSLV-315 (SEQ ID NO: 14) has the configuration RNase1 N34S/N76S/N88S-$(Gly_4Ser)_3$-HSA, wherein a mutant, human RNase1 N34S/N76S/N88S domain (SEQ ID NO: 2) is operably coupled via a $(Gly_4Ser)_3$ linker (SEQ ID NO: 12) to the N-terminus of wild-type HSA (SEQ ID NO: 6). The asparagines residues at positions 34, 76, and 88 of SEQ ID NO: 1 (potential acceptors of N-linked glycosylation) are mutated to serine. Albumin is a naturally aglycosylated protein.

RSLV-316 (SEQ ID NO: 15) has the configuration HSA-(Gly_4Ser)3-RNase1 N34S/N76S/N88S, wherein a mutant, human RNase1 domain N34S/N76S/N88S is operably coupled via a $(Gly_4Ser)_3$ linker to the C-terminus of wild-type HSA.

RSLV-317 (SEQ ID NO: 16) has the configuration DNase1 N18S/N106S/A114F-$(Gly_4Ser)_3$-HSA, wherein a mutant, human DNase1 N18S/N106S/A114F domain (SEQ ID NO: 5) is operably coupled via a $(Gly_4Ser)_3$ linker to the N-terminus of wild-type HSA. The asparagine residues at positions 40 and 128 are potential acceptors of N-linked glycosylation.

RSLV-318 (SEQ ID NO: 17) has the configuration HSA-$(Gly_4Ser)_3$-DNase1 N18S/N106S/A114F, wherein a mutant, human DNase1 domain N18S/N106S/A114F is operably coupled via a $(Gly_4Ser)_3$ linker to the C-terminus of wild-type HSA.

RSLV-319 (SEQ ID NO: 18) has the configuration RNase1 N34S/N76S/N88S-$(Gly_4Ser)_3$-HSA-$(Gly_4Ser)_3$-DNase1 A114F, wherein a mutant, human RNase1 N34S/N76S/N88S domain is operably coupled via a first $(Gly_4Ser)_3$ sequence to the N-terminus of wild-type HSA, and a mutant, human DNase1 A114F domain (SEQ ID NO: 4) is operably coupled via a second $(Gly_4Ser)_3$ sequence to the C-terminus of wild-type HSA.

RSLV-320 (SEQ ID NO: 19) has the configuration DNase1 A114F-$(Gly_4Ser)_3$-HSA-$(Gly_4Ser)_3$-RNase1 N34S/N76S/N88S, wherein a mutant, human DNase1 A114F domain is operably coupled via a first $(Gly_4Ser)_3$ sequence to the N-terminus of wild-type HSA, and a mutant, human RNase1 N34S/N76S/N88S domain is operably coupled via a second $(Gly_4Ser)_3$ sequence to the C-terminus of wild-type HSA.

RSLV-321 (SEQ ID NO: 20) has the configuration RNase1 N34S/N76S/N88S-$(Gly_4Ser)_3$-HSA-$(Gly_4Ser)_3$-DNase1 N18S/N106S/A114F, wherein a mutant, human RNase1 N34S/N76S/N88S domain is operably coupled via a first $(Gly_4Ser)_3$ sequence to the N-terminus of wild-type HSA, and a mutant, human DNase1 N18S/N106S/A114F domain is operably coupled via a second $(Gly_4Ser)_3$ sequence to the C-terminus of wild-type HSA.

RSLV-322 (SEQ ID NO: 21) has the configuration DNase1 N18S/N106S/A114F-$(Gly_4Ser)_3$-HSA-$(Gly_4Ser)_3$-RNase1 N34S/N76S/N88S, wherein a mutant, human DNase1 N18S/N106S/A114F domain is operably coupled via a first $(Gly_4Ser)_3$ sequence

to the N-terminus of wild-type HSA, and a mutant, human RNase1 N34S/N76S/N88S domain is operably coupled via a second $(Gly_4Ser)_3$ sequence to the C-terminus of wild-type HSA.

The hybrid nuclease-albumin molecules with altered glycosylation described above can also be generated using conventional cloning techniques well-known in the art, for example, by preparing modular cassettes of each component of the hybrid nuclease-albumin molecule (e.g., nuclease domain, linker domain, HSA) with compatible restriction enzyme sites to allow for shuttling and domain swapping. A polynucleotide encoding each component of the hybrid nuclease-albumin molecule (e.g., RNase, DNase, HSA) can be readily obtained by amplifying the component of interest using polymerase chain reaction (PCR) from an appropriate cDNA library. For example, the full length nucleotide sequences of human RNase1, DNase1, and HSA can be amplified from random primed and oligo dT primed cDNA derived from commercially available human pancreatic total RNA (Ambion/Applied Biosystems, Austin, TX) using sequence specific 5' and 3' primers based on published sequences of the component being amplified (or as shown in Table 1). PCR amplicons are purified by agarose gel electrophoresis and subsequent application to QIAquick gel purification columns. Purified amplicons are cloned into a convenient vector for subcloning and subsequent domain swapping and shuttling (e.g., pC42.1 TOPO cloning vector; Invitrogen, Carlsbad, CA). Polynucleotides encoding mutant nuclease domains or HSA variants are generated by introducing mutations into the domain of interest using commercially available kits (e.g., QuickChange™ site-directed mutagenesis kit; Stratagene), or overlap extension PCR to introduce mutations at desired positions, followed by DNA sequencing to confirm that the intended mutations are introduced. Linkers (e.g., $(Gly_4Ser)_3$ linkers) can be generated by overlap PCR using routine methods, or through direct synthesis using commercially available services, and designed to have overhangs or be blunt to facilitate subsequent cloning to allow for fusion with other domains of interest.

A similar approach can be taken to generate hybrid nuclease-PK molecules with altered glycosylation which have PK moieties other than albumin. Although albumin is naturally aglycosylated, other PK moieties, such as transferrin and Fc, can be engineered to lack

potential N-glycosylation sites as discussed *supra*. When the PK moiety is PEG, routine art-recognized methods, such as those described *supra*, can be used conjugate PEG to the nuclease domain(s).

Human RNase1 contains 3 sites of N-linked glycosylation corresponding to Asn^{54}, Asn^{96}, and Asn^{108} of RSLV-132 (numbering based on consecutive numbering including a 20 amino acid heterologous leader). In addition, the Fc region of RSLV-132 contains a single N-linked glycosylation site at Asn^{231}(numbering based on consecutive numbering of RSLV-132 including a 20 amino acid heterologous leader). Studies of human RNase1 have indicated that all 3 N-linked sites can be glycosylated and processed to a heterogeneous array of complex-type structures with fucosylated/sialylated biantennary structures dominating. The single N-linked site within the Fc domain of RSLV-132 also is expected to be glycosylated and processed to fucosylated complex-type structures based on studies of native IgG molecules.

To assess the impact of removing the N-linked oligosaccharides on RNase activity, two variants of RSLV-132 were generated (Figure 2). AG1-RSLV-132 (also referred to as "AG1" or "RSLV-132 AG1") was engineered to lack the 3 N-linked sites in the RNase domain; the three asparagine residues were mutated to serine residues. Similarly, AG2-RSLV-132 (also referred to as "AG2" or "RSLV-132 AG2") was engineered to lack the 3 N-linked sites in the RNase domain and the single N-linked site within the Fc domain (Figure 2).

AG1-RSLV-132 (SEQ ID NO: 71 and 72) has the configuration RNase1 N54S/N96S/N108S operably coupled to the N-terminus of a mutant human IgG1 Fc domain (SCC, P238S, P331S), wherein a mutant, human RNase1 having mutations at N-linked glycosylation sites N54 (N34), N96 (N76) and N108 (N88) is operably coupled to the N-terminus of a mutant human IgG1 Fc domain having a hinge region mutation (SCC) and P238S and P331S mutations. The sites of N-linked glycosylation are numbered consecutively in AG1-RSLV-132 including a 20 amino acid heterologous leader. Accordingly, N54, N96, and N108 correspond to N34, N76 and N88 in human

RNase1 without a leader sequence as shown in SEQ ID NO: 2 and N62, N104 and N116 in human RNase1 (P07998) including the 28 amino acid native leader sequence.

AG2-RSLV-132 (SEQ ID NO: 73 and 74) has the configuration RNase1 N54S/N96S/N108S operably coupled to the N-terminus of a mutant human IgG1 Fc domain (SCC, P238S, P331S, N231S), wherein a mutant, human RNase1 having mutations at N-linked glycosylation sites N54 (N34), N96 (N76) and N108 (N88) is operably coupled to the N-terminus of a mutant human IgG1 Fc domain having a hinge region mutation (SCC) and P238S and P331S mutations. The sites of N-linked glycosylation are numbered consecutively in AG1-RSLV-132 including a 20 amino acid heterologous leader. Accordingly, N54, N96, and N108 correspond to N34, N76 and N88 in human RNase1 without a leader sequence as shown in SEQ ID NO:11 and N62, N104 and N116 in human RNase1 (P07998) including the 28 amino acid native leader sequence. The site of N-linked glycosylation is numbered consecutively in AG2-RSLV-132 so N231S corresponds to N297S in human IgG1 Fc according to Kabat numbering and N83 according to numbering in SEQ ID NO:11.

EXAMPLE 2

Transient expression of mammalian cell lines with nuclease-Fc molecules with altered glycosylation

Expression

For transient expression, pcDNA3.1 expression vectors were constructed encoding AG1-RSLV-132 and AG2-RSLV-132 and these were employed to transiently transfect CHO-S cells (FreeStyle™ MAX CHO-S cells Life Technologies). On the day of transfection, CHO-S cells were seeded at a density of $1x10^6$ cells/ml in 500 ml of FreeStyle CHO Expression Medium supplemented to a final concentration of 8 mM L-glutamine. 625 μg of plasmid DNA was diluted into OptiPRO SFM to a total volume of 10 mls. In a separate tube, 625 ml of FreeStyle MAX Transfection Reagent was diluted into OptiPRO SFM to a total volume of 10 mls. The diluted FreeStyle MAX Transfection Reagent was immediately added to the diluted DNA solution to obtain a total volume of 20 mls. This

DNA-Freestyle MAX solution was mixed gently and incubated for 10 minutes after which the mixture was added to the cell suspension while slowly swirling the flask. Transfected cultures were incubated at 37°C in the presence of 8% CO_2 on an orbital shaker platform rotating at 135 rpm. Conditioned media were harvested on day 5 post-transfection.

Purification

Harvested conditioned media from the individual cultures were passed over a column of Protein A-Sepharose (1 ml total bed volume) after which the column was washed with 10 ml of PBS. The column was then washed with 10 ml PBS supplemented to 2M NaCl, and then re-equilibrated with PBS. When the absorbance (OD_{280}) of the eluate achieved a stable base line value, the column was eluted with 10 mM sodium formate, pH 3.5, and individual 1 ml fractions were collected and immediately adjusted to pH 7 with 2 M L-Arginine, pH 10.0. Fractions corresponding to the low pH-induced peak of OD_{280} positive material were pooled and concentrated 3x using a 20K MWCO concentrator (Pierce; Catalog # 89886 A). To further purify the constructs, the concentrated protein A pools were subjected to Superdex 200 10/30 column chromatography using a running buffer composed of 100 mM Na_2SO_4, 100 mM NaOAc, pH 6.0. Positive fractions, based upon OD_{280} and Coomassie stained gels, were pooled and final protein concentrations were determined based on OD_{280} values and an extinction coefficient of 46922 M^{-1} cm^{-1}.

Larger Scale Expression and Purification

In order to obtain larger quantities of AG1-RSLV-132, the GeneArt clone (in pcDNA3.1$^+$ vector) was sent to BRI (National Research Council Canada) where it was reinserted into their proprietary vector and transfected into their own version of CHO cell hosts. The secreted protein product was purified by Protein A-Sepharose chromatography and dialyzed into 20 mM sodium citrate, pH 6, 200 mM trehalose, 50 mM L-arginine. The material provided by BRI was determined to be 8.42 mg/ml (based on OD_{280}).

EXAMPLE 3

Transient expression of mammalian cell lines with nuclease-albumin molecules with altered glycosylation

pcDNA3.1$^+$ mammalian expression vectors were constructed encoding RSLV-308, RSLV-310, RSLV-319 and RSLV-320 and transiently expressed in CHO cells using FreeStyle™ MAX Reagent, e.g., CHO-S cells (e.g., FeeStyle™ CHO-S cells, Life Technologies). A two-step column chromatography purification scheme was developed and employed to prepare highly enriched preparations of the nuclease-HSA fusion constructs. In particular, the two-step purification process included: (1) sequential ion exchange (Q Sepharose Fast Flow resin) and (2) size exclusion (Superdex 200 10/30) chromatography. All chromatography steps were conducted using a Biorad BioLogic FPLC system.

RSLV-308 (SEQ ID NO: 93) has the configuration RNase1-$(Gly_4Ser)_3$-HSA-$(Gly_4Ser)_3$-DNase1 A114F, wherein a wild-type, human RNase1 domain is operably coupled via a first $(Gly_4Ser)_3$ sequence to the N-terminus of wild-type HSA, and a mutant, human DNase1 A114F domain is operably coupled via a second $(Gly_4Ser)_3$ sequence to the C-terminus of wild-type HSA. The nucleic acid sequence of RSLV-308 is set forth in SEQ ID NO: 97.

RSLV-310 (SEQ ID NO: 94) has the configuration DNase1 A114F-$(Gly_4Ser)_3$-HSA-$(Gly_4Ser)_3$-RNase1, wherein a mutant, human DNase1 A114F domain is operably coupled via a first $(Gly_4Ser)_3$ sequence to the N-terminus of wild-type HSA, and a wild-type, human RNase1 domain is operably coupled via a second $(Gly_4Ser)_3$ sequence to the C-terminus of wild-type HSA. The nucleic acid sequence of RSLV-310 is set forth in SEQ ID NO: 98.

RSLV-319 (SEQ ID NO: 18) has the configuration RNase1 N34S/N76S/N88S-$(Gly_4Ser)_3$-HSA-$(Gly_4Ser)_3$-DNase1 A114F, wherein a mutant, human RNase1 N34S/N76S/N88S domain is operably coupled via a first $(Gly_4Ser)_3$ sequence to the N-

terminus of wild-type HSA, and a mutant, human DNase1 A114F domain (SEQ ID NO: 4) is operably coupled via a second $(Gly_4Ser)3$ sequence to the C-terminus of wild-type HSA.

RSLV-320 (SEQ ID NO: 19) has the configuration DNase1 A114F-$(Gly_4Ser)_3$-HSA-$(Gly_4Ser)_3$-RNase1 N34S/N76S/N88S, wherein a mutant, human DNase1 A114F domain is operably coupled via a first $(Gly_4Ser)_3$ sequence to the N-terminus of wild-type HSA, and a mutant, human RNase1 N34S/N76S/N88S domain is operably coupled via a second $(Gly_4Ser)_3$ sequence to the C-terminus of wild-type HSA.

Expression of the hybrid nuclease-albumin molecules was assayed by standard Western Blot analysis. Expression of each of the generated hybrid nuclease-albumin molecules was observed at the expected size.

EXAMPLE 4

SDS gel electrophoresis of nuclease-Fc molecules with altered glycosylation

The small scale purified RSLV-132 aglycosylated variants were subjected to SDS gel electrophoresis in the presence and absence of reducing agents (dithiothreitol), and compared to glycosylated RSLV-132 Reference Material. The gels were stained with Coomassie blue to visualize the proteins. Under non-reducing conditions, AG1-RSLV-132 (AG1) and AG2-RSLV-132 (AG2) migrated faster than RSLV-132 itself. Moreover, the Coomassie staining bands corresponding to AG1-RSLV-132 and AG2-RSLV-132 were less heterogeneous (*i.e.*, more homogeneous) than the broad staining band observed with RSLV-132 (Figure 3A). Following reduction, RSLV-132 migrated as a quartet of Coomassie staining bands. In contrast, reduced AG1-RSLV-132 and AG2-RSLV-132 migrated as single species (Figure 3A). These observed differences in electrophoretic behavior are consistent with the removal of N-linked oligosaccharides from AG1-RSLV-132 and AG2-RSLV-132.

EXAMPLE 5

Nuclease activity of purified hybrid nuclease-Fc molecules with altered glycosylation

RNase activity assessment in the RNaseAlert® format

RNase activity assays of three variants (RSLV-132, AG1-RSLV-132 and AG2-RSLV-132) were performed using the RNaseAlert® assay format. In this analysis, RSLV-132 and the lab scale preparations of AG1-RSLV-132 and AG2-RSLV-132 were compared to a standard curve generated with RSLV-132. As indicated in Figure 3B, the three preparations showed similar catalytic activities toward the quenched, fluorescent substrate employed in the RNaseAlert® kit. Thus, removal of N-linked oligosaccharides did not affect the inherent RNase activity of AG1-RSLV-132 and AG2-RSLV-132.

Version 3 RNase activity assessment

The version 3 activity assay format was designed as an endpoint assay, rather than a kinetic analysis required by the version 1 activity format (RNaseAlert®). The mixed substrate burden in the version 3 format consists of a small amount of a quenched, fluorescent oligonucleotide (consisting of 5 total nucleotides) and an excess of unlabeled low molecular weight poly(I:C), a synthetic double-stranded RNA. In this format, both substrates are assumed to be hydrolyzed by the RNase domain of RSLV-132 but the large excess of poly(I:C) slows hydrolysis of the quenched, fluorescent oligonucleotide due to competition; as a result, there is minimal (<15%) overall hydrolysis of the fluorescent signal oligonucleotide under the conditions and time course (60 min) of the assay. Three different preparations of AG1-RSLV-132 were profiled in the version 3 format: (1) AG1-RSLV-132 (Fremont) produced on a small scale by transfection of CHO-S cells and purified by Protein-A Sepharose chromatography, (2) AG1- RSLV-132 (AG1) Supernatant (BRI) produced as a Protein A-isolate of the CHO cell conditioned medium obtained following BRI's larger scale expression of AG1-RSLV-132 (isolated by Protein A-agarose chromatography), and (3) AG1-RSLV-132 (AG1) Purified (BRI) represents the large scale fully purified product generated by BRI. Each sample was tested over a range of four concentrations in the version 3 assay format. The mean specific activity (U/mg)

was calculated and shown as a function of the preparation. For comparision, an RSLV-132 reference standard was included as a bench mark. As shown in Figure 4, all three AG1-RSLV-132 preparations yielded reduced specific activity values relative to the RSLV-132 reference.

hTLR3 HEK Blue cell-based activity assay

When cultured in the presence of poly(I:C), HEK Blue cells engineered to express human Toll-like receptor 3 (TLR3) secrete an alkaline phosphatase (SEAP) that is readily detected in the conditioned medium using a colorometric substrate. TLR3-transfected cells are commercially available from Invivogen. Aliquots of hTLR3 HEK Blue cell growth media containing a fixed concentration of poly(I:C) (400 ng/ml) were preincubated with the indicated concentrations of RSLV-132 (lot# P3084776) or AG1-RSLV-132 (AG1) for 45 min. The pretreated mixtures then were added to wells of a 96-well plate containing hTLR3 HEK Blue cells and incubated overnight at 37°C. Where indicated in Figure 5 (circles and right side-up triangles), RSLV-132 or AG1-RSLV-132 was added to the cells in the absence of poly(I:C). The overnight conditioned media subsequently were harvested and assessed for SEAP activity; the amount of activity detected is indicated (A_{620}) as a function of fusion construct concentration. Two of the three assays performed are shown. During the 45-min preincubation, the constructs are 5-fold higher than indicated on the X-axis; a 5-fold dilution occurs when the reaction mixtures subsequently are diluted into the cell culture wells. Concentrations of RSLV-132 and AG1-RSLV-132 are calculated on the basis of monomeric subunits.

As shown in Figure 5, pre-incubation of a fixed concentration of poly(I:C) with RSLV-132 prior to addition to hTLR3 HEK Blue cell cultures reduced SEAP output in a concentration-dependent manner. The ability of RSLV-132 to degrade poly(I:C) is notable because RNase1 is the most active member of the human RNase A family with respect to degrading double stranded (including poly(I:C)) RNA (6,7). In a side-by-side comparison, AG1-RSLV-132 yielded a similar dose response curve to RSLV-132 with respect to inhibition of poly(I:C)-induced SEAP output from hTLR3 HEK Blue cells (Figure 5). Mean estimated IC_{50} values (n=3) were $1.76x10^{-10}$ +/- $3.2x10^{-11}$ M and $1.42x10^{-10}$ +/-

$3.8x10^{-11}$ M for RSLV-132 and AG1-RSLV-132, respectively. Thus, despite a low apparent activity in the version 3 assay format, AG1-RSLV-132 appears to be equally capable to RSLV-132 at degrading poly(I:C).

The observed lack of activity in the version 3 assay format may reflect that AG1-RSLV-132 prefers poly(I:C) as a substrate over the quenched, fluorescent oligonucleotide. This preference would lead to reduced hydrolysis of the fluorescent reporter molecule and the appearance of low catalytic activity in the version 3 assay format. Absence of negatively charged N-linked oligosaccharides may enhance binding of large negatively-charged oligosaccharides such as poly(I:C) to AG1-RSLV-132.

EXAMPLE 6

Nuclease activity of purified hybrid nuclease-albumin molecules with altered glycosylation

To assess the relative catalytic activity of nuclease-albumin constructs, four different variants were produced and profiled side-by-side using either the RNaseAlert® or DNaseAlert® substrate kits. Two of the HSA constructs contained fully glycosylated RNase and DNase domains; RSLV-308 contained RNase at the N-terminus and DNase at the C-terminus whereas RSLV-310 had the opposite orientation. The other two constructs contained mutations to prevent N-linked glycosylation within the RNase domains; RSLV-319 contained the mutated RNase domain at the N-terminus and DNase domain at the C-terminus whereas RSLV-320 had the opposite orientation. In this analysis, rates of hydrolysis were calculated for each substrate and compared to the activity of RSLV-133. The observed RNase activities of the RSLV-300 series constructs were consistently less than RSLV-133. However, when expressed as a percentage of the RSLV-133 activity, RSLV-319 and RSLV-320, which possess mutations in the RNase domain to reduce N-linked glycosylation, demonstrated RNase activity comparable to their glycosylated counterparts (RSLV-308 and RSLV-310) in the RNaseAlert format (Table 2). Likewise, all four HSA constructs showed reduced activity relative to RSLV-133 in the DNaseAlert

assay format but the two variants possessing mutations to prevent N-linked glycosylation (RSLV-319 and RSLV-320) were comparable to their wild-type counterparts (RSLV-308 and RSLV-310; Table 2)

Table 2: Comparison of RNase and DNase activities of RSLV-300 constructs using Alert Formats

Construct	% RNase Activity relative to RSLV-133	% DNase Activity relative to RSLV-133
RSLV-308	25%	8%
RSLV-310	31%	5%
RSLV-319	20%	18%
RSLV-320	25%	6%
RSLV-133	100%	100%

EXAMPLE 7

Size Exclusion Chromatography

Chromatographic behavior of AG1-RSLV-132 was assessed by size exclusion chromatography. A TSK-Gel G3000 SW_{XL} column (Tosoh Biosciences LLC; 7.8 mm X 30 cm; 5 m particle size) was equilibrated in 0.1 M Na_2SO_4, pH 6. 758 mg of AG1-RSLV-132 were loaded onto the column. The purified AG1-RSLV-132 protein eluted primarily as a single uniform peak with an elution time of 8.62 min and represented >97% of the total recovered A_{280} absorbance (Figure 6A, Top panel). Based on comparison to the elution of the molecular weight standards, this would equate to an apparent MW of 100 kDa. The small peak at 11.5 min is due to trehalose within the buffer (Figure 6A, Top panel). The elution time of AG1-RSLV-132 is greater than observed historically with RSLV-132 on the same column (8.2-8.3 min corresponding to an apparent MW of 130 kDa), consistent with AG1-RSLV-132 being smaller as a result of absence of N-linked oligosaccharides. The width of the major peak observed with AG1-RSLV-132 also appeared less than that observed with RSLV-132 itself analyzed under

similar conditions (Figure 6B); this is expected based on the absence of N-linked oligosaccharides mitigating molecular heterogeneity.

AG1-RSLV-132 eluted as a highly symmetrical peak with an apparent MW of 100 kDa consistent with an Fc dimer, and there was little evidence of aggregates. As such, absence of the 3 N-linked oligosaccharides from the RNase domain did not appear to impact protein folding.

EXAMPLE 8

Comparison of AG1-RSLV-132 and RSLV-132 PK profiles following intravenous administration to Cynomolgus monkeys

A monkey PK study comparing RSLV-132 and AG1-RSLV-132 (Ricerca Study #031391) was conducted. This study consisted of two cohorts (n=3 animals/cohort): one received RSLV-132 at 30 mg/kg and the other received AG1-RSLV-132 at 30 mg/kg. Both constructs were formulated in 20 mM sodium citrate, pH 6, 200 mM trehalose, 50 mM L-arginine and administered via an IV bolus injection. At pre-specified times post-injection (Figure 7), serum samples were harvested and assessed for the presence of the construct using a competitive ELISA assay (analysis conducted by ABC Labs). It is assumed, though not experimentally defined, that RSLV-132 and AG1-RSLV-132 compete equally with the reuthenium-labeled RSLV-132 probe used in the competitive ELISA. The curves in Figure 7 show the mean serum concentration observed (n=3/group) over the 12 day time period.

A summary of study findings are presented in Table 3. Immediately after injection, the observed serum levels of AG1-RSLV-132 were elevated above those observed in animals receiving RSLV-132. This is represented by mean C_0 values being estimated as 918 μg/ml for AG1-RSLV-132 and 221 μg/ml for RSLV-132. As a result of the enhanced serum levels achieved immediately post-injection, overall exposure throughout the 12 day evaluation period was higher for AG1-RSLV-132 (4460 +/- 2440 hr*μg/ml) than for

RSLV-132 (3320 +/- 1570 hr*μg/ml). However, as shown in Figure 7, terminal half-lives of the two constructs appear very similar. Individual animal RSLV-132 and AG1-RSLV-132 (AG1) concentration versus time profiles following a single 30 mg/kg IV bolus dose to male Cynomolgus monkeys is graphically depicted in Figure 8.

Following IV administration to Cynomolgus monkeys, AG1-RSLV-132 yielded higher (approximately 4-fold) initial serum concentrations than did RSLV-132. This difference may indicate that some RSLV-132 molecules, by virtue of the composition of their associated N-linked oligosaccharides, bind to cells and/or tissues and are rapidly removed from the circulation. Cells and tissues are known to express lectin-like receptors on their surfaces which recognize specific carbohydrate structures (8). Since AG1-RSLV-132 lacks many of the N-linked oligosaccharides found on RSLV-132, it may be less prone to this type of serum removal mechanism. Alternatively, sialylated, negatively-charged N-linked oligosaccharides associated with RSLV-132 may mediate ionic interactions with cells and/or tissues; since AG1-RSLV-132 lacks many of these sialylated oligosaccharides, it may be less subject to such interactions. The increased serum concentrations observed early after IV bolus injection of AG1-RSLV-132 allowed it to achieve a higher overall exposure than RSLV-132 over the course of the 12 day analysis period. However, the terminal half-lives of the two constructs appeared similar.

Table 3. Summary of pharmacokinetics of RSLV-132 and AG1-RSLV-132 (RSLV-132 AG1) following intravenous administration to male Cynomolgus Monkeys

Group	Test Article	Animal ID	C_0 (µg/mL)	C_{max} (µg/mL)	AUC_{0-t} (hr*µg/mL)
1	RSLV-132	131204	195	191	2570
		131236	278	291	5130
		131256	190	189	2270
		N	**3**	**3**	**3**
		Mean	**221**	**223**	**3320**
		SD	**49.5**	**58.2**	**1570**
2	RSLV-132 AG1	131206	1110	1050	7280
		131218	806	696	3170
		131257	833	703	2930
		N	**3**	**3**	**3**
		Mean	**918**	**818**	**4460**
		SD	**171**	**204**	**2440**

Table 4: Monkey serum concentration (mg/mL) of RSLV-132 and AG1-RSLV-132 (RSLV-132 AG1) versus time following a single 30 mg/Kg IV bolus dose.

	RSLV-132 (Group 1)			RSLV-132 AG1 (Group 2)		
Time (hr)	N	Mean (µg/ml)	SD (µg/ml)	N	Mean (µg/ml)	SD (µg/ml)
0.000	0	BQL	--	0	BQL	--
0.166	3	219	50.8	3	818	204
1	3	216	65.0	3	477	277
6	3	76.3	20.2	3	133	72.2
24	3	19.7	11.0	3	16.4	7.58
48	3	10.6	7.79	3	4.77	3.92
96	3	3.23	1.20	3	2.98	1.82
144	3	5.57	5.65	3	2.87	2.23
288	3	2.82	1.06	3	1.75	1.34

BQL = below quantitation limit

EXAMPLE 9

Analysis of enzyme kinetics

Efficacy of hybrid nuclease-albumin molecules with altered glycosylation *in vitro*

Effects of hybrid nuclease-albumin molecules with altered glycosylation on cytokine expression

Human PBMCs are isolated from healthy volunteers and lupus patients and cultured. The cells are treated with various stimulatory TLR ligands, costimulatory antibodies, immune complexes, and normal or autoimmune sera, with or without the hybrid nuclease-albumin molecules with altered glycosylation described herein, such as the hybrid nuclease-albumin molecules with altered glycosylation of Example 1. Culture supernatant is collected at various time points (e.g., 6 hrs, 12 hrs, 24 hrs, 48 hrs, etc) and levels of a panel of cytokines, including human IL-6, IL-8, IL-10, IL-4, IFN-gamma, IFN-alpha and TNF-alpha are measured using commercially available ELISA kits from, e.g., Thermo Fisher Scientific, Inc. Effective hybrid nuclease-albumin molecules with altered glycosylation are expected to reduce the levels of cytokines produced by stimulated PBMCs relative to controls.

Effects of hybrid nuclease-albumin molecules with altered glycosylation on lymphocyte activation receptor expression

Human PBMCs are isolated from healthy volunteers and lupus patients and cultured. The cells are treated with various stimulatory TLR ligands, costimulatory antibodies, immune complexes, and normal or autoimmune sera, with or without the hybrid nuclease-albumin molecules with altered glycosylation described herein, such as the hybrid nuclease-albumin molecules with altered glycosylation of Example 1. Cells are then subjected to multi-color flow cytometry to measure the expression of lymphocyte activation receptors CD5, CD23, CD69, CD80, CD86, and CD25 at various time points (e.g., 6 hrs, 12 hrs, 24

hrs, 48 hrs, etc.) after stimulation using routine art-recognized methods. Suitable antibodies for these receptors are commercially available from, e.g., BD/PharMingen. Effective hybrid nuclease-albumin molecules with altered glycosylation are expected to reduce the expression of the lymphocyte activation receptors in stimulated PBMCs relative to controls.

Effects of hybrid nuclease-albumin molecules with altered glycosylation on plasmacytoid dendritic cell (pDC) interferon output

pDCs from healthy volunteers are isolated using art-recognized methods or commercially available kits, such as the EasySep™ Human EpCAM Positive Selection Kit (StemCell Technologies, Inc.). Isolated pDCs are cultured in, e.g., 96-well flat-bottom plates, at a densities ranging from 5 x 10^4 to 2.5 x 10^5/well in 0.1 ml in an appropriate medium (e.g., complete RPMI medium containing 10% FBS, 2 mM glutamine, 55 μM β-mercaptoethanol, 1 mM sodium pyruvate, 100 U/ml penicillin, and 100 μg/ml streptomycin). Cultured pDCs are activated by adding sera or plasma from individual SLE patients diluted with culture medium at a 1:5 ratio, and 0.1 ml of these samples are added to the cell-containing wells (final patient serum concentration is 10%). Cultures are incubated at 37°C for 40 hr, after which the conditioned media is harvested and assessed for IFNα content using a commercially available ELISA kit. Serum samples obtained from healthy volunteers are used as controls. To assess the impact of the hybrid nuclease-albumin constructs, SLE patient sera or plasma is pretreated with the hybrid nuclease-albumin constructs (1-10 μg/ml) with altered glycosylation for 30 min and added to the pDC cultures. Effective hybrid nuclease-albumin molecules are expected to reduce the quantity of IFNα produced as a result of degrading the nucleic acid-containing ICs.

The assays described above are equally applicable to hybrid nuclease-PK molecules with altered glycosylation having different PK moieties and are tailored accordingly.

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While the invention has been particularly shown and described with reference to a preferred embodiment and various alternate embodiments, it will be understood by persons skilled in the relevant art that various changes in form and details can be made therein without departing from the spirit and scope of the invention.

All references, issued patents and patent applications cited within the body of the instant specification are hereby incorporated by reference in their entirety, for all purposes.

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Table 1. Summary of sequences

SEQ ID NO	Description	Sequence
1	Mature wild type human RNase1	KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRNMTQ GRCKPVNTFVHEPLVDVQNVCFQEKVTCKNGQGNCYK SNSSMHITDCRLTNGSRYPNCAYRTSPKERHIIVACE GSPYVPVHFDASVEDST
2	Mature human RNase1 N34S/N76S/N88S	KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRSMTQ GRCKPVNTFVHEPLVDVQNVCFQEKVTCKNGQGNCYK SSSSMHITDCRLTSGSRYPNCAYRTSPKERHIIVACE GSPYVPVHFDASVEDST
3	Mature wild type human DNase1	LKIAAFNIQTFGETKMSNATLVSYIVQILSRYDIALV QEVRDSHLTAVGKLLDNLNQDAPDTYHYVVSEPLGRN SYKERYLFVYRPDQVSAVDSYYYDDGCEPCGNDTFNR EPAIVRFFSRFTEVREFAIVPLHAAPGDAVAEIDALY DVYLDVQEKWGLEDVMLMGDFNAGCSYVRPSQWSSIR LWTSPTFQWLIPDSADTTATPTHCAYDRIVVAGMLLR GAVVPDSALPFNFQAAYGLSDQLAQAISDHYPVEVML K
4	Human DNase1 A114F	LKIAAFNIQTFGETKMSNATLVSYIVQILSRYDIALV QEVRDSHLTAVGKLLDNLNQDAPDTYHYVVSEPLGRN SYKERYLFVYRPDQVSAVDSYYYDDGCEPCGNDTFNR EPFIVRFFSRFTEVREFAIVPLHAAPGDAVAEIDALY DVYLDVQEKWGLEDVMLMGDFNAGCSYVRPSQWSSIR LWTSPTFQWLIPDSADTTATPTHCAYDRIVVAGMLLR GAVVPDSALPFNFQAAYGLSDQLAQAISDHYPVEVML K
5	Human DNase1 N18S/N106S/A114F	LKIAAFNIQTFGETKMSSATLVSYIVQILSRYDIALV QEVRDSHLTAVGKLLDNLNQDAPDTYHYVVSEPLGRN SYKERYLFVYRPDQVSAVDSYYYDDGCEPCGSDTFNR EPFIVRFFSRFTEVREFAIVPLHAAPGDAVAEIDALY DVYLDVQEKWGLEDVMLMGDFNAGCSYVRPSQWSSIR LWTSPTFQWLIPDSADTTATPTHCAYDRIVVAGMLLR GAVVPDSALPFNFQAAYGLSDQLAQAISDHYPVEVML K
6	Human serum albumin (HSA; mature)	DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFE DHVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKL CTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPN LPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPY FYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDEL RDEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQR FPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRAD LAKYICENQDSISSKLKECCEKPLLEKSHCIAEVEND EMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYE YARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECY AKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNAL LVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAK RMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESL VNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSE KERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFV EKCCKADDKETCFAEEGKKLVAASQAALGL
7	Human serum transferrin (HST; mature)	VPDKTVRWCAVSEHEATKCQSFRDHMKSVIPSDGPSV ACVKKASYLDCIRAIAANEADAVTLDAGLVYDAYLAP NNLKPVVAEFYGSKEDPQTFYYAVAVVKKDSGFQMNQ

		LRGKKSCHTGLGRSAGWNIPIGLLYCDLPEPRKPLEK AVANFFSGSCAPCADGTDFPQLCQLCPGCGCSTLNQY FGYSGAFKCLKDGAGDVAFVKHSTIFENLANKADRDQ YELLCLDNTRKPVDEYKDCHLAQVPSHTVVARSMGGK EDLIWELLNQAQEHFGKDKSKEFQLFSSPHGKDLLFK DSAHGFLKVPPRMDAKMYLGYEYVTAIRNLREGTCPE APTDECKPVKWCALSHHERLKCDEWSVNSVGKIECVS AETTEDCIAKIMNGEADAMSLDGGFVYIAGKCGLVPV LAENYNKSDNCEDTPEAGYFAIAVVKKSASDLTWDNL KGKKSCHTAVGRTAGWNIPMGLLYNKINHCRFDEFFS EGCAPGSKKDSSLCKLCMGSGLNLCEPNNKEGYYGYT GAFRCLVEKGDVAFVKHQTVPQNTGGKNPDPWAKNLN EKDYELLCLDGTRKPVEEYANCHLARAPNHAVVTRKD KEACVHKILRQQQHLFGSNVTDCSGNFCLFRSETKDL LFRDDTVCLAKLHDRNTYEKYLGEEYVKAVGNLRKCS TSSLLEACTFRRP
8	HST N413S/N611S	VPDKTVRWCAVSEHEATKCQSFRDHMKSVIPSDGPSV ACVKKASYLDCIRAIAANEADAVTLDAGLVYDAYLAP NNLKPVVAEFYGSKEDPQTFYYAVAVVKKDSGFQMNQ LRGKKSCHTGLGRSAGWNIPIGLLYCDLPEPRKPLEK AVANFFSGSCAPCADGTDFPQLCQLCPGCGCSTLNQY FGYSGAFKCLKDGAGDVAFVKHSTIFENLANKADRDQ YELLCLDNTRKPVDEYKDCHLAQVPSHTVVARSMGGK EDLIWELLNQAQEHFGKDKSKEFQLFSSPHGKDLLFK DSAHGFLKVPPRMDAKMYLGYEYVTAIRNLREGTCPE APTDECKPVKWCALSHHERLKCDEWSVNSVGKIECVS AETTEDCIAKIMNGEADAMSLDGGFVYIAGKCGLVPV LAENYSKSDNCEDTPEAGYFAIAVVKKSASDLTWDNL KGKKSCHTAVGRTAGWNIPMGLLYNKINHCRFDEFFS EGCAPGSKKDSSLCKLCMGSGLNLCEPNNKEGYYGYT GAFRCLVEKGDVAFVKHQTVPQNTGGKNPDPWAKNLN EKDYELLCLDGTRKPVEEYANCHLARAPNHAVVTRKD KEACVHKILRQQQHLFGSSVTDCSGNFCLFRSETKDL LFRDDTVCLAKLHDRNTYEKYLGEEYVKAVGNLRKCS TSSLLEACTFRRP
9	HST S12A/N413S/N611S	VPDKTVRWCAVAEHEATKCQSFRDHMKSVIPSDGPSV ACVKKASYLDCIRAIAANEADAVTLDAGLVYDAYLAP NNLKPVVAEFYGSKEDPQTFYYAVAVVKKDSGFQMNQ LRGKKSCHTGLGRSAGWNIPIGLLYCDLPEPRKPLEK AVANFFSGSCAPCADGTDFPQLCQLCPGCGCSTLNQY FGYSGAFKCLKDGAGDVAFVKHSTIFENLANKADRDQ YELLCLDNTRKPVDEYKDCHLAQVPSHTVVARSMGGK EDLIWELLNQAQEHFGKDKSKEFQLFSSPHGKDLLFK DSAHGFLKVPPRMDAKMYLGYEYVTAIRNLREGTCPE APTDECKPVKWCALSHHERLKCDEWSVNSVGKIECVS AETTEDCIAKIMNGEADAMSLDGGFVYIAGKCGLVPV LAENYSKSDNCEDTPEAGYFAIAVVKKSASDLTWDNL KGKKSCHTAVGRTAGWNIPMGLLYNKINHCRFDEFFS EGCAPGSKKDSSLCKLCMGSGLNLCEPNNKEGYYGYT GAFRCLVEKGDVAFVKHQTVPQNTGGKNPDPWAKNLN EKDYELLCLDGTRKPVEEYANCHLARAPNHAVVTRKD KEACVHKILRQQQHLFGSSVTDCSGNFCLFRSETKDL LFRDDTVCLAKLHDRNTYEKYLGEEYVKAVGNLRKCS TSSLLEACTFRRP
10	Human wild type IgG1 Fc	LEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK

		TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGK
11	Human Fc N83S	LEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYSSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGK
12	$(Gly_4Ser)3$	GGGGSGGGGSGGGGS
13	VK3LP	METPAQLLFLLLLWLPDTTG
14	RSLV-315 (RNase I N34S/N76S/N88S-linker-HSA)	METPAQLLFLLLLWLPDTTGKESRAKKFQRQHMDSDS SPSSSSTYCNQMMRRRSMTQGRCKPVNTFVHEPLVDV QNVCFQEKVTCKNGQGNCYKSSSSMHITDCRLTSGSR YPNCAYRTSPKERHIIVACEGSPYVPVHFDASVEDST GGGGSGGGGSGGGGSDAHKSEVAHRFKDLGEENFKAL VLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADESA ENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEP ERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEET FLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQ AADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFG ERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTE CCHGDLLECADDRADLAKYICENQDSISSKLKECCEK PLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKN YAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYET TLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNC ELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRN LGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEK TPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFN AETFTFHADICTLSEKERQIKKQTALVELVKHKPKAT KEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVA ASQAALGL
15	RSLV-316 (HSA-linker-RNase1 N34S/N76S/N88S)	METPAQLLFLLLLWLPDTTGDAHKSEVAHRFKDLGEE NFKALVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCV ADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNPNLPRLVRPEVDVMCTAFH DNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAF TECCQAADKAACLLPKLDELRDEGKASSAKQRLKCAS LQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISSKLK ECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESK DVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLA KTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNL IKQNCELFEQLGEYKFQNALLVRYTKKVPQVSTPTLV EVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLC VLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYV PKEFNAETFTFHADICTLSEKERQIKKQTALVELVKH KPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEG KKLVAASQAALGLGGGGSGGGGSGGGGSKESRAKKFQ RQHMDSDSSPSSSSTYCNQMMRRRSMTQGRCKPVNTF VHEPLVDVQNVCFQEKVTCKNGQGNCYKSSSSMHITD CRLTSGSRYPNCAYRTSPKERHIIVACEGSPYVPVHF DASVEDST

16	RSLV-317 (DNase1 N18S/N106S/A114F-linker-HSA)	METPAQLLFLLLLWLPDTTGLKIAAFNIQTFGETKMS SATLVSYIVQILSRYDIALVQEVRDSHLTAVGKLLDN LNQDAPDTYHYVVSEPLGRNSYKERYLFVYRPDQVSA VDSYYYDDGCEPCGSDTFNREPFIVRFFSRFTEVREF AIVPLHAAPGDAVAEIDALYDVYLDVQEKWGLEDVML MGDFNAGCSYVRPSQWSSIRLWTSPTFQWLIPDSADT TATPTHCAYDRIVVAGMLLRGAVVPDSALPFNFQAAY GLSDQLAQAISDHYPVEVMLKGGGGSGGGGSGGGGSD AHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFED HVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLC TVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNL PRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYF YAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELR DEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQRF PKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADL AKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDE MPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEY ARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYA KVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALL VRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKR MPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLV NRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEK ERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVE KCCKADDKETCFAEEGKKLVAASQAALGL
17	RSLV-318 (HSA-linker-DNase1 N18S/N106S/A114F)	METPAQLLFLLLLWLPDTTGDAHKSEVAHRFKDLGEE NFKALVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCV ADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNPNLPRLVRPEVDVMCTAFH DNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAF TECCQAADKAACLLPKLDELRDEGKASSAKQRLKCAS LQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISSKLK ECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESK DVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLA KTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNL IKQNCELFEQLGEYKFQNALLVRYTKKVPQVSTPTLV EVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLC VLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYV PKEFNAETFTFHADICTLSEKERQIKKQTALVELVKH KPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEG KKLVAASQAALGLGGGGSGGGGSGGGGSLKIAAFNIQ TFGETKMSSATLVSYIVQILSRYDIALVQEVRDSHLT AVGKLLDNLNQDAPDTYHYVVSEPLGRNSYKERYLFV YRPDQVSAVDSYYYDDGCEPCGSDTFNREPFIVRFFS RFTEVREFAIVPLHAAPGDAVAEIDALYDVYLDVQEK WGLEDVMLMGDFNAGCSYVRPSQWSSIRLWTSPTFQW LIPDSADTTATPTHCAYDRIVVAGMLLRGAVVPDSAL PFNFQAAYGLSDQLAQAISDHYPVEVMLK
18	RSLV-319 (RNase1 N34S/N76S/N88S-linker-HSA-linker-DNase1 A114F)	METPAQLLFLLLLWLPDTTGKESRAKKFQRQHMDSDS SPSSSSTYCNQMMRRRSMTQGRCKPVNTFVHEPLVDV QNVCFQEKVTCKNGQGNCYKSSSSMHITDCRLTSGSR YPNCAYRTSPKERHIIVACEGSPYVPVHFDASVEDST GGGGSGGGGSGGGGSDAHKSEVAHRFKDLGEENFKAL VLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADESA ENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEP ERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEET

		FLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQ AADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFG ERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTE CCHGDLLECADDRADLAKYICENQDSISSKLKECCEK PLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKN YAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYET TLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNC ELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRN LGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEK TPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFN AETFTFHADICTLSEKERQIKKQTALVELVKHKPKAT KEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVA ASQAALGLGGGGSGGGGSGGGGSLKIAAFNIQTFGET KMSNATLVSYIVQILSRYDIALVQEVRDSHLTAVGKL LDNLNQDAPDTYHYVVSEPLGRNSYKERYLFVYRPDQ VSAVDSYYYDDGCEPCGNDTFNREPFIVRFFSRFTEV REFAIVPLHAAPGDAVAEIDALYDVYLDVQEKWGLED VMLMGDFNAGCSYVRPSQWSSIRLWTSPTFQWLIPDS ADTTATPTHCAYDRIVVAGMLLRGAVVPDSALPFNFQ AAYGLSDQLAQAISDHYPVEVMLK
19	RSLV-320 (DNase1 A114F-linker-HSA-linker-RNase1 N34S/N76S/N88S)	METPAQLLFLLLLWLPDTTGLKIAAFNIQTFGETKMS NATLVSYIVQILSRYDIALVQEVRDSHLTAVGKLLDN LNQDAPDTYHYVVSEPLGRNSYKERYLFVYRPDQVSA VDSYYYDDGCEPCGNDTFNREPFIVRFFSRFTEVREF AIVPLHAAPGDAVAEIDALYDVYLDVQEKWGLEDVML MGDFNAGCSYVRPSQWSSIRLWTSPTFQWLIPDSADT TATPTHCAYDRIVVAGMLLRGAVVPDSALPFNFQAAY GLSDQLAQAISDHYPVEVMLKGGGGSGGGGSGGGGSD AHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFED HVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLC TVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNL PRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYF YAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELR DEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQRF PKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADL AKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDE MPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEY ARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYA KVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALL VRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKR MPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLV NRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEK ERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVE KCCKADDKETCFAEEGKKLVAASQAALGLGGGGSGGG GSGGGGSKESRAKKFQRQHMDSDSSPSSSSTYCNQMM RRRSMTQGRCKPVNTFVHEPLVDVQNVCFQEKVTCKN GQGNCYKSSSSMHITDCRLTSGSRYPNCAYRTSPKER HIIVACEGSPYVPVHFDASVEDST
20	RSLV-321 (RNase1 N34S/N76S/N88S-linker-HSA-linker-DNase1 N18S/N106S/A114F)	METPAQLLFLLLLWLPDTTGKESRAKKFQRQHMDSDS SPSSSSTYCNQMMRRRSMTQGRCKPVNTFVHEPLVDV QNVCFQEKVTCKNGQGNCYKSSSSMHITDCRLTSGSR YPNCAYRTSPKERHIIVACEGSPYVPVHFDASVEDST GGGGSGGGGSGGGGSDAHKSEVAHRFKDLGEENFKAL VLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADESA ENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEP ERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEET

		FLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQ AADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFG ERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTE CCHGDLLECADDRADLAKYICENQDSISSKLKECCEK PLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKN YAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYET TLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNC ELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRN LGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEK TPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFN AETFTFHADICTLSEKERQIKKQTALVELVKHKPKAT KEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVA ASQAALGLGGGGSGGGGSGGGGSLKIAAFNIQTFGET KMSSATLVSYIVQILSRYDIALVQEVRDSHLTAVGKL LDNLNQDAPDTYHYVVSEPLGRNSYKERYLFVYRPDQ VSAVDSYYYDDGCEPCGSDTFNREPFIVRFFSRFTEV REFAIVPLHAAPGDAVAEIDALYDVYLDVQEKWGLED VMLMGDFNAGCSYVRPSQWSSIRLWTSPTFQWLIPDS ADTTATPTHCAYDRIVVAGMLLRGAVVPDSALPFNFQ AAYGLSDQLAQAISDHYPVEVMLK
21	RSLV-322 (DNase1 N18S/N106S/A114F- linker-HSA-linker-RNase1 N34S/N76S/N88S)	METPAQLLFLLLLWLPDTTGLKIAAFNIQTFGETKMS SATLVSYIVQILSRYDIALVQEVRDSHLTAVGKLLDN LNQDAPDTYHYVVSEPLGRNSYKERYLFVYRPDQVSA VDSYYYDDGCEPCGSDTFNREPFIVRFFSRFTEVREF AIVPLHAAPGDAVAEIDALYDVYLDVQEKWGLEDVML MGDFNAGCSYVRPSQWSSIRLWTSPTFQWLIPDSADT TATPTHCAYDRIVVAGMLLRGAVVPDSALPFNFQAAY GLSDQLAQAISDHYPVEVMLKGGGGSGGGGSGGGGSD AHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFED HVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLC TVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNL PRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYF YAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELR DEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQRF PKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADL AKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDE MPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEY ARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYA KVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALL VRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKR MPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLV NRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEK ERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVE KCCKADDKETCFAEEGKKLVAASQAALGLGGGGSGGG GSGGGGSKESRAKKFQRQHMDSDSSPSSSSTYCNQMM RRRSMTQGRCKPVNTFVHEPLVDVQNVCFQEKVTCKN GQGNCYKSSSSMHITDCRLTSGSRYPNCAYRTSPKER HIIVACEGSPYVPVHFDASVEDST
22	Human albumin (precursor)	MKWVTFISLLFLFSSAYSRGVFRRDAHKSEVAHRFKD LGEENFKALVLIAFAQYLQQCPFEDHVKLVNEVTEFA KTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEM ADCCAKQEPERNECFLQHKDDNPNLPRLVRPEVDVMC TAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRY KAAFTECCQAADKAACLLPKLDELRDEGKASSAKQRL KCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLV TDLTKVHTECCHGDLLECADDRADLAKYICENQDSIS

		SKLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADF VESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLL LRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEE PQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQVST PTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVL NQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVD ETYVPKEFNAETFTFHADICTLSEKERQIKKQTALVE LVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCF AEEGKKLVAASQAALGL
23	Human transferrin (precursor)	MRLAVGALLVCAVLGLCLAVPDKTVRWCAVSEHEATK CQSFRDHMKSVIPSDGPSVACVKKASYLDCIRAIAAN EADAVTLDAGLVYDAYLAPNNLKPVVAEFYGSKEDPQ TFYYAVAVVKKDSGFQMNQLRGKKSCHTGLGRSAGWN IPIGLLYCDLPEPRKPLEKAVANFFSGSCAPCADGTD FPQLCQLCPGCGCSTLNQYFGYSGAFKCLKDGAGDVA FVKHSTIFENLANKADRDQYELLCLDNTRKPVDEYKD CHLAQVPSHTVVARSMGGKEDLIWELLNQAQEHFGKD KSKEFQLFSSPHGKDLLFKDSAHGFLKVPPRMDAKMY LGYEYVTAIRNLREGTCPEAPTDECKPVKWCALSHHE RLKCDEWSVNSVGKIECVSAETTEDCIAKIMNGEADA MSLDGGFVYIAGKCGLVPVLAENYNKSDNCEDTPEAG YFAIAVVKKSASDLTWDNLKGKKSCHTAVGRTAGWNI PMGLLYNKINHCRFDEFFSEGCAPGSKKDSSLCKLCM GSGLNLCEPNNKEGYYGYTGAFRCLVEKGDVAFVKHQ TVPQNTGGKNPDPWAKNLNEKDYELLCLDGTRKPVEE YANCHLARAPNHAVVTRKDKEACVHKILRQQQHLFGS NVTDCSGNFCLFRSETKDLLFRDDTVCLAKLHDRNTY EKYLGEEYVKAVGNLRKCSTSSLLEACTFRRP
24	Human DNase1 precursor	MRGMKLLGALLALAALLQGAVSLKIAAFNIQTFGETK MSNATLVSYIVQILSRYDIALVQEVRDSHLTAVGKLL DNLNQDAPDTYHYVVSEPLGRNSYKERYLFVYRPDQV SAVDSYYYDDGCEPCGNDTFNREPAIVRFFSRFTEVR EFAIVPLHAAPGDAVAEIDALYDVYLDVQEKWGLEDV MLMGDFNAGCSYVRPSQWSSIRLWTSPTFQWLIPDSA DTTATPTHCAYDRIVVAGMLLRGAVVPDSALPFNFQA AYGLSDQLAQAISDHYPVEVMLK
25	Mature human DNase1 G105R	LKIAAFNIQTFGETKMSNATLVSYIVQILSRYDIALV QEVRDSHLTAVGKLLDNLNQDAPDTYHYVVSEPLGRN SYKERYLFVYRPDQVSAVDSYYYDDGCEPCRNDTFNR EPAIVRFFSRFTEVREFAIVPLHAAPGDAVAEIDALY DVYLDVQEKWGLEDVMLMGDFNAGCSYVRPSQWSSIR LWTSPTFQWLIPDSADTTATPTHCAYDRIVVAGMLLR GAVVPDSALPFNFQAAYGLSDQLAQAISDHYPVEVML K
26	Human DNase1 N18S/A114F	LKIAAFNIQTFGETKMSSATLVSYIVQILSRYDIALV QEVRDSHLTAVGKLLDNLNQDAPDTYHYVVSEPLGRN SYKERYLFVYRPDQVSAVDSYYYDDGCEPCGNDTFNR EPFIVRFFSRFTEVREFAIVPLHAAPGDAVAEIDALY DVYLDVQEKWGLEDVMLMGDFNAGCSYVRPSQWSSIR LWTSPTFQWLIPDSADTTATPTHCAYDRIVVAGMLLR GAVVPDSALPFNFQAAYGLSDQLAQAISDHYPVEVML K
27	Human DNase1 N106S/A114F	LKIAAFNIQTFGETKMSNATLVSYIVQILSRYDIALV QEVRDSHLTAVGKLLDNLNQDAPDTYHYVVSEPLGRN SYKERYLFVYRPDQVSAVDSYYYDDGCEPCGSDTFNR EPFIVRFFSRFTEVREFAIVPLHAAPGDAVAEIDALY

		DVYLDVQEKWGLEDVMLMGDFNAGCSYVRPSQWSSIR LWTSPTFQWLIPDSADTTATPTHCAYDRIVVAGMLLR GAVVPDSALPFNFQAAYGLSDQLAQAISDHYPVEVML K
28	Mature human DNase1L3	MRICSFNVRSFGESKQEDKNAMDVIVKVIKRCDIILV MEIKDSNNRICPILMEKLNRNSRRGITYNYVISSRLG RNTYKEQYAFLYKEKLVSVKRSYHYHDYQDGDADVFS REPFVVWFQSPHTAVKDFVIIPLHTTPETSVKEIDEL VEVYTDVKHRWKAENFIFMGDFNAGCSYVPKKAWKNI RLRTDPRFVWLIGDQEDTTVKKSTNCAYDRIVLRGQE IVSSVVPKSNSVFDFQKAYKLTEEEALDVSDHFPVEF KLQSSRAFTNSKKSVTLRKKTKSKRS
29	Human Trex1	MGPGARRQGRIVQGRPEMCFCPPPTPLPPLRILTLGT HTPTPCSSPGSAAGTYPTMGSQALPPGPMQTLIFFDM EATGLPFSQPKVTELCLLAVHRCALESPPTSQGPPPT VPPPPRVVDKLSLCVAPGKACSPAASEITGLSTAVLA AHGRQCFDDNLANLLLAFLRRQPQPWCLVAHNGDRYD FPLLQAELAMLGLTSALDGAFCVDSITALKALERASS PSEHGPRKSYSLGSIYTRLYGQSPPDSHTAEGDVLAL LSICQWRPQALLRWVDAHARPFGTIRPMYGVTASART KPRPSAVTTTAHLATTRNTSPSLGESRGTKDLPPVKD PGALSREGLLAPLGLLAILTLAVATLYGLSLATPGE
30	Human DNase2 alpha	MIPLLLAALLCVPAGALTCYGDSGQPVDWFVVYKLPA LRGSGEAAQRGLQYKYLDESSGGWRDGRALINSPEGA VGRSLQPLYRSNTSQLAFLLYNDQPPQPSKAQDSSMR GHTKGVLLLDHDGGFWLVHSVPNFPPPASSAAYSWPH SACTYGQTLLCVSFPFAQFSKMGKQLTYTYPWVYNYQ LEGIFAQEFPDLENVVKGHHVSQEPWNSSITLTSQAG AVFQSFAKFSKFGDDLYSGWLAAALGTNLQVQFWHKT VGILPSNCSDIWQVLNVNQIAFPGPAGPSFNSTEDHS KWCVSPKGPWTCVGDMNRNQGEEQRGGGTLCAQLPAL WKAFQPLVKNYQPCNGMARKPSRAYKI
31	Human DNase2 beta	MKQKMMARLLRTSFALLFLGLFGVLGAATISCRNEEG KAVDWFTFYKLPKRQNKESGETGLEYLYLDSTTRSWR KSEQLMNDTKSVLGRTLQQLYEAYASKSNNTAYLIYN DGVPKPVNYSRKYGHTKGLLLWNRVQGFWLIHSIPQF PPIPEEGYDYPPTGRRNGQSGICITFKYNQYEAIDSQ LLVCNPNVYSCSIPATFHQELIHMPQLCTRASSSEIP GRLLTTLQSAQGQKFLHFAKSDSFLDDIFAAWMAQRL KTHLLTETWQRKRQELPSNCSLPYHVYNIKAIKLSRH SYFSSYQDHAKWCISQKGTKNRWTCIGDLNRSPHQAF RSGGFICTQNWQIYQAFQGLVLYYESCK
32	Mouse DNase1L3	MSLHPASPRLASLLLFILALHDTLALRLCSFNVRSFG ASKKENHEAMDIIVKIIKRCDLILLMEIKDSSNNICP MLMEKLNGNSRRSTTYNYVISSRLGRNTYKEQYAFVY KEKLVSVKTKYHYHDYQDGDTDVFSREPFVVWFHSPF TAVKDFVIVPLHTTPETSVKEIDELVDVYTDVRSQWK TENFIFMGDFNAGCSYVPKKAWQNIRLRTDPKFVWLI GDQEDTTVKKSTSCAYDRIVLCGQEIVNSVVPRSSGV FDFQKAYDLSEEEALDVSDHFPVEFKLQSSRAFTNNR KSVSLKKRKKGNRS
33	Human RNase1 precursor	MALEKSLVRLLLLVLILLVLGWVQPSLGKESRAKKFQ RQHMDSDSSPSSSSTYCNQMMRRRNMTQGRCKPVNTF VHEPLVDVQNVCFQEKVTCKNGQGNCYKSNSSMHITD CRLTNGSRYPNCAYRTSPKERHIIVACEGSPYVPVHF DASVEDST

34	Human RNase1 N34S	KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRSMTQ GRCKPVNTFVHEPLVDVQNVCFQEKVTCKNGQGNCYK SNSSMHITDCRLTNGSRYPNCAYRTSPKERHIIVACE GSPYVPVHFDASVEDST
35	Human RNase1 N76S	KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRNMTQ GRCKPVNTFVHEPLVDVQNVCFQEKVTCKNGQGNCYK SSSSMHITDCRLTNGSRYPNCAYRTSPKERHIIVACE GSPYVPVHFDASVEDST
36	Human Rnase1 N88S	KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRNMTQ GRCKPVNTFVHEPLVDVQNVCFQEKVTCKNGQGNCYK SNSSMHITDCRLTSGSRYPNCAYRTSPKERHIIVACE GSPYVPVHFDASVEDST
37	Human RNase1 N34S/N76S	KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRSMTQ GRCKPVNTFVHEPLVDVQNVCFQEKVTCKNGQGNCYK SSSSMHITDCRLTNGSRYPNCAYRTSPKERHIIVACE GSPYVPVHFDASVEDST
38	Human RNase1 N34S/N88S	KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRSMTQ GRCKPVNTFVHEPLVDVQNVCFQEKVTCKNGQGNCYK SNSSMHITDCRLTSGSRYPNCAYRTSPKERHIIVACE GSPYVPVHFDASVEDST
39	Human Rnase1 N76S/N88S	KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRNMTQ GRCKPVNTFVHEPLVDVQNVCFQEKVTCKNGQGNCYK SSSSMHITDCRLTSGSRYPNCAYRTSPKERHIIVACE GSPYVPVHFDASVEDST
40	Human Fc domain SCC	LEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGK
41	Human Fc domain SSS	LEPKSSDKTHTSPPSPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGK
42	HST N413S	VPDKTVRWCAVSEHEATKCQSFRDHMKSVIPSDGPSV ACVKKASYLDCIRAIAANEADAVTLDAGLVYDAYLAP NNLKPVVAEFYGSKEDPQTFYYAVAVVKKDSGFQMNQ LRGKKSCHTGLGRSAGWNIPIGLLYCDLPEPRKPLEK AVANFFSGSCAPCADGTDFPQLCQLCPGCGCSTLNQY FGYSGAFKCLKDGAGDVAFVKHSTIFENLANKADRDQ YELLCLDNTRKPVDEYKDCHLAQVPSHTVVARSMGGK EDLIWELLNQAQEHFGKDKSKEFQLFSSPHGKDLLFK DSAHGFLKVPPRMDAKMYLGYEYVTAIRNLREGTCPE APTDECKPVKWCALSHHERLKCDEWSVNSVGKIECVS AETTEDCIAKIMNGEADAMSLDGGFVYIAGKCGLVPV LAENYSKSDNCEDTPEAGYFAIAVVKKSASDLTWDNL KGKKSCHTAVGRTAGWNIPMGLLYNKINHCRFDEFFS EGCAPGSKKDSSLCKLCMGSGLNLCEPNNKEGYYGYT GAFRCLVEKGDVAFVKHQTVPQNTGGKNPDPWAKNLN EKDYELLCLDGTRKPVEEYANCHLARAPNHAVVTRKD KEACVHKILRQQQHLFGSNVTDCSGNFCLFRSETKDL LFRDDTVCLAKLHDRNTYEKYLGEEYVKAVGNLRKCS

		TSSLLEACTFRRP
43	HST N611S	VPDKTVRWCAVSEHEATKCQSFRDHMKSVIPSDGPSV ACVKKASYLDCIRAIAANEADAVTLDAGLVYDAYLAP NNLKPVVAEFYGSKEDPQTFYYAVAVVKKDSGFQMNQ LRGKKSCHTGLGRSAGWNIPIGLLYCDLPEPRKPLEK AVANFFSGSCAPCADGTDFPQLCQLCPGCGCSTLNQY FGYSGAFKCLKDGAGDVAFVKHSTIFENLANKADRDQ YELLCLDNTRKPVDEYKDCHLAQVPSHTVVARSMGGK EDLIWELLNQAQEHFGKDKSKEFQLFSSPHGKDLLFK DSAHGFLKVPPRMDAKMYLGYEYVTAIRNLREGTCPE APTDECKPVKWCALSHHERLKCDEWSVNSVGKIECVS AETTEDCIAKIMNGEADAMSLDGGFVYIAGKCGLVPV LAENYNKSDNCEDTPEAGYFAIAVVKKSASDLTWDNL KGKKSCHTAVGRTAGWNIPMGLLYNKINHCRFDEFFS EGCAPGSKKDSSLCKLCMGSGLNLCEPNNKEGYYGYT GAFRCLVEKGDVAFVKHQTVPQNTGGKNPDPWAKNLN EKDYELLCLDGTRKPVEEYANCHLARAPNHAVVTRKD KEACVHKILRQQQHLFGSSVTDCSGNFCLFRSETKDL LFRDDTVCLAKLHDRNTYEKYLGEEYVKAVGNLRKCS TSSLLEACTFRRP
44	Linker	LEA(EAAAK)$_4$ALEA(EAAAK)$_4$ALE
45	Linker	GGSG
46	Linker	GSAT
47	RSLV-315 (w/o leader) (RNase I N34S/N76S/N88S-linker-HSA; w/o leader)	KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRSMTQ GRCKPVNTFVHEPLVDVQNVCFQEKVTCKNGQGNCYK SSSSMHITDCRLTSGSRYPNCAYRTSPKERHIIVACE GSPYVPVHFDASVEDSTGGGGSGGGGSGGGGSDAHKS EVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKL VNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVAT LRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLV RPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPE LLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGK ASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYI CENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPAD LPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRH PDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFD EFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYT KKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCA EDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRP CFSALEVDETYVPKEFNAETFTFHADICTLSEKERQI KKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCK ADDKETCFAEEGKKLVAASQAALGL
48	RSLV-316 (w/o leader) (HSA-linker-RNase1 N34S/N76S/N88S; w/o leader)	DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFE DHVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKL CTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPN LPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPY FYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDEL RDEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQR FPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRAD LAKYICENQDSISSKLKECCEKPLLEKSHCIAEVEND EMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYE YARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECY AKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNAL LVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAK RMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESL

		VNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSE KERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFV EKCCKADDKETCFAEEGKKLVAASQAALGLGGGGSGG GGSGGGGSKESRAKKFQRQHMDSDSSPSSSSTYCNQM MRRRSMTQGRCKPVNTFVHEPLVDVQNVCFQEKVTCK NGQGNCYKSSSSMHITDCRLTSGSRYPNCAYRTSPKE RHIIVACEGSPYVPVHFDASVEDST
49	RSLV-317 (w/o leader) (DNase1 N18S/N106S/A114F-linker-HSA; w/o leader)	LKIAAFNIQTFGETKMSSATLVSYIVQILSRYDIALV QEVRDSHLTAVGKLLDNLNQDAPDTYHYVVSEPLGRN SYKERYLFVYRPDQVSAVDSYYYDDGCEPCGSDTFNR EPFIVRFFSRFTEVREFAIVPLHAAPGDAVAEIDALY DVYLDVQEKWGLEDVMLMGDFNAGCSYVRPSQWSSIR LWTSPTFQWLIPDSADTTATPTHCAYDRIVVAGMLLR GAVVPDSALPFNFQAAYGLSDQLAQAISDHYPVEVML KGGGGSGGGGSGGGGSDAHKSEVAHRFKDLGEENFKA LVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADES AENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQE PERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEE TFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECC QAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKF GERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHT ECCHGDLLECADDRADLAKYICENQDSISSKLKECCE KPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCK NYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYE TTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQN CELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSR NLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHE KTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEF NAETFTFHADICTLSEKERQIKKQTALVELVKHKPKA TKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLV AASQAALGL
50	RSLV-318 (w/o leader) (HSA-linker-DNase1 N18S/N106S/A114F; w/o leader)	DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFE DHVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKL CTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPN LPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPY FYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDEL RDEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQR FPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRAD LAKYICENQDSISSKLKECCEKPLLEKSHCIAEVEND EMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYE YARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECY AKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNAL LVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAK RMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESL VNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSE KERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFV EKCCKADDKETCFAEEGKKLVAASQAALGLGGGGSGG GGSGGGGSLKIAAFNIQTFGETKMSSATLVSYIVQIL SRYDIALVQEVRDSHLTAVGKLLDNLNQDAPDTYHYV VSEPLGRNSYKERYLFVYRPDQVSAVDSYYYDDGCEP CGSDTFNREPFIVRFFSRFTEVREFAIVPLHAAPGDA VAEIDALYDVYLDVQEKWGLEDVMLMGDFNAGCSYVR PSQWSSIRLWTSPTFQWLIPDSADTTATPTHCAYDRI VVAGMLLRGAVVPDSALPFNFQAAYGLSDQLAQAISD HYPVEVMLK
51	RSLV-319 (w/o leader)	KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRSMTQ

	(RNase1 N34S/N76S/N88S-linker-HSA-linker-DNase1 A114F; w/o leader)	GRCKPVNTFVHEPLVDVQNVCFQEKVTCKNGQGNCYK SSSSMHITDCRLTSGSRYPNCAYRTSPKERHIIVACE GSPYVPVHFDASVEDSTGGGGSGGGGSGGGGSDAHKS EVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKL VNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVAT LRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLV RPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPE LLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGK ASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYI CENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPAD LPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRH PDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFD EFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYT KKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCA EDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRP CFSALEVDETYVPKEFNAETFTFHADICTLSEKERQI KKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCK ADDKETCFAEEGKKLVAASQAALGLGGGGSGGGGSGG GGSLKIAAFNIQTFGETKMSNATLVSYIVQILSRYDI ALVQEVRDSHLTAVGKLLDNLNQDAPDTYHYVVSEPL GRNSYKERYLFVYRPDQVSAVDSYYYDDGCEPCGNDT FNREPFIVRFFSRFTEVREFAIVPLHAAPGDAVAEID ALYDVYLDVQEKWGLEDVMLMGDFNAGCSYVRPSQWS SIRLWTSPTFQWLIPDSADTTATPTHCAYDRIVVAGM LLRGAVVPDSALPFNFQAAYGLSDQLAQAISDHYPVE VMLK
52	RSLV-320 (w/o leader) (DNase1 A114F-linker-HSA-linker-RNase1 N34S/N76S/N88S; w/o leader)	LKIAAFNIQTFGETKMSNATLVSYIVQILSRYDIALV QEVRDSHLTAVGKLLDNLNQDAPDTYHYVVSEPLGRN SYKERYLFVYRPDQVSAVDSYYYDDGCEPCGNDTFNR EPFIVRFFSRFTEVREFAIVPLHAAPGDAVAEIDALY DVYLDVQEKWGLEDVMLMGDFNAGCSYVRPSQWSSIR LWTSPTFQWLIPDSADTTATPTHCAYDRIVVAGMLLR GAVVPDSALPFNFQAAYGLSDQLAQAISDHYPVEVML KGGGGSGGGGSGGGGSDAHKSEVAHRFKDLGEENFKA LVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADES AENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQE PERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEE TFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECC QAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKF GERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHT ECCHGDLLECADDRADLAKYICENQDSISSKLKECCE KPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCK NYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYE TTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQN CELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSR NLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHE KTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEF NAETFTFHADICTLSEKERQIKKQTALVELVKHKPKA TKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLV AASQAALGLGGGGSGGGGSGGGGSKESRAKKFQRQHM DSDSSPSSSSTYCNQMMRRRSMTQGRCKPVNTFVHEP LVDVQNVCFQEKVTCKNGQGNCYKSSSSMHITDCRLT SGSRYPNCAYRTSPKERHIIVACEGSPYVPVHFDASV EDST
53	RSLV-321 (w/o leader)	KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRSMTQ

	(RNase1 N34S/N76S/N88S-linker-HSA-linker-DNase1 N18S/N106S/A114F; w/o leader)	GRCKPVNTFVHEPLVDVQNVCFQEKVTCKNGQGNCYK SSSSMHITDCRLTSGSRYPNCAYRTSPKERHIIVACE GSPYVPVHFDASVEDSTGGGGSGGGGSGGGGSDAHKS EVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKL VNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVAT LRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLV RPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPE LLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGK ASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYI CENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPAD LPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRH PDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFD EFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYT KKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCA EDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRP CFSALEVDETYVPKEFNAETFTFHADICTLSEKERQI KKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCK ADDKETCFAEEGKKLVAASQAALGLGGGGSGGGGSGG GGSLKIAAFNIQTFGETKMSSATLVSYIVQILSRYDI ALVQEVRDSHLTAVGKLLDNLNQDAPDTYHYVVSEPL GRNSYKERYLFVYRPDQVSAVDSYYYDDGCEPCGSDT FNREPFIVRFFSRFTEVREFAIVPLHAAPGDAVAEID ALYDVYLDVQEKWGLEDVMLMGDFNAGCSYVRPSQWS SIRLWTSPTFQWLIPDSADTTATPTHCAYDRIVVAGM LLRGAVVPDSALPFNFQAAYGLSDQLAQAISDHYPVE VMLK
54	RSLV-322 (w/o leader) (DNase1 N18S/N106S/A114F-linker-HSA-linker-RNase1 N34S/N76S/N88S; w/o leader)	LKIAAFNIQTFGETKMSSATLVSYIVQILSRYDIALV QEVRDSHLTAVGKLLDNLNQDAPDTYHYVVSEPLGRN SYKERYLFVYRPDQVSAVDSYYYDDGCEPCGSDTFNR EPFIVRFFSRFTEVREFAIVPLHAAPGDAVAEIDALY DVYLDVQEKWGLEDVMLMGDFNAGCSYVRPSQWSSIR LWTSPTFQWLIPDSADTTATPTHCAYDRIVVAGMLLR GAVVPDSALPFNFQAAYGLSDQLAQAISDHYPVEVML KGGGGSGGGGSGGGGSDAHKSEVAHRFKDLGEENFKA LVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADES AENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQE PERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEE TFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECC QAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKF GERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHT ECCHGDLLECADDRADLAKYICENQDSISSKLKECCE KPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCK NYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYE TTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQN CELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSR NLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHE KTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEF NAETFTFHADICTLSEKERQIKKQTALVELVKHKPKA TKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLV AASQAALGLGGGGSGGGGSGGGGSKESRAKKFQRQHM DSDSSPSSSSTYCNQMMRRRSMTQGRCKPVNTFVHEP LVDVQNVCFQEKVTCKNGQGNCYKSSSSMHITDCRLT SGSRYPNCAYRTSPKERHIIVACEGSPYVPVHFDASV EDST
55	Gaussia luciferase signal	MGVKVLFALICIAVAEA

	peptide	
56	linker	LEA(EAAAK)$_4$ALEA(EAAAK)$_4$
57	NLG linker	VDGAAASPVNVSSPSVQDI
58	O-linked glycosylation consensus	CXXGG-T/S-C
59	O-linked glycosylation consensus	NST-E/D-A
60	O-linked glycosylation consensus	NITQS
61	O-linked glycosylation consensus	QSTQS
62	O-linked glycosylation consensus	D/EFT-R/K-V
63	O-linked glycosylation consensus	C-E/D-SN
64	O-linked glycosylation consensus	GGSC-K/R
65	Human Fc domain P238S, P331S	LEPKSCDKTHTCPPCPAPELLGGSSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPASIEKTISKAKGQPREPQVYTLPPSRDELTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGK
66	Human Fc domain SCC, P238S, P331S	LEPKSSDKTHTCPPCPAPELLGGSSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPASIEKTISKAKGQPREPQVYTLPPSRDELTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGK
67	Human Fc domain SSS, P238S, P331S	LEPKSSDKTHTSPPSPAPELLGGSSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPASIEKTISKAKGQPREPQVYTLPPSRDELTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGK
68	Human Fc domain P238S, P331S, N297S	LEPKSCDKTHTCPPCPAPELLGGSSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYSSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPASIEKTISKAKGQPREPQVYTLPPSRDELTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGK
69	Human Fc domain SCC, P238S, P331S, N297S	LEPKSSDKTHTCPPCPAPELLGGSSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYSSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPASIEKTISKAKGQPREPQVYTLPPSRDELTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGK
70	Human Fc domain SSS, P238S, P331S, N297S	LEPKSSDKTHTSPPSPAPELLGGSSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYSSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPASIEKTISKAKGQPREPQVYTLPPSRDELTKNQ

		VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGK
71	AG1-RSLV-132 huVK3LP-hRNase1 (N54S/N96S/N108S)-hIgG1Fc (SCC, P238S, P331S)	METPAQLLFLLLLWLPDTTGKESRAKKFQRQHMDSDS SPSSSSTYCNQMMRRRSMTQGRCKPVNTFVHEPLVDV QNVCFQEKVTCKNGQGNCYKSSSSMHITDCRLTSGSR YPNCAYRTSPKERHIIVACEGSPYVPVHFDASVEDST LEPKSSDKTHTCPPCPAPELLGGSSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPASIEKTISKAKGQPREPQVYTLPPSRDELTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGK
72	AG1-RSLV-132 (no leader) hRNase1(N34S/N76S/N88S)- hIgG1Fc (SCC, P238S, P331S)	KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRSMTQ GRCKPVNTFVHEPLVDVQNVCFQEKVTCKNGQGNCYK SSSSMHITDCRLTSGSRYPNCAYRTSPKERHIIVACE GSPYVPVHFDASVEDSTLEPKSSDKTHTCPPCPAPEL LGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPASIEKTISKAKGQPRE PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK
73	AG2-RSLV-132 huVK3LP-hRNase1 (N54S/N96S/N108S)-hIgG1Fc (SCC, P238S, P331S, N297S)	METPAQLLFLLLLWLPDTTGKESRAKKFQRQHMDSDS SPSSSSTYCNQMMRRRSMTQGRCKPVNTFVHEPLVDV QNVCFQEKVTCKNGQGNCYKSSSSMHITDCRLTSGSR YPNCAYRTSPKERHIIVACEGSPYVPVHFDASVEDST LEPKSSDKTHTCPPCPAPELLGGSSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYSSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPASIEKTISKAKGQPREPQVYTLPPSRDELTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGK
74	AG2-RSLV-132 (no leader) hRNase1(N34S/N76S/N88S)- hIgG1Fc (SCC, P238S, P331S, N297S)	KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRSMTQ GRCKPVNTFVHEPLVDVQNVCFQEKVTCKNGQGNCYK SSSSMHITDCRLTSGSRYPNCAYRTSPKERHIIVACE GSPYVPVHFDASVEDSTLEPKSSDKTHTCPPCPAPEL LGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYSSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPASIEKTISKAKGQPRE PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK
75	HSA-linker-DNase E13R/N74K/A114F/ T205K/N18S/N106S	METPAQLLFLLLLWLPDTTGDAHKSEVAHRFKDLGEE NFKALVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCV ADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNPNLPRLVRPEVDVMCTAFH DNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAF TECCQAADKAACLLPKLDELRDEGKASSAKQRLKCAS LQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISSKLK ECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESK DVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLA KTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNL IKQNCELFEQLGEYKFQNALLVRYTKKVPQVSTPTLV EVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLC

		VLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYV PKEFNAETFTFHADICTLSEKERQIKKQTALVELVKH KPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEG KKLVAASQAALGLGGGGSGGGGSGGGGSLKIAAFNIQ TFGRTKMSSATLVSYIVQILSRYDIALVQEVRDSHLT AVGKLLDNLNQDAPDTYHYVVSEPLGRKSYKERYLFV YRPDQVSAVDSYYYDDGCEPCGSDTFNREPFIVRFFS RFTEVREFAIVPLHAAPGDAVAEIDALYDVYLDVQEK WGLEDVMLMGDFNAGCSYVRPSQWSSIRLWTSPTFQW LIPDSADTTAKPTHCAYDRIVVAGMLLRGAVVPDSAL PFNFQAAYGLSDQLAQAISDHYPVEVMLK
76	RSLV-330 (DNase E13R/N74K/A114F/ T205K/N18S/N106S-linker- HSA)	METPAQLLFLLLLWLPDTTGLKIAAFNIQTFGRTKMS SATLVSYIVQILSRYDIALVQEVRDSHLTAVGKLLDN LNQDAPDTYHYVVSEPLGRKSYKERYLFVYRPDQVSA VDSYYYDDGCEPCGSDTFNREPFIVRFFSRFTEVREF AIVPLHAAPGDAVAEIDALYDVYLDVQEKWGLEDVML MGDFNAGCSYVRPSQWSSIRLWTSPTFQWLIPDSADT TAKPTHCAYDRIVVAGMLLRGAVVPDSALPFNFQAAY GLSDQLAQAISDHYPVEVMLKGGGGSGGGGSGGGGSD AHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFED HVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLC TVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNL PRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYF YAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELR DEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQRF PKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADL AKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDE MPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEY ARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYA KVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALL VRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKR MPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLV NRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEK ERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVE KCCKADDKETCFAEEGKKLVAASQAALGL
77	RSLV-331 (RNase-linker-HSA-linker- DNase E13R/N74K/A114F/ T205K/N18S/N106S)	METPAQLLFLLLLWLPDTTGKESRAKKFQRQHMDSDS SPSSSSTYCNQMMRRRNMTQGRCKPVNTFVHEPLVDV QNVCFQEKVTCKNGQGNCYKSNSSMHITDCRLTNGSR YPNCAYRTSPKERHIIVACEGSPYVPVHFDASVEDST GGGGSGGGGSGGGGSDAHKSEVAHRFKDLGEENFKAL VLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADESA ENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEP ERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEET FLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQ AADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFG ERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTE CCHGDLLECADDRADLAKYICENQDSISSKLKECCEK PLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKN YAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYET TLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNC ELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRN LGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEK TPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFN AETFTFHADICTLSEKERQIKKQTALVELVKHKPKAT KEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVA ASQAALGLGGGGSGGGGSGGGGSLKIAAFNIQTFGRT KMSSATLVSYIVQILSRYDIALVQEVRDSHLTAVGKL LDNLNQDAPDTYHYVVSEPLGRKSYKERYLFVYRPDQ

		VSAVDSYYYDDGCEPCGSDTFNREPFIVRFFSRFTEV REFAIVPLHAAPGDAVAEIDALYDVYLDVQEKWGLED VMLMGDFNAGCSYVRPSQWSSIRLWTSPTFQWLIPDS ADTTAKPTHCAYDRIVVAGMLLRGAVVPDSALPFNFQ AAYGLSDQLAQAISDHYPVEVMLK
78	RSLV-332 (DNase E13R/N74K/A114F/ T205K/N18S/N106S- linker-HSA-linker- RNase)	METPAQLLFLLLLWLPDTTGLKIAAFNIQTFGRTKMS SATLVSYIVQILSRYDIALVQEVRDSHLTAVGKLLDN LNQDAPDTYHYVVSEPLGRKSYKERYLFVYRPDQVSA VDSYYYDDGCEPCGSDTFNREPFIVRFFSRFTEVREF AIVPLHAAPGDAVAEIDALYDVYLDVQEKWGLEDVML MGDFNAGCSYVRPSQWSSIRLWTSPTFQWLIPDSADT TAKPTHCAYDRIVVAGMLLRGAVVPDSALPFNFQAAY GLSDQLAQAISDHYPVEVMLKGGGGSGGGGSGGGGSD AHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFED HVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLC TVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNL PRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYF YAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELR DEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQRF PKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADL AKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDE MPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEY ARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYA KVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALL VRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKR MPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLV NRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEK ERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVE KCCKADDKETCFAEEGKKLVAASQAALGLGGGGSGGG GSGGGGSKESRAKKFQRQHMDSDSSPSSSSTYCNQMM RRRNMTQGRCKPVNTFVHEPLVDVQNVCFQEKVTCKN GQGNCYKSNSSMHITDCRLTNGSRYPNCAYRTSPKER HIIVACEGSPYVPVHFDASVEDST
79	HSA-linker-DNase E13R/N74K/A114F/ T205K/N18S/N106S; w/o leader	DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFE DHVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKL CTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPN LPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPY FYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDEL RDEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQR FPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRAD LAKYICENQDSISSKLKECCEKPLLEKSHCIAEVEND EMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYE YARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECY AKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNAL LVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAK RMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESL VNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSE KERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFV EKCCKADDKETCFAEEGKKLVAASQAALGLGGGGSGG GGSGGGGSLKIAAFNIQTFGRTKMSSATLVSYIVQIL SRYDIALVQEVRDSHLTAVGKLLDNLNQDAPDTYHYV VSEPLGRKSYKERYLFVYRPDQVSAVDSYYYDDGCEP CGSDTFNREPFIVRFFSRFTEVREFAIVPLHAAPGDA VAEIDALYDVYLDVQEKWGLEDVMLMGDFNAGCSYVR PSQWSSIRLWTSPTFQWLIPDSADTTAKPTHCAYDRI VVAGMLLRGAVVPDSALPFNFQAAYGLSDQLAQAISD HYPVEVMLK
80	RSLV-330 (DNase E13R/N74K/A114F/	LKIAAFNIQTFGRTKMSSATLVSYIVQILSRYDIALV QEVRDSHLTAVGKLLDNLNQDAPDTYHYVVSEPLGRK

	T205K/N18S/N106S-linker-HSA; w/o leader)	SYKERYLFVYRPDQVSAVDSYYYDDGCEPCGSDTFNR EPFIVRFFSRFTEVREFAIVPLHAAPGDAVAEIDALY DVYLDVQEKWGLEDVMLMGDFNAGCSYVRPSQWSSIR LWTSPTFQWLIPDSADTTAKPTHCAYDRIVVAGMLLR GAVVPDSALPFNFQAAYGLSDQLAQAISDHYPVEVML KGGGGSGGGGSGGGGSDAHKSEVAHRFKDLGEENFKA LVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADES AENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQE PERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEE TFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECC QAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKF GERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHT ECCHGDLLECADDRADLAKYICENQDSISSKLKECCE KPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCK NYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYE TTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQN CELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSR NLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHE KTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEF NAETFTFHADICTLSEKERQIKKQTALVELVKHKPKA TKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLV AASQAALGL
81	RSLV-331 (RNase-linker-HSA-linker-DNase E13R/N74K/A114F/T205K/N18S/N106S; w/o leader)	KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRNMTQ GRCKPVNTFVHEPLVDVQNVCFQEKVTCKNGQGNCYK SNSSMHITDCRLTNGSRYPNCAYRTSPKERHIIVACE GSPYVPVHFDASVEDSTGGGGSGGGGSGGGGSDAHKS EVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKL VNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVAT LRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLV RPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPE LLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGK ASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYI CENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPAD LPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRH PDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFD EFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYT KKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCA EDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRP CFSALEVDETYVPKEFNAETFTFHADICTLSEKERQI KKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCK ADDKETCFAEEGKKLVAASQAALGLGGGGSGGGGSGG GGSLKIAAFNIQTFGRTKMSSATLVSYIVQILSRYDI ALVQEVRDSHLTAVGKLLDNLNQDAPDTYHYVVSEPL GRKSYKERYLFVYRPDQVSAVDSYYYDDGCEPCGSDT FNREPFIVRFFSRFTEVREFAIVPLHAAPGDAVAEID ALYDVYLDVQEKWGLEDVMLMGDFNAGCSYVRPSQWS SIRLWTSPTFQWLIPDSADTTAKPTHCAYDRIVVAGM LLRGAVVPDSALPFNFQAAYGLSDQLAQAISDHYPVE VMLK
82	RSLV-332 (DNase E13R/N74K/A114F/T205K/N18S/N106S-linker-HSA-linker-RNase; w/o leader)	LKIAAFNIQTFGRTKMSSATLVSYIVQILSRYDIALV QEVRDSHLTAVGKLLDNLNQDAPDTYHYVVSEPLGRK SYKERYLFVYRPDQVSAVDSYYYDDGCEPCGSDTFNR EPFIVRFFSRFTEVREFAIVPLHAAPGDAVAEIDALY DVYLDVQEKWGLEDVMLMGDFNAGCSYVRPSQWSSIR LWTSPTFQWLIPDSADTTAKPTHCAYDRIVVAGMLLR

		GAVVPDSALPFNFQAAYGLSDQLAQAISDHYPVEVML KGGGGSGGGGSGGGGSDAHKSEVAHRFKDLGEENFKA LVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADES AENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQE PERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEE TFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECC QAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKF GERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHT ECCHGDLLECADDRADLAKYICENQDSISSKLKECCE KPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCK NYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYE TTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQN CELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSR NLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHE KTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEF NAETFTFHADICTLSEKERQIKKQTALVELVKHKPKA TKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLV AASQAALGLGGGGSGGGGSGGGGSKESRAKKFQRQHM DSDSSPSSSSTYCNQMMRRRNMTQGRCKPVNTFVHEP LVDVQNVCFQEKVTCKNGQGNCYKSNSSMHITDCRLT NGSRYPNCAYRTSPKERHIIVACEGSPYVPVHFDASV EDST
83	HSA-linker-DNase E13R/N74K/A114F/ T205K/N18S/N106S nucleic acid	ATGGAAACCCCTGCCCAGCTGCTGTTCCTGCTGCTGC TGTGGCTGCCCGACACCACCGGCGATGCCCACAAGTC TGAGGTGGCCCACCGGTTCAAGGACCTGGGCGAGGAA AACTTCAAGGCCCTGGTGCTGATCGCCTTCGCCCAGT ACCTGCAGCAGTGCCCCTTCGAGGACCACGTGAAGCT GGTGAACGAAGTGACCGAGTTCGCCAAGACCTGCGTG GCCGACGAGTCCGCCGAGAACTGCGACAAGAGCCTGC ACACCCTGTTCGGCGACAAGCTGTGCACCGTGGCCAC CCTGCGGGAAACCTACGGCGAGATGGCCGACTGCTGC GCCAAGCAAGAACCCGAGCGGAACGAGTGCTTCCTGC AGCACAAGGACGACAACCCCAACCTGCCCCGGCTGGT CCGACCCGAGGTGGACGTGATGTGCACCGCCTTCCAC GACAACGAGGAAACCTTCCTGAAGAAGTACCTGTACG AGATCGCCAGACGGCACCCCTACTTCTACGCCCCCGA GCTGCTGTTTTTCGCCAAGCGGTACAAGGCCGCCTTC ACCGAGTGCTGCCAGGCCGCCGATAAGGCCGCCTGCC TGCTGCCTAAGCTGGACGAGCTGAGGGACGAGGGCAA GGCCTCCTCTGCCAAGCAGCGGCTGAAGTGCGCCTCC CTGCAGAAGTTCGGCGAGCGGGCCTTTAAGGCCTGGG CCGTGGCTCGGCTGTCCCAGAGATTCCCCAAGGCCGA GTTTGCCGAGGTGTCCAAGCTGGTGACAGACCTGACC AAGGTGCACACCGAGTGTTGTCACGGCGACCTGCTGG AATGCGCCGACGACAGAGCCGACCTGGCCAAGTACAT CTGCGAGAACCAGGACTCCATCTCCTCCAAGCTGAAA GAGTGCTGCGAGAAGCCCCTGCTGGAAAAGTCCCACT GTATCGCCGAGGTGGAAAACGACGAGATGCCCGCCGA CCTGCCTTCCCTGGCCGCCGACTTCGTGGAATCCAAG GACGTGTGCAAGAACTACGCCGAGGCCAAGGATGTGT TCCTGGGCATGTTCCTGTACGAGTACGCTCGGCGGCA CCCCGACTACTCCGTGGTGCTGCTGCTGAGACTGGCC AAGACCTACGAGACAACCCTGGAAAAGTGCTGCGCCG CTGCCGACCCCCACGAGTGCTACGCCAAGGTGTTCGA CGAGTTCAAGCCTCTGGTGGAAGAACCCCAGAACCTG ATCAAGCAGAACTGCGAGCTGTTCGAGCAGCTGGGCG AGTACAAGTTCCAGAACGCCCTGCTGGTCCGATACAC CAAGAAAGTGCCCCAGGTGTCCACCCCCACCCTGGTG GAAGTGTCCCGGAACCTGGGCAAAGTGGGCTCCAAGT GCTGCAAGCACCCTGAGGCCAAGCGGATGCCCTGCGC CGAGGACTACCTGAGCGTGGTGCTGAACCAGCTGTGC

		GTGCTGCACGAAAAGACCCCCGTGTCCGACAGAGTGA CCAAGTGCTGTACCGAGTCCCTGGTGAACAGACGGCC CTGCTTCTCCGCCCTGGAAGTGGACGAGACATACGTG CCCAAAGAGTTCAACGCCGAGACATTCACCTTCCACG CCGACATCTGCACCCTGTCCGAGAAAGAGCGGCAGAT CAAGAAACAGACCGCACTGGTGGAACTGGTGAAACAC AAGCCCAAGGCCACCAAAGAACAGCTGAAGGCCGTGA TGGACGACTTCGCCGCCTTTGTGGAAAAGTGTTGCAA GGCCGACGACAAAGAGACATGCTTCGCCGAAGAGGGC AAGAAACTGGTGGCCGCTTCTCAGGCTGCTCTGGGCC TGGGAGGCGGAGGATCTGGGGGAGGCGGAAGCGGAGG GGGCGGATCTCTGAAGATCGCCGCCTTCAACATCCAG ACCTTCGGCCGGACCAAGATGTCCAGCGCTACCCTGG TGTCCTACATCGTGCAGATCCTGTCCAGATACGATAT CGCCCTGGTGCAAGAAGTGCGGGACTCCCACCTGACC GCCGTGGGCAAGCTGCTGGACAACCTGAACCAGGACG CCCCCGACACCTACCACTACGTGGTGTCTGAGCCCCT GGGCCGGAAGTCCTACAAAGAAAGATACCTGTTCGTG TACCGGCCCGACCAGGTGTCCGCCGTGGACTCCTACT ACTACGACGACGGCTGCGAGCCCTGCGGCAGCGACAC CTTCAACCGCGAGCCCTTCATCGTGCGGTTCTTCAGC CGGTTCACCGAAGTGCGCGAGTTTGCCATCGTGCCCC TGCACGCTGCTCCAGGCGACGCCGTGGCTGAGATCGA CGCCCTGTACGACGTGTACCTGGATGTGCAAGAAAAG TGGGGCCTGGAAGATGTGATGCTGATGGGCGACTTCA ACGCCGGCTGCTCCTACGTGCGGCCCTCCCAGTGGTC CTCCATCCGGCTGTGGACCAGCCCCACCTTCCAGTGG CTGATCCCCGACTCCGCCGATACCACCGCCAAGCCCA CCCACTGTGCCTACGACAGAATCGTGGTGGCCGGCAT GCTGCTGAGGGGCGCTGTGGTGCCTGACTCCGCCCTG CCATTCAATTTTCAAGCCGCCTACGGCCTGTCCGACC AGCTGGCCCAGGCCATCTCCGACCACTACCCCGTGGA AGTGATGCTGAAG
84	RSLV-330 nucleic acid	ATGGAAACCCCTGCCCAGCTGCTGTTCCTGCTGCTGC TGTGGCTGCCCGACACCACCGGCCTGAAGATCGCCGC CTTCAACATCCAGACCTTCGGCCGGACCAAGATGTCC AGCGCCACCCTGGTGTCCTACATCGTGCAGATCCTGT CCAGATACGATATCGCCCTGGTGCAAGAAGTGCGGGA CTCCCACCTGACCGCCGTGGGCAAGCTGCTGGACAAC CTGAACCAGGACGCCCCCGACACCTACCACTACGTGG TGTCTGAGCCCCTGGGCCGGAAGTCCTACAAAGAAAG ATACCTGTTCGTGTACCGGCCCGACCAGGTGTCCGCC GTGGACTCCTACTACTACGACGACGGCTGCGAGCCCT GCGGCAGCGACACCTTCAACCGCGAGCCCTTCATCGT GCGGTTCTTCAGCCGGTTCACCGAAGTGCGCGAGTTC GCCATCGTGCCCCTGCATGCTGCTCCAGGCGACGCCG TGGCCGAGATCGACGCCCTGTACGACGTGTACCTGGA CGTGCAAGAAAAGTGGGGCCTGGAAGATGTGATGCTG ATGGGCGACTTCAACGCCGGCTGCTCCTACGTGCGGC CCTCCCAGTGGTCCTCCATCCGGCTGTGGACCTCCCC CACCTTCCAGTGGCTGATCCCCGACTCCGCCGATACC ACCGCCAAGCCCACCCACTGCGCCTACGACAGAATCG TGGTGGCCGGCATGCTGCTGAGAGGCGCCGTGGTGCC TGACTCCGCCCTGCCATTCAATTTTCAAGCCGCCTAC GGCCTGTCCGACCAGCTGGCCCAGGCCATCTCCGACC ACTACCCCGTGGAAGTGATGCTGAAGGGGGGAGGCGG ATCTGGCGGAGGGGGAAGTGGCGGCGGAGGCTCTGAT GCCCACAAGTCTGAGGTGGCCCACCGGTTCAAGGACC TGGGCGAGGAAAACTTCAAGGCCCTGGTGCTGATCGC CTTCGCCCAGTACCTGCAGCAGTGCCCCTTCGAGGAC

		CACGTGAAGCTGGTGAACGAAGTGACCGAGTTTGCCA AGACCTGCGTGGCCGACGAGTCCGCCGAGAACTGCGA CAAGTCCCTGCACACCCTGTTCGGCGACAAGCTGTGC ACCGTGGCCACCCTGCGGGAAACCTACGGCGAGATGG CCGACTGCTGCGCCAAGCAAGAACCCGAGCGGAACGA GTGCTTCCTGCAGCACAAGGACGACAACCCCAACCTG CCCCGGCTGGTCCGACCCGAGGTGGACGTGATGTGCA CCGCCTTCCACGACAACGAGGAAACCTTCCTGAAGAA GTACCTGTACGAGATCGCCAGACGGCACCCCTACTTC TACGCCCCCGAGCTGCTGTTTTTCGCCAAGCGGTACA AGGCCGCCTTCACCGAGTGCTGCCAGGCCGCCGATAA GGCCGCCTGCCTGCTGCCTAAGCTGGACGAGCTGAGG GACGAGGGCAAGGCCTCCTCTGCCAAGCAGCGGCTGA AGTGCGCCTCCCTGCAGAAGTTCGGCGAGCGGGCCTT TAAGGCCTGGGCCGTGGCCCGGCTGTCCCAGAGATTC CCTAAGGCCGAGTTCGCCGAGGTGTCCAAGCTGGTGA CAGACCTGACCAAGGTGCACACCGAGTGTTGTCACGG CGACCTGCTGGAATGCGCCGACGACAGGGCCGACCTG GCCAAGTACATCTGCGAGAACCAGGACTCCATCTCCT CCAAGCTGAAAGAGTGCTGCGAGAAGCCCCTGCTGGA AAAGTCCCACTGTATCGCTGAGGTGGAAAACGACGAG ATGCCCGCCGACCTGCCTTCCCTGGCCGCCGACTTCG TGGAATCCAAGGACGTGTGCAAGAACTACGCCGAGGC CAAGGATGTGTTCCTGGGCATGTTCCTGTACGAGTAC GCTCGGCGGCACCCCGACTACTCCGTGGTGCTGCTGC TGAGACTGGCCAAGACCTACGAGACAACCCTGGAAAA GTGCTGCGCCGCTGCCGACCCCCACGAGTGCTACGCC AAGGTGTTCGACGAGTTCAAGCCTCTGGTGGAAGAAC CCCAGAACCTGATCAAGCAGAACTGCGAGCTGTTCGA GCAGCTGGGCGAGTACAAGTTCCAGAACGCCCTGCTG GTCCGATACACCAAGAAAGTGCCCCAGGTGTCCACCC CTACCCTGGTGGAAGTGTCCCGGAACCTGGGCAAAGT GGGCTCCAAGTGCTGCAAGCACCCTGAGGCCAAGCGG ATGCCCTGCGCCGAGGACTACCTGAGCGTGGTGCTGA ACCAGCTGTGCGTGCTGCACGAAAAGACCCCCGTGTC CGACAGAGTGACCAAGTGCTGTACCGAGTCCCTGGTG AACAGACGGCCCTGCTTCTCCGCCCTGGAAGTGGACG AGACATACGTGCCCAAAGAGTTCAACGCCGAGACATT CACCTTCCACGCCGACATCTGCACCCTGTCCGAGAAA GAGCGGCAGATCAAGAAACAGACCGCACTGGTGGAAC TGGTGAAACACAAGCCCAAGGCCACCAAAGAACAGCT GAAGGCCGTGATGGACGACTTCGCCGCCTTTGTGGAA AAGTGTTGCAAGGCCGACGACAAAGAGACATGCTTCG CCGAAGAGGGCAAGAAACTGGTGGCCGCCTCTCAGGC TGCTCTGGGCCTG
85	RSLV-331 nucleic acid	ATGGAAACCCCTGCCCAGCTGCTGTTCCTGCTGCTGC TGTGGCTGCCCGACACCACCGGCAAAGAGTCCCGGGC CAAGAAGTTCCAGCGGCAGCACATGGACTCCGACTCC AGCCCCTCCAGCTCCTCCACCTACTGCAACCAGATGA TGCGGCGGAGAAACATGACCCAGGGCCGGTGCAAGCC CGTGAACACCTTCGTGCACGAGCCCCTGGTGGACGTG CAGAACGTGTGTTTTCAAGAAAAAGTCACATGCAAGA ACGGCCAGGGCAACTGCTACAAGTCCAACTCCTCCAT GCACATCACCGACTGCCGGCTGACCAACGGCTCCAGA TACCCCAACTGCGCCTACCGGACCTCCCCCAAAGAAC GGCACATCATCGTGGCCTGCGAGGGCTCCCCTTACGT

		GCCCGTGCACTTCGACGCCTCCGTGGAAGATTCTACC GGCGGAGGCGGATCTGGCGGCGGAGGAAGTGGCGGGG GAGGCTCTGATGCCCACAAGTCTGAGGTGGCCCACCG GTTCAAGGACCTGGGCGAGGAAAACTTCAAGGCCCTG GTGCTGATCGCCTTCGCCCAGTACCTGCAGCAGTGCC CCTTCGAGGACCACGTGAAGCTGGTGAACGAAGTGAC CGAGTTCGCCAAGACCTGCGTGGCCGACGAGTCCGCC GAGAACTGCGACAAGAGCCTGCACACCCTGTTCGGCG ACAAGCTGTGCACCGTGGCCACCCTGCGGGAAACCTA CGGCGAGATGGCCGACTGCTGCGCCAAGCAAGAACCC GAGCGGAACGAGTGCTTCCTGCAGCACAAGGACGACA ACCCCAACCTGCCCCGGCTGGTCCGACCCGAGGTGGA CGTGATGTGCACCGCCTTCCACGACAACGAGGAAACC TTCCTGAAGAAGTACCTGTACGAGATCGCCAGACGGC ACCCCTACTTCTACGCCCCCGAGCTGCTGTTTTTCGC CAAGCGGTACAAGGCCGCCTTCACCGAGTGCTGCCAG GCCGCCGATAAGGCCGCCTGCCTGCTGCCTAAGCTGG ACGAGCTGAGGGACGAGGGCAAGGCCTCCTCTGCCAA GCAGCGGCTGAAGTGCGCCTCCCTGCAGAAGTTCGGC GAGCGGGCCTTTAAGGCCTGGGCCGTGGCTCGGCTGT CCCAGAGATTCCCCAAGGCCGAGTTTGCCGAGGTGTC CAAGCTGGTGACAGACCTGACCAAGGTGCACACCGAG TGTTGTCACGGCGACCTGCTGGAATGCGCCGACGACA GAGCCGACCTGGCCAAGTACATCTGCGAGAACCAGGA CTCCATCTCCTCCAAGCTGAAAGAGTGCTGCGAGAAG CCCCTGCTGGAAAAGTCCCACTGTATCGCCGAGGTGG AAAACGACGAGATGCCCGCCGACCTGCCTTCCCTGGC CGCCGACTTCGTGGAATCCAAGGACGTGTGCAAGAAC TACGCCGAGGCCAAGGATGTGTTCCTGGGCATGTTCC TGTACGAGTACGCTCGGCGGCACCCCGACTACTCCGT GGTGCTGCTGCTGAGACTGGCCAAGACCTACGAGACA ACCCTGGAAAAGTGCTGCGCCGCTGCCGACCCCCACG AGTGCTACGCCAAGGTGTTCGACGAGTTCAAGCCTCT GGTGGAAGAACCCCAGAACCTGATCAAGCAGAACTGC GAGCTGTTCGAGCAGCTGGGCGAGTACAAGTTCCAGA ACGCCCTGCTGGTCCGATACACCAAGAAAGTGCCCCA GGTGTCCACCCCCACCCTGGTGGAAGTGTCCCGGAAC CTGGGCAAAGTGGGCTCCAAGTGCTGCAAGCACCCTG AGGCCAAGCGGATGCCCTGCGCCGAGGACTACCTGAG CGTGGTGCTGAACCAGCTGTGCGTGCTGCACGAAAAG ACCCCCGTGTCCGACAGAGTGACCAAGTGCTGTACCG AGTCCCTGGTGAACAGACGGCCCTGCTTCTCCGCCCT GGAAGTGGACGAGACATACGTGCCCAAAGAGTTCAAC GCCGAGACATTCACCTTCCACGCCGACATCTGCACCC TGTCCGAGAAAGAGCGGCAGATCAAGAAACAGACCGC ACTGGTGGAACTGGTGAAACACAAGCCCAAGGCCACC AAAGAACAGCTGAAGGCCGTGATGGACGACTTCGCCG CCTTTGTGGAAAAGTGTTGCAAGGCCGACGACAAAGA GACATGCTTCGCCGAAGAGGGCAAGAAACTGGTGGCC GCTTCTCAGGCTGCTCTGGGCCTGGGAGGCGGAGGAT CTGGGGGAGGCGGAAGCGGAGGGGGCGGATCTCTGAA GATCGCCGCCTTCAACATCCAGACCTTCGGCCGGACC AAGATGTCCAGCGCTACCCTGGTGTCCTACATCGTGC AGATCCTGTCCAGATACGATATCGCCCTGGTGCAAGA AGTGCGGGACTCCCACCTGACCGCCGTGGGCAAGCTG CTGGACAACCTGAACCAGGACGCCCCCGACACCTACC

		ACTACGTGGTGTCTGAGCCCCTGGGCCGGAAGTCCTA CAAAGAAAGATACCTGTTCGTGTACCGGCCCGACCAG GTGTCCGCCGTGGACTCCTACTACTACGACGACGGCT GCGAGCCCTGCGGCAGCGACACCTTCAACCGCGAGCC CTTCATCGTGCGGTTCTTCAGCCGGTTCACCGAAGTG CGCGAGTTTGCCATCGTGCCCCTGCACGCTGCTCCAG GCGACGCCGTGGCTGAGATCGACGCCCTGTACGACGT GTACCTGGATGTGCAAGAAAAGTGGGGCCTGGAAGAT GTGATGCTGATGGGCGACTTCAACGCCGGCTGCTCCT ACGTGCGGCCCTCCCAGTGGTCCTCCATCCGGCTGTG GACCAGCCCCACCTTCCAGTGGCTGATCCCCGACTCC GCCGATACCACCGCCAAGCCCACCCACTGTGCCTACG ACAGAATCGTGGTGGCCGGCATGCTGCTGAGGGGCGC TGTGGTGCCTGACTCCGCCCTGCCATTCAATTTTCAA GCCGCCTACGGCCTGTCCGACCAGCTGGCCCAGGCCA TCTCCGACCACTACCCCGTGGAAGTGATGCTGAAG
86	RSLV-332 nucleic acid	ATGGAAACCCCTGCCCAGCTGCTGTTCCTGCTGCTGC TGTGGCTGCCCGACACCACCGGCCTGAAGATCGCCGC CTTCAACATCCAGACCTTCGGCCGGACCAAGATGTCC AGCGCCACCCTGGTGTCCTACATCGTGCAGATCCTGT CCAGATACGATATCGCCCTGGTGCAAGAAGTGCGGGA CTCCCACCTGACCGCCGTGGGCAAGCTGCTGGACAAC CTGAACCAGGACGCCCCCGACACCTACCACTACGTGG TGTCTGAGCCCCTGGGCCGGAAGTCCTACAAAGAAAG ATACCTGTTCGTGTACCGGCCCGACCAGGTGTCCGCC GTGGACTCCTACTACTACGACGACGGCTGCGAGCCCT GCGGCAGCGACACCTTCAACCGCGAGCCCTTCATCGT GCGGTTCTTCAGCCGGTTCACCGAAGTGCGCGAGTTC GCCATCGTGCCCCTGCATGCTGCTCCAGGCGACGCCG TGGCCGAGATCGACGCCCTGTACGACGTGTACCTGGA CGTGCAAGAAAAGTGGGGCCTGGAAGATGTGATGCTG ATGGGCGACTTCAACGCCGGCTGCTCCTACGTGCGGC CCTCCCAGTGGTCCTCCATCCGGCTGTGGACCTCCCC CACCTTCCAGTGGCTGATCCCCGACTCCGCCGATACC ACCGCCAAGCCCACCCACTGCGCCTACGACAGAATCG TGGTGGCCGGCATGCTGCTGAGAGGCGCCGTGGTGCC TGACTCCGCCCTGCCATTCAATTTTCAAGCCGCCTAC GGCCTGTCCGACCAGCTGGCCCAGGCCATCTCCGACC ACTACCCCGTGGAAGTGATGCTGAAGGGGGGAGGCGG ATCTGGCGGAGGGGGAAGTGGCGGCGGAGGCTCTGAT GCCCACAAGTCTGAGGTGGCCCACCGGTTCAAGGACC TGGGCGAGGAAAACTTCAAGGCCCTGGTGCTGATCGC CTTCGCCCAGTACCTGCAGCAGTGCCCCTTCGAGGAC CACGTGAAGCTGGTGAACGAAGTGACCGAGTTTGCCA AGACCTGCGTGGCCGACGAGTCCGCCGAGAACTGCGA CAAGTCCCTGCACACCCTGTTCGGCGACAAGCTGTGC ACCGTGGCCACCCTGCGGGAAACCTACGGCGAGATGG CCGACTGCTGCGCCAAGCAAGAACCCGAGCGGAACGA GTGCTTCCTGCAGCACAAGGACGACAACCCCAACCTG CCCCGGCTGGTCCGACCCGAGGTGGACGTGATGTGCA CCGCCTTCCACGACAACGAGGAAACCTTCCTGAAGAA GTACCTGTACGAGATCGCCAGACGGCACCCCTACTTC TACGCCCCCGAGCTGCTGTTTTTCGCCAAGCGGTACA AGGCCGCCTTCACCGAGTGCTGCCAGGCCGCCGATAA GGCCGCCTGCCTGCTGCCTAAGCTGGACGAGCTGAGG GACGAGGGCAAGGCCTCCTCTGCCAAGCAGCGGCTGA

		AGTGCGCCTCCCTGCAGAAGTTCGGCGAGCGGGCCTT TAAGGCCTGGGCCGTGGCCCGGCTGTCCCAGAGATTC CCTAAGGCCGAGTTCGCCGAGGTGTCCAAGCTGGTGA CAGACCTGACCAAGGTGCACACCGAGTGTTGTCACGG CGACCTGCTGGAATGCGCCGACGACAGGGCCGACCTG GCCAAGTACATCTGCGAGAACCAGGACTCCATCTCCT CCAAGCTGAAAGAGTGCTGCGAGAAGCCCCTGCTGGA AAAGTCCCACTGTATCGCTGAGGTGGAAAACGACGAG ATGCCCGCCGACCTGCCTTCCCTGGCCGCCGACTTCG TGGAATCCAAGGACGTGTGCAAGAACTACGCCGAGGC CAAGGATGTGTTCCTGGGCATGTTCCTGTACGAGTAC GCTCGGCGGCACCCCGACTACTCCGTGGTGCTGCTGC TGAGACTGGCCAAGACCTACGAGACAACCCTGGAAAA GTGCTGCGCCGCTGCCGACCCCCACGAGTGCTACGCC AAGGTGTTCGACGAGTTCAAGCCTCTGGTGGAAGAAC CCCAGAACCTGATCAAGCAGAACTGCGAGCTGTTCGA GCAGCTGGGCGAGTACAAGTTCCAGAACGCCCTGCTG GTCCGATACACCAAGAAAGTGCCCCAGGTGTCCACCC CTACCCTGGTGGAAGTGTCCCGGAACCTGGGCAAAGT GGGCTCCAAGTGCTGCAAGCACCCTGAGGCCAAGCGG ATGCCCTGCGCCGAGGACTACCTGAGCGTGGTGCTGA ACCAGCTGTGCGTGCTGCACGAAAAGACCCCCGTGTC CGACAGAGTGACCAAGTGCTGTACCGAGTCCCTGGTG AACAGACGGCCCTGCTTCTCCGCCCTGGAAGTGGACG AGACATACGTGCCCAAAGAGTTCAACGCCGAGACATT CACCTTCCACGCCGACATCTGCACCCTGTCCGAGAAA GAGCGGCAGATCAAGAAACAGACCGCACTGGTGGAAC TGGTGAAACACAAGCCCAAGGCCACCAAAGAACAGCT GAAGGCCGTGATGGACGACTTCGCCGCCTTTGTGGAA AAGTGTTGCAAGGCCGACGACAAAGAGACATGCTTCG CCGAAGAGGGCAAGAAACTGGTGGCCGCCTCTCAGGC TGCTCTGGGCCTGGGAGGCGGAGGATCTGGCGGAGGC GGCTCTGGCGGGGGAGGCAGCAAAGAGTCCCGGGCCA AGAAGTTCCAGCGGCAGCACATGGACTCCGACTCCAG CCCCTCCAGCTCCTCCACCTACTGCAACCAGATGATG CGGCGGAGAAACATGACCCAGGGCCGGTGCAAGCCCG TGAACACCTTCGTGCACGAGCCCCTGGTGGATGTGCA GAACGTGTGTTTTCAAGAAAAAGTCACATGCAAGAAC GGCCAGGGCAACTGCTACAAGAGCAACTCCTCCATGC ACATCACCGACTGCCGGCTGACCAACGGCTCCAGATA CCCCAACTGTGCCTACCGGACCTCCCCTAAAGAACGG CACATCATCGTGGCCTGCGAGGGCTCCCCTTACGTGC CCGTGCACTTCGACGCCTCCGTGGAAGATTCCACC
87	Mature human DNase1 E13R/N74K/A114F/ T205K	LKIAAFNIQTFGRTKMSNATLVSYIVQILSRYDIALV QEVRDSHLTAVGKLLDNLNQDAPDTYHYVVSEPLGRK SYKERYLFVYRPDQVSAVDSYYYDDGCEPCGNDTFNR EPFIVRFFSRFTEVREFAIVPLHAAPGDAVAEIDALY DVYLDVQEKWGLEDVMLMGDFNAGCSYVRPSQWSSIR LWTSPTFQWLIPDSADTTAKPTHCAYDRIVVAGMLLR GAVVPDSALPFNFQAAYGLSDQLAQAISDHYPVEVML K
88	Mature human DNase1 E13R/N74K/A114F/ T205K/N18S/N106S	LKIAAFNIQTFGRTKMSSATLVSYIVQILSRYDIALV QEVRDSHLTAVGKLLDNLNQDAPDTYHYVVSEPLGRK SYKERYLFVYRPDQVSAVDSYYYDDGCEPCGSDTFNR EPFIVRFFSRFTEVREFAIVPLHAAPGDAVAEIDALY DVYLDVQEKWGLEDVMLMGDFNAGCSYVRPSQWSSIR

		LWTSPTFQWLIPDSADTTAKPTHCAYDRIVVAGMLLR GAVVPDSALPFNFQAAYGLSDQLAQAISDHYPVEVML K
89	AG1-RSLV-132 nucleic acid	ATGGAAACCCCTGCCCAGCTGCTGTTCCTGCTGCTG CTGTGGCTGCCTGACACCACCGGCAAAGAGAGCCGG GCCAAGAAGTTCCAGCGGCAGCACATGGACAGCGAC AGCAGCCCTAGCAGCTCCAGCACCTACTGCAACCAG ATGATGCGGAGGCGGAGCATGACCCAGGGCAGATGC AAGCCCGTGAACACCTTTGTGCACGAGCCCCTGGTG GACGTGCAGAACGTGTGTTTTCAAGAGAAAGTGACC TGCAAGAACGGCCAGGGCAACTGCTACAAGAGCAGC TCCTCCATGCACATCACCGACTGCAGACTGACCAGC GGCTCCAGATACCCCAACTGCGCCTACCGGACCAGC CCCAAAGAACGGCACATCATCGTGGCCTGCGAGGGC AGCCCTTACGTGCCCGTGCACTTTGATGCCAGCGTG GAAGATAGCACCCTGGAACCCAAGTCCAGCGACAAG ACCCACACCTGTCCCCCTTGTCCTGCCCCTGAACTG CTGGGCGGCAGCAGCGTGTTCCTGTTCCCCCCAAAG CCCAAGGACACCCTGATGATCAGCCGGACCCCCGAA GTGACATGCGTGGTGGTGGATGTGTCCCACGAGGAC CCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTG GAAGTGCACAACGCCAAGACCAAGCCCAGAGAGGAA CAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTG ACCGTGCTGCACCAGGACTGGCTGAATGGCAAAGAG TACAAGTGCAAGGTGTCCAACAAGGCCCTGCCCGCC AGCATCGAGAAAACCATCAGCAAGGCCAAGGGCCAG CCCCGCGAACCCCAGGTGTACACACTGCCCCCTAGC AGGGACGAGCTGACCAAGAACCAGGTGTCCCTGACC TGCCTCGTGAAGGGCTTCTACCCCTCCGATATCGCC GTGGAATGGGAGAGCAACGGCCAGCCCGAGAACAAC TACAAGACCACCCCCCCTGTGCTGGACTCCGACGGC TCATTCTTCCTGTACAGCAAGCTGACAGTGGACAAG AGCCGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGC GTGATGCACGAAGCCCTGCACAACCACTACACCCAG AAGTCCCTGAGCCTGAGCCCCGGCAAA
90	AG2-RSLV-132 nucleic acid	ATGGAAACCCCTGCCCAGCTGCTGTTCCTGCTGCTG CTGTGGCTGCCTGACACCACCGGCAAAGAGAGCCGG GCCAAGAAGTTCCAGCGGCAGCACATGGACAGCGAC AGCAGCCCTAGCAGCTCCAGCACCTACTGCAACCAG ATGATGCGGAGGCGGAGCATGACCCAGGGCAGATGC AAGCCCGTGAACACCTTTGTGCACGAGCCCCTGGTG GACGTGCAGAACGTGTGTTTTCAAGAGAAAGTGACC TGCAAGAACGGCCAGGGCAACTGCTACAAGAGCAGC TCCTCCATGCACATCACCGACTGCAGACTGACCAGC GGCTCCAGATACCCCAACTGCGCCTACCGGACCAGC CCCAAAGAACGGCACATCATCGTGGCCTGCGAGGGC AGCCCTTACGTGCCCGTGCACTTTGATGCCAGCGTG GAAGATAGCACCCTGGAACCCAAGTCCAGCGACAAG ACCCACACCTGTCCCCCTTGTCCTGCCCCTGAACTG CTGGGCGGCAGCAGCGTGTTCCTGTTCCCCCCAAAG CCCAAGGACACCCTGATGATCAGCCGGACCCCCGAA GTGACATGCGTGGTGGTGGATGTGTCCCACGAGGAC CCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTG GAAGTGCACAACGCCAAGACCAAGCCCAGAGAGGAA

		CAGTACAGCTCCACCTACCGGGTGGTGTCCGTGCTG ACAGTGCTGCACCAGGACTGGCTGAATGGCAAAGAG TACAAGTGCAAGGTGTCCAACAAGGCCCTGCCCGCC AGCATCGAGAAAACCATCAGCAAGGCCAAGGGCCAG CCCCGCGAACCCCAGGTGTACACACTGCCCCCTAGC AGGGACGAGCTGACCAAGAACCAGGTGTCCCTGACC TGCCTCGTGAAGGGCTTCTACCCCTCCGATATCGCC GTGGAATGGGAGAGCAACGGCCAGCCCGAGAACAAC TACAAGACCACCCCCCCTGTGCTGGACTCCGACGGC TCATTCTTCCTGTACAGCAAGCTGACCGTGGACAAG AGCCGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGC GTGATGCACGAAGCCCTGCACAACCACTACACCCAG AAGTCCCTGAGCCTGAGCCCCGGCAAA
91	RSLV-319 nucleic acid	ATGGAAACCCCTGCCCAGCTGCTGTTCCTGCTGCTGC TGTGGCTGCCCGACACCACCGGCAAAGAGTCCCGGGC CAAGAAGTTCCAGCGGCAGCACATGGACTCCGACTCC AGCCCCTCCAGCTCCTCCACCTACTGCAACCAGATGA TGCGGCGGAGATCCATGACCCAGGGCCGGTGCAAGCC CGTGAACACCTTCGTGCACGAGCCCCTGGTGGACGTG CAGAACGTGTGTTTTCAAGAAAAAGTGACCTGCAAGA ACGGCCAGGGCAACTGCTACAAGTCCTCCTCCTCCAT GCACATCACCGACTGCCGGCTGACCTCCGGCTCCAGA TACCCCAACTGCGCCTACCGGACCTCCCCCAAAGAAC GGCACATCATCGTGGCCTGCGAGGGCTCCCCTTACGT GCCCGTGCACTTCGACGCCTCCGTGGAAGATTCTACC GGCGGAGGCGGATCTGGCGGCGGAGGAAGTGGCGGGG GAGGCTCTGATGCCCACAAGTCTGAGGTGGCCCACCG GTTCAAGGACCTGGGCGAGGAAAACTTCAAGGCCCTG GTGCTGATCGCCTTCGCCCAGTACCTGCAGCAGTGCC CCTTCGAGGACCACGTGAAGCTGGTGAACGAAGTGAC CGAGTTCGCCAAGACCTGCGTGGCCGACGAGTCCGCC GAGAACTGCGACAAGAGCCTGCACACCCTGTTCGGCG ACAAGCTGTGCACCGTGGCCACCCTGCGGGAAACCTA CGGCGAGATGGCCGACTGCTGCGCCAAGCAAGAACCC GAGCGGAACGAGTGCTTCCTGCAGCACAAGGACGACA ACCCCAACCTGCCCCGGCTGGTCCGACCCGAGGTGGA CGTGATGTGCACCGCCTTCCACGACAACGAGGAAACC TTCCTGAAGAAGTACCTGTACGAGATCGCCAGACGGC ACCCCTACTTCTACGCCCCCGAGCTGCTGTTTTTCGC CAAGCGGTACAAGGCCGCCTTCACCGAGTGCTGCCAG GCCGCCGATAAGGCCGCCTGCCTGCTGCCTAAGCTGG ACGAGCTGAGGGACGAGGGCAAGGCCTCCTCTGCCAA GCAGCGGCTGAAGTGCGCCTCCCTGCAGAAGTTCGGC GAGCGGGCCTTTAAGGCCTGGGCCGTGGCTCGGCTGT CCCAGAGATTCCCCAAGGCCGAGTTTGCCGAGGTGTC CAAGCTGGTGACAGACCTGACCAAGGTGCACACCGAG TGTTGTCACGGCGACCTGCTGGAATGCGCCGACGACA GAGCCGACCTGGCCAAGTACATCTGCGAGAACCAGGA CTCCATCTCCTCCAAGCTGAAAGAGTGCTGCGAGAAG CCCCTGCTGGAAAAGTCCCACTGTATCGCCGAGGTGG AAAACGACGAGATGCCCGCCGACCTGCCTTCCCTGGC CGCCGACTTCGTGGAATCCAAGGACGTGTGCAAGAAC TACGCCGAGGCCAAGGATGTGTTCCTGGGCATGTTCC TGTACGAGTACGCTCGGCGGCACCCCGACTACTCCGT GGTGCTGCTGCTGAGACTGGCCAAGACCTACGAGACA ACCCTGGAAAAGTGCTGCGCCGCTGCCGACCCCCACG AGTGCTACGCCAAGGTGTTCGACGAGTTCAAGCCTCT GGTGGAAGAACCCCAGAACCTGATCAAGCAGAACTGC GAGCTGTTCGAGCAGCTGGGCGAGTACAAGTTCCAGA ACGCCCTGCTGGTCCGATACACCAAGAAAGTGCCCCA GGTGTCCACCCCCACCCTGGTGGAAGTGTCCCGGAAC CTGGGCAAAGTGGGCTCCAAGTGCTGCAAGCACCCTG

		AGGCCAAGCGGATGCCCTGCGCCGAGGACTACCTGAG CGTGGTGCTGAACCAGCTGTGCGTGCTGCACGAAAAG ACCCCCGTGTCCGACAGAGTGACCAAGTGCTGTACCG AGTCCCTGGTGAACAGACGGCCCTGCTTCTCCGCCCT GGAAGTGGACGAGACATACGTGCCCAAAGAGTTCAAC GCCGAGACATTCACCTTCCACGCCGACATCTGCACCC TGTCCGAGAAAGAGCGGCAGATCAAGAAACAGACCGC ACTGGTGGAACTGGTGAAACACAAGCCCAAGGCCACC AAAGAACAGCTGAAGGCCGTGATGGACGACTTCGCCG CCTTTGTGGAAAAGTGTTGCAAGGCCGACGACAAAGA GACATGCTTCGCCGAAGAGGGCAAGAAACTGGTGGCC GCTTCTCAGGCTGCTCTGGGCCTGGGAGGCGGAGGAT CTGGGGGAGGCGGAAGCGGAGGGGGCGGATCTCTGAA GATCGCCGCCTTCAACATCCAGACCTTCGGCGAGACA AAGATGTCCAACGCTACCCTGGTGTCCTACATCGTGC AGATCCTGTCCAGATACGATATCGCCCTGGTGCAAGA AGTGCGGGACTCCCACCTGACCGCCGTGGGCAAGCTG CTGGACAACCTGAACCAGGACGCCCCCGACACCTACC ACTACGTGGTGTCTGAGCCCCTGGGCCGGAACTCCTA CAAAGAAAGATACCTGTTCGTGTACCGGCCCGACCAG GTGTCCGCCGTGGACTCCTACTACTACGACGACGGCT GCGAGCCCTGCGGCAACGACACCTTCAACCGCGAGCC CTTCATCGTGCGGTTCTTCAGCCGGTTCACCGAAGTG CGCGAGTTTGCCATCGTGCCCCTGCACGCTGCTCCAG GCGACGCCGTGGCTGAGATCGACGCCCTGTACGACGT GTACCTGGATGTGCAAGAAAAGTGGGGCCTGGAAGAT GTGATGCTGATGGGCGACTTCAACGCCGGCTGCTCCT ACGTGCGGCCCTCCCAGTGGTCCTCCATCCGGCTGTG GACCAGCCCCACCTTCCAGTGGCTGATCCCCGACTCC GCCGATACCACCGCCACCCCTACCCACTGTGCCTACG ACAGAATCGTGGTGGCCGGCATGCTGCTGAGGGGCGC TGTGGTGCCTGACTCCGCCCTGCCATTCAATTTTCAA GCCGCCTACGGCCTGTCCGACCAGCTGGCCCAGGCCA TCTCCGACCACTACCCCGTGGAAGTGATGCTGAAGTG ACTCGAG
92	RSLV-320 nucleic acid	ATGGAAACCCCTGCCCAGCTGCTGTTCCTGCTGCTGC TGTGGCTGCCCGACACCACCGGCCTGAAGATCGCCGC CTTCAACATCCAGACCTTCGGCGAGACAAAGATGTCC AACGCCACCCTGGTGTCCTACATCGTGCAGATCCTGT CCAGATACGATATCGCCCTGGTGCAAGAAGTGCGGGA CTCCCACCTGACCGCCGTGGGCAAGCTGCTGGACAAC CTGAACCAGGACGCCCCCGACACCTACCACTACGTGG TGTCTGAGCCCCTGGGCCGGAACTCCTACAAAGAAAG ATACCTGTTCGTGTACCGGCCCGACCAGGTGTCCGCC GTGGACTCCTACTACTACGACGACGGCTGCGAGCCCT GCGGCAACGACACCTTCAACCGCGAGCCCTTCATCGT GCGGTTCTTCAGCCGGTTCACCGAAGTGCGCGAGTTC GCCATCGTGCCCCTGCATGCTGCTCCAGGCGACGCCG TGGCCGAGATCGACGCCCTGTACGACGTGTACCTGGA CGTGCAAGAAAAGTGGGGCCTGGAAGATGTGATGCTG ATGGGCGACTTCAACGCCGGCTGCTCCTACGTGCGGC CCTCCCAGTGGTCCTCCATCCGGCTGTGGACCTCCCC CACCTTCCAGTGGCTGATCCCCGACTCCGCCGATACC ACCGCCACCCCTACCCACTGCGCCTACGACAGAATCG TGGTGGCCGGCATGCTGCTGAGAGGCGCCGTGGTGCC TGACTCCGCCCTGCCATTCAATTTTCAAGCCGCCTAC GGCCTGTCCGACCAGCTGGCCCAGGCCATCTCCGACC ACTACCCCGTGGAAGTGATGCTGAAGGGGGGAGGCGG ATCTGGCGGAGGGGGAAGTGGCGGCGGAGGCTCTGAT GCCCACAAGTCTGAGGTGGCCCACCGGTTCAAGGACC TGGGCGAGGAAAACTTCAAGGCCCTGGTGCTGATCGC CTTCGCCCAGTACCTGCAGCAGTGCCCCTTCGAGGAC CACGTGAAGCTGGTGAACGAAGTGACCGAGTTTGCCA AGACCTGCGTGGCCGACGAGTCCGCCGAGAACTGCGA

		CAAGTCCCTGCACACCCTGTTCGGCGACAAGCTGTGC ACCGTGGCCACCCTGCGGGAAACCTACGGCGAGATGG CCGACTGCTGCGCCAAGCAAGAACCCGAGCGGAACGA GTGCTTCCTGCAGCACAAGGACGACAACCCCAACCTG CCCCGGCTGGTCCGACCCGAGGTGGACGTGATGTGCA CCGCCTTCCACGACAACGAGGAAACCTTCCTGAAGAA GTACCTGTACGAGATCGCCAGACGGCACCCCTACTTC TACGCCCCCGAGCTGCTGTTTTTCGCCAAGCGGTACA AGGCCGCCTTCACCGAGTGCTGCCAGGCCGCCGATAA GGCCGCCTGCCTGCTGCCTAAGCTGGACGAGCTGAGG GACGAGGGCAAGGCCTCCTCTGCCAAGCAGCGGCTGA AGTGCGCCTCCCTGCAGAAGTTCGGCGAGCGGGCCTT TAAGGCCTGGGCCGTGGCTCGGCTGTCCCAGAGATTC CCTAAGGCCGAGTTCGCCGAGGTGTCCAAGCTGGTGA CAGACCTGACCAAGGTGCACACCGAGTGTTGTCACGG CGACCTGCTGGAATGCGCCGACGACAGGGCCGACCTG GCCAAGTACATCTGCGAGAACCAGGACTCCATCTCCT CCAAGCTGAAAGAGTGCTGCGAGAAGCCCCTGCTGGA AAAGTCCCACTGTATCGCTGAGGTGGAAAACGACGAG ATGCCCGCCGACCTGCCTTCCCTGGCCGCCGACTTCG TGGAATCCAAGGACGTGTGCAAGAACTACGCCGAGGC CAAGGATGTGTTCCTGGGCATGTTCCTGTACGAGTAC GCTCGGCGGCACCCCGACTACTCCGTGGTGCTGCTGC TGAGACTGGCCAAGACCTACGAGACAACCCTGGAAAA GTGCTGCGCCGCTGCCGACCCCCACGAGTGCTACGCC AAGGTGTTCGACGAGTTCAAGCCTCTGGTGGAAGAAC CCCAGAACCTGATCAAGCAGAACTGCGAGCTGTTCGA GCAGCTGGGCGAGTACAAGTTCCAGAACGCCCTGCTG GTCCGATACACCAAGAAAGTGCCCCAGGTGTCCACCC CAACCCTGGTGGAAGTGTCCCGGAATCTGGGCAAAGT GGGCTCCAAGTGCTGCAAGCACCCTGAGGCCAAGCGG ATGCCCTGCGCCGAGGACTACCTGAGCGTGGTGCTGA ACCAGCTGTGCGTGCTGCACGAAAAGACCCCCGTGTC CGACAGAGTGACCAAGTGCTGTACCGAGTCCCTGGTG AACAGACGGCCCTGCTTCTCCGCCCTGGAAGTGGACG AGACATACGTGCCCAAAGAGTTCAACGCCGAGACATT CACCTTCCACGCCGACATCTGCACCCTGTCCGAGAAA GAGCGGCAGATCAAGAAACAGACCGCACTGGTGGAAC TGGTGAAACACAAGCCCAAGGCCACCAAAGAACAGCT GAAGGCCGTGATGGACGACTTCGCCGCCTTTGTGGAA AAGTGTTGCAAGGCCGACGACAAAGAGACATGCTTCG CCGAAGAGGGCAAGAAACTGGTGGCCGCCTCTCAGGC TGCTCTGGGCCTGGGAGGCGGAGGATCTGGCGGAGGC GGCTCTGGCGGGGGAGGCAGCAAAGAGTCCCGGGCCA AGAAGTTCCAGCGGCAGCACATGGACTCCGACTCCAG CCCCTCCAGCTCCTCCACCTACTGCAACCAGATGATG CGGCGGAGATCCATGACCCAGGGCCGGTGCAAGCCCG TGAACACCTTCGTGCACGAGCCCCTGGTGGATGTGCA GAACGTGTGTTTTCAAGAAAAAGTCACATGCAAGAAC GGCCAGGGCAACTGCTACAAGTCTAGCTCCTCCATGC ACATCACCGACTGCCGGCTGACCTCCGGCTCCAGATA CCCCAACTGTGCCTACCGGACCTCCCCTAAAGAACGG CACATCATCGTGGCCTGCGAGGGCTCCCCTTACGTGC CCGTGCACTTCGACGCCTCCGTGGAAGATTCCACCTG ACTCGAG
93	RSLV-308 (RNase-linker-HSA-linker- DNase A114F)	METPAQLLFLLLLWLPDTTGKESRAKKFQRQHMDSDS SPSSSSTYCNQMMRRRNMTQGRCKPVNTFVHEPLVDV QNVCFQEKVTCKNGQGNCYKSNSSMHITDCRLTNGSR YPNCAYRTSPKERHIIVACEGSPYVPVHFDASVEDST GGGGSGGGGSGGGGSDAHKSEVAHRFKDLGEENFKAL VLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADESA ENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEP ERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEET

		FLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQ AADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFG ERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTE CCHGDLLECADDRADLAKYICENQDSISSKLKECCEK PLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKN YAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYET TLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNC ELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRN LGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEK TPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFN AETFTFHADICTLSEKERQIKKQTALVELVKHKPKAT KEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVA ASQAALGLGGGGSGGGGSGGGGSLKIAAFNIQTFGET KMSNATLVSYIVQILSRYDIALVQEVRDSHLTAVGKL LDNLNQDAPDTYHYVVSEPLGRNSYKERYLFVYRPDQ VSAVDSYYYDDGCEPCGNDTFNREPFIVRFFSRFTEV REFAIVPLHAAPGDAVAEIDALYDVYLDVQEKWGLED VMLMGDFNAGCSYVRPSQWSSIRLWTSPTFQWLIPDS ADTTATPTHCAYDRIVVAGMLLRGAVVPDSALPFNFQ AAYGLSDQLAQAISDHYPVEVMLK
94	RSLV-310 (DNase1 A114F-linker-HSA-linker-RNase1)	METPAQLLFLLLLWLPDTTGLKIAAFNIQTFGETKMS NATLVSYIVQILSRYDIALVQEVRDSHLTAVGKLLDN LNQDAPDTYHYVVSEPLGRNSYKERYLFVYRPDQVSA VDSYYYDDGCEPCGNDTFNREPFIVRFFSRFTEVREF AIVPLHAAPGDAVAEIDALYDVYLDVQEKWGLEDVML MGDFNAGCSYVRPSQWSSIRLWTSPTFQWLIPDSADT TATPTHCAYDRIVVAGMLLRGAVVPDSALPFNFQAAY GLSDQLAQAISDHYPVEVMLKGGGGSGGGGSGGGGSD AHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFED HVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLC TVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNL PRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYF YAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELR DEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQRF PKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADL AKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDE MPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEY ARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYA KVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALL VRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKR MPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLV NRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEK ERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVE KCCKADDKETCFAEEGKKLVAASQAALGLGGGGSGGG GSGGGGSKESRAKKFQRQHMDSDSSPSSSSTYCNQMM RRRNMTQGRCKPVNTFVHEPLVDVQNVCFQEKVTCKN GQGNCYKSNSSMHITDCRLTNGSRYPNCAYRTSPKER HIIVACEGSPYVPVHFDASVEDST
95	RSLV-308 (RNase-linker-HSA-linker-DNase A114F; w/o leader)	KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRNMTQ GRCKPVNTFVHEPLVDVQNVCFQEKVTCKNGQGNCYK SNSSMHITDCRLTNGSRYPNCAYRTSPKERHIIVACE GSPYVPVHFDASVEDSTGGGGSGGGGSGGGGSDAHKS EVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKL VNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVAT LRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLV RPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPE

		LLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGK ASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYI CENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPAD LPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRH PDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFD EFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYT KKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCA EDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRP CFSALEVDETYVPKEFNAETFTFHADICTLSEKERQI KKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCK ADDKETCFAEEGKKLVAASQAALGLGGGGSGGGGSGG GGSLKIAAFNIQTFGETKMSNATLVSYIVQILSRYDI ALVQEVRDSHLTAVGKLLDNLNQDAPDTYHYVVSEPL GRNSYKERYLFVYRPDQVSAVDSYYYDDGCEPCGNDT FNREPFIVRFFSRFTEVREFAIVPLHAAPGDAVAEID ALYDVYLDVQEKWGLEDVMLMGDFNAGCSYVRPSQWS SIRLWTSPTFQWLIPDSADTTATPTHCAYDRIVVAGM LLRGAVVPDSALPFNFQAAYGLSDQLAQAISDHYPVE VMLK
96	RSLV-310 (DNase1 A114F-linker-HSA-linker-RNase1; w/o leader)	LKIAAFNIQTFGETKMSNATLVSYIVQILSRYDIALV QEVRDSHLTAVGKLLDNLNQDAPDTYHYVVSEPLGRN SYKERYLFVYRPDQVSAVDSYYYDDGCEPCGNDTFNR EPFIVRFFSRFTEVREFAIVPLHAAPGDAVAEIDALY DVYLDVQEKWGLEDVMLMGDFNAGCSYVRPSQWSSIR LWTSPTFQWLIPDSADTTATPTHCAYDRIVVAGMLLR GAVVPDSALPFNFQAAYGLSDQLAQAISDHYPVEVML KGGGGSGGGGSGGGGSDAHKSEVAHRFKDLGEENFKA LVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADES AENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQE PERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEE TFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECC QAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKF GERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHT ECCHGDLLECADDRADLAKYICENQDSISSKLKECCE KPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCK NYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYE TTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQN CELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSR NLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHE KTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEF NAETFTFHADICTLSEKERQIKKQTALVELVKHKPKA TKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLV AASQAALGLGGGGSGGGGSGGGGSKESRAKKFQRQHM DSDSSPSSSSTYCNQMMRRRNMTQGRCKPVNTFVHEP LVDVQNVCFQEKVTCKNGQGNCYKSNSSMHITDCRLT NGSRYPNCAYRTSPKERHIIVACEGSPYVPVHFDASV EDST
97	RSLV-308 nucleic acid	ATGGAAACCCCTGCCCAGCTGCTGTTCCTGCTGCTGC TGTGGCTGCCCGACACCACCGGCAAAGAGTCCCGGGC CAAGAAGTTCCAGCGGCAGCACATGGACTCCGACTCC AGCCCCTCCAGCTCCTCCACCTACTGCAACCAGATGA TGCGGCGGAGAAACATGACCCAGGGCCGGTGCAAGCC CGTGAACACCTTCGTGCACGAGCCCCTGGTGGACGTG CAGAACGTGTGTTTTCAAGAAAAAGTCACTTGCAAGA ACGGCCAGGGCAACTGCTACAAGTCCAACTCCTCCAT

		GCACATCACCGACTGCCGGCTGACCAACGGCTCCAGA TACCCCAACTGCGCCTACCGGACCTCCCCCAAAGAAC GGCACATCATCGTGGCCTGCGAGGGCTCCCCTTACGT GCCCGTGCACTTCGACGCCTCCGTGGAAGATTCCACC GGCGGAGGCGGATCTGGCGGCGGAGGTTCTGGTGGCG GCGGATCTGACGCCCACAAGTCCGAGGTGGCCCACCG GTTCAAGGACCTGGGCGAGGAAAACTTCAAGGCCCTG GTGCTGATCGCCTTCGCCCAGTACCTGCAGCAGTGCC CCTTCGAGGACCACGTGAAGCTGGTCAACGAAGTGAC CGAGTTCGCCAAGACCTGCGTGGCCGACGAGTCCGCC GAGAACTGCGACAAGAGCCTGCACACCCTGTTCGGCG ACAAGCTGTGCACCGTGGCCACCCTGCGGGAAACCTA CGGCGAGATGGCCGACTGCTGCGCCAAGCAGGAACCC GAGCGGAACGAGTGCTTCCTGCAGCACAAGGACGACA ACCCCAACCTGCCCCGGCTGGTCCGACCTGAGGTGGA CGTGATGTGCACCGCCTTCCACGACAACGAGGAAACC TTCCTGAAGAAGTACCTGTACGAGATCGCCAGACGGC ACCCCTACTTCTACGCCCCCGAGCTGCTGTTTTTCGC CAAGCGGTACAAGGCCGCCTTCACCGAGTGCTGCCAG GCCGCCGATAAGGCCGCCTGCCTGCTGCCTAAGCTGG ACGAGCTGCGGGACGAGGGCAAGGCCTCCTCCGCCAA GCAGAGACTGAAGTGCGCCTCCCTGCAGAAGTTCGGC GAGCGGGCCTTTAAGGCCTGGGCCGTGGCCCGGCTGT CTCAGAGATTCCCCAAGGCCGAGTTTGCCGAGGTGTC CAAGCTGGTCACCGACCTGACCAAGGTGCACACCGAG TGTTGTCACGGCGACCTGCTGGAATGCGCCGACGACA GAGCCGACCTGGCCAAGTACATCTGCGAGAACCAGGA CTCCATCTCCTCCAAGCTGAAAGAGTGCTGCGAGAAG CCCCTGCTGGAAAAGTCCCACTGTATCGCCGAGGTGG AAAACGACGAGATGCCCGCCGACCTGCCTTCCCTGGC CGCCGACTTCGTGGAATCCAAGGACGTGTGCAAGAAC TACGCCGAGGCCAAGGATGTGTTCCTGGGCATGTTCC TGTACGAGTACGCTCGGCGGCACCCCGACTACTCCGT GGTGCTGCTGCTGAGACTGGCCAAGACCTACGAGACA ACCCTGGAAAAGTGCTGCGCCGCTGCCGACCCCCACG AGTGCTACGCCAAGGTGTTCGACGAGTTCAAGCCTCT GGTGGAAGAACCCCAGAACCTGATCAAGCAGAACTGC GAGCTGTTCGAGCAGCTGGGCGAGTACAAGTTCCAGA ACGCCCTGCTGGTCCGATACACCAAGAAAGTGCCCCA GGTGTCCACCCCCACCCTGGTGGAAGTGTCCCGGAAC CTGGGCAAAGTGGGCTCCAAGTGCTGCAAGCACCCTG AGGCCAAGCGGATGCCCTGCGCCGAGGACTACCTGAG CGTGGTGCTGAACCAGCTGTGCGTGCTGCACGAAAAG ACCCCCGTGTCCGACAGAGTGACCAAGTGCTGTACCG AGTCCCTGGTCAACAGACGGCCCTGCTTCTCCGCCCT GGAAGTGGACGAGACATACGTGCCCAAAGAGTTCAAC GCCGAGACATTCACCTTCCACGCCGACATCTGCACCC TGTCCGAGAAAGAGCGGCAGATCAAGAAACAGACCGC CCTGGTCGAGCTGGTCAAGCACAAGCCCAAGGCCACC AAAGAACAGCTGAAGGCCGTGATGGACGACTTCGCCG CCTTCGTCGAGAAGTGTTGCAAGGCCGACGACAAAGA GACATGCTTCGCCGAAGAGGGCAAGAAACTGGTGGCC GCCTCTCAGGCCGCTCTGGGACTGGGAGGCGGAGGAA GTGGTGGCGGAGGTAGCGGAGGTGGCGGCTCCCTGAA GATCGCCGCCTTTAACATCCAGACCTTCGGCGAGACA AAGATGTCCAACGCTACCCTGGTGTCCTACATCGTGC

		AGATCCTGTCCAGATACGATATCGCCCTGGTGCAGGA AGTGCGGGACTCCCACCTGACCGCCGTGGGCAAGCTG CTGGACAACCTGAACCAGGACGCCCCCGACACCTACC ACTACGTGGTGTCCGAGCCTCTGGGCCGGAACTCCTA CAAAGAAAGATACCTGTTCGTGTACCGGCCCGACCAG GTGTCCGCCGTGGAC TCCTACTACTACGACGACGGCTGCGAGCCCTGCGGCA ACGACACCTTCAACCGCGAGCCCTTCATCGTGCGGTT CTTCAGCCGGTTCACCGAAGTCCGCGAGTTTGCCATC GTGCCCCTGCACGCTGCTCCAGGCGACGCCGTGGCTG AGATCGACGCCCTGTACGACGTGTACCTGGATGTGCA GGAAAAGTGGGGCCTGGAAGATGTGATGCTGATGGGC GACTTCAACGCCGGCTGCTCCTACGTGCGGCCCTCCC AGTGGTCCTCCATCCGGCTGTGGACCAGCCCCACCTT CCAGTGGCTGATCCCCGACTCCGCCGATACCACCGCC ACCCCTACCCACTGTGCCTACGACCGGATCGTGGTGG CCGGCATGCTGCTGAGGGGTGCCGTGGTGCCTGACTC CGCCCTGCCATTCAATTTTCAAGCCGCCTACGGCCTG TCCGACCAGCTGGCCCAGGCCATCTCCGACCACTACC CCGTGGAAGTGATGCTGAAGTGATGACTCGAG
98	RSLV-310 nucleic acid	ATGGAAACCCCTGCCCAGCTGCTGTTCCTGCTGCTGC TGTGGCTGCCCGACACCACCGGCCTGAAGATCGCCGC CTTCAACATCCAGACCTTCGGCGAGACAAAGATGTCC AACGCCACCCTGGTGTCCTACATCGTGCAGATCCTGT CCAGATACGATATCGCCCTGGTGCAGGAAGTGCGGGA CTCCCACCTGACCGCCGTGGGCAAGCTGCTGGACAAC CTGAACCAGGACGCCCCCGACACCTACCACTACGTGG TGTCCGAGCCCCTGGGCCGGAACTCCTACAAAGAAAG ATACCTGTTCGTGTACCGGCCCGACCAGGTGTCCGCC GTGGACTCCTACTACTACGACGACGGCTGCGAGCCCT GCGGCAACGACACCTTCAACCGCGAGCCCTTCATCGT GCGGTTCTTCAGCCGGTTCACCGAAGTCCGCGAGTTC GCCATCGTGCCCCTGCATGCTGCTCCAGGCGACGCCG TGGCCGAGATCGACGCCCTGTACGACGTGTACCTGGA CGTGCAGGAAAAGTGGGGCCTGGAAGATGTGATGCTG ATGGGCGACTTCAACGCCGGCTGCTCCTACGTGCGGC CCTCCCAGTGGTCCTCCATCCGGCTGTGGACCTCCCC CACCTTCCAGTGGCTGATCCCCGACTCCGCCGATACC ACCGCCACCCCTACCCACTGCGCCTACGACAGAATCG TGGTGGCCGGCATGCTGCTGAGAGGCGCCGTGGTGCC TGACTCCGCCCTGCCATTCAATTTTCAAGCCGCCTAC GGCCTGTCCGACCAGCTGGCCCAGGCCATCTCCGACC ACTACCCCGTGGAAGTGATGCTGAAGGGGGGAGGCGG ATCTGGCGGCGGAGGTTCTGGTGGCGGCGGATCTGAC GCCCACAAGTCCGAGGTGGCCCACCGGTTCAAGGACC TGGGCGAGGAAAACTTCAAGGCCCTGGTGCTGATCGC CTTCGCCCAGTACCTGCAGCAGTGCCCCTTCGAGGAC CACGTGAAGCTGGTCAACGAAGTGACCGAGTTTGCCA AGACCTGCGTGGCCGACGAGTCCGCCGAGAACTGCGA CAAGTCCCTGCACACCCTGTTCGGCGACAAGCTGTGC ACCGTGGCCACCCTGCGGGAAACCTACGGCGAGATGG CCGACTGCTGCGCCAAGCAGGAACCCGAGCGGAACGA GTGCTTCCTGCAGCACAAGGACGACAACCCCAACCTG CCCCGGCTGGTCCGACCTGAGGTGGACGTGATGTGCA CCGCCTTCCACGACAACGAGGAAACCTTCCTGAAGAA GTACCTGTACGAGATCGCCAGACGGCACCCCTACTTC

		TACGCCCCCGAGCTGCTGTTTTTCGCCAAGCGGTACA AGGCCGCCTTCACCGAGTGCTGCCAGGCCGCCGATAA GGCCGCCTGCCTGCTGCCTAAGCTGGACGAGCTGCGG GACGAGGGCAAGGCCTCCTCCGCCAAGCAGAGACTGA AGTGCGCCTCCCTGCAGAAGTTCGGCGAGCGGGCCTT TAAGGCCTGGGCCGTGGCCCGGCTGTCTCAGAGATTC CCCAAGGCCGAGTTCGCCGAGGTGTCCAAGCTGGTCA CCGACCTGACCAAGGTGCACACCGAGTGTTGTCACGG CGACCTGCTGGAATGCGCCGACGACAGGGCCGACCTG GCCAAGTACATCTGCGAGAACCAGGACTCCATCTCCT CCAAGCTGAAAGAGTGCTGCGAGAAGCCCCTGCTGGA AAAGTCCCACTGTATCGCTGAGGTGGAAAACGACGAG ATGCCCGCCGACCTGCCTTCCCTGGCCGCCGACTTCG TGGAATCCAAGGACGTGTGCAAGAACTACGCCGAGGC CAAGGATGTGTTCCTGGGCATGTTCCTGTACGAGTAC GCTCGGCGGCACCCCGACTACTCCGTGGTGCTGCTGC TGAGACTGGCCAAGACCTACGAGACAACCCTGGAAAA GTGCTGCGCCGCTGCCGACCCCCACGAGTGCTACGCC AAGGTGTTCGACGAGTTCAAGCCTCTGGTGGAAGAAC CCCAGAACCTGATCAAGCAGAACTGCGAGCTGTTCGA GCAGCTGGGCGAGTACAAGTTCCAGAACGCCCTGCTG GTCCGATACACCAAGAAAGTGCCCCAGGTGTCCACCC CAACCCTGGTGGAAGTGTCCCGGAACCTGGGCAAAGT GGGCTCCAAGTGCTGCAAGCACCCTGAGGCCAAGCGG ATGCCCTGCGCCGAGGACTACCTGAGCGTGGTGCTGA ACCAGCTGTGCGTGCTGCACGAAAAGACCCCCGTGTC CGACAGAGTGACCAAGTGCTGTACCGAGTCCCTGGTC AACAGACGGCCCTGCTTCTCCGCCCTGGAAGTGGACG AGACATACGTGCCCAAAGAGTTCAACGCCGAGACATT CACCTTCCACGCCGACATCTGCACCCTGTCCGAGAAA GAGCGGCAGATCAAGAAACAGACCGCCCTGGTCGAGC TGGTCAAGCACAAGCCCAAGGCCACCAAAGAACAGCT GAAGGCCGTGATGGACGACTTCGCCGCCTTTGTGGAA AAGTGTTGCAAGGCC GACGACAAAGAGACATGCTTCGCCGAAGAGGGCAAGA AACTGGTGGCCGCCTCTCAGGCCGCTCTGGGACTGGG AGGCGGAGGAAGTGGTGGCGGAGGTAGTGGCGGAGGC GGCTCCAAAGAGTCCCGGGCCAAGAAGTTCCAGCGGC AGCACATGGACTCCGACTCCAGCCCCTCCAGCTCCTC CACCTACTGCAACCAGATGATGCGGCGGAGAAACATG ACCCAGGGCCGGTGCAAGCCCGTGAACACCTTCGTGC ACGAGCCACTGGTGGATGTGCAGAACGTGTGTTTTCA AGAAAAAGTCACTTGCAAGAACGGCCAGGGCAACTGC TACAAGAGCAACTCCTCCATGCACATCACCGACTGCC GGCTGACCAACGGCTCCAGATACCCCAACTGTGCCTA CCGGACCTCCCCTAAAGAACGGCACATCATCGTGGCC TGCGAGGGCTCCCCTTACGTGCCCGTGCACTTCGACG CCTCCGTGGAAGATTCCACCTGATGACTCGAG
99	RSLV-132 huVK3LP-wthRNase-SCC- mthIgG1 P238S P331S	metpaqllfllllwlpdttgkesrakkfqrqhmdsds spssstycnqmmrrrnmtqgrckpvntfvheplvdv qnvcfqekvtckngqgncyksnssmhitdcrltngsr ypncayrtspkerhiivacegspyvpvhfdasvedst lepkssdkthtcppcpapellggssvflfppkpkdtl misrtpevtcvvvdvshedpevkfnwyvdgvevhnak tkpreeqynstyrvvsvltvlhqdwlngkeykckvsn kalpasiektiskakgqprepqvytlppsrdeltknq

		vsltclvkgfypsdiavewesngqpennykttppvld sdgsfflyskltvdksrwqqgnvfscsvmhealhnhy tqkslslspgk
100	RSLV132: huVK3LP-wthRNase-SCC- mthIgG1 P238S P331S	atggaaaccctgcccagctgctgttcctgctgctgc tgtggctgcctgacaccaccggcaaagagtcccgggc caagaagttccagcggcagcacatggactccgactcc agcccttccagctcctccacctactgcaaccagatga tgcggcggagaaacatgacccagggccggtgcaagcc cgtgaacacctttgtgcacgagcccctggtggacgtg cagaacgtgtgttttcaagagaaagtgacctgcaaga acggccagggcaactgctacaagtccaactcctccat gcacatcaccgactgccggctgaccaacggctccaga taccccaactgcgcctaccggacctcccccaaagaac ggcacatcatcgtggcctgcgagggctctccttacgt gcccgtgcacttcgacgcctccgtggaagattccacc ctggaacccaagtcctccgacaagacccacacctgtc ccccttgtcctgcccctgaactgctgggcggctcctc cgtgttcctgttcccccccaaagcccaaggacaccctg atgatctcccggaccccccgaagtgacatgcgtggtgg tggatgtgtcccacgaggaccctgaagtgaagttcaa ttggtacgtggacggggtggaagtgcacaacgccaag accaagcccagagaggaacagtacaacagcacctacc gggtggtgtccgtgctgaccgtgctgcaccaggattg gctgaacggaaaagagtacaagtgcaaggtgtccaac aaggccctgcccgcctccatcgaaaagaccatctcca aggccaagggccagccccgggaaccccaggtgtacac actgccccctagcagggacgagctgaccaagaaccag gtgtccctgacctgcctcgtgaagggcttctacccct ccgatatcgccgtggaatgggagtccaacggccagcc tgagaacaactacaagaccacccccccctgtgctggac agcgacggctcattcttcctgtactccaagctgacag tggacaagtcccggtggcagcagggcaacgtgttctc ctgctccgtgatgcacgaggctctgcacaaccactac acccagaagtccctgtccctgagccccggcaaatga
101	RSLV-133 huVK3LP-hRNAseWT-SCC- mthIgG1-P238S-P331S-NLG- hDNAse 105/114	metpaqllfllllwlpdttgkesrakkfqrqhmdsds spsssstycnqmmrrrnmtqgrckpvntfvheplvdv qnvcfqekvtckngqgncyksnssmhitdcrltngsr ypncayrtspkerhiivacegspyvpvhfdasvedst lepkssdkthtcppcpapellggssvflfppkpkdtl misrtpevtcvvvdvshedpevkfnwyvdgvevhnak tkpreeqynstyrvvsvltvlhqdwlngkeykckvsn kalpasiektiskakgqprepqvytlppsrdeltknq vsltclvkgfypsdiavewesngqpennykttppvld sdgsfflyskltvdksrwqqgnvfscsvmhealhnhy tqkslslspgkvdgasspvnvsspsvqdilkiaafni qtfgetkmsnatlvsyivqilsrydialvqevrdshl tavgklldnlnqdapdtyhyvvseplgrnsykerylf vyrpdqvsavdsyyyddgcepcrndtfnrepfivrff srftevrefaivplhaapgdavaeidalydvyldvqe kwgledvmlmgdfnagcsyvrpsqwssirlwtsptfq wlipdsadttatpthcaydrivvagmllrgavvpdsa lpfnfqaayglsdqlaqaisdhypvevmlk
102	RSLV133: huVK3LP-hRNAseWT-SCC- mthIgG1-P238S-P331S-NLG- hDNAse 105/114	atggaaaccctgcccagctgctgttcctgctgctgc tgtggctgcccgacaccaccggcaaagagagccgggc caagaagttccagcggcagcacatggacagcgacagc agccccagcagctccagcacctactgcaaccagatga

		tgcggcggagaaacatgacccagggccggtgcaagcc cgtgaacaccttcgtgcacgagcccctggtggacgtg cagaacgtgtgttttcaagaaaaagtgacctgcaaga acggccagggcaactgctacaagagcaacagcagcat gcacatcaccgactgccggctgaccaacggcagcaga taccccaactgcgcctaccggaccagccccaaagaac ggcacatcatcgtggcctgcgagggcagcccttacgt gcccgtgcactttgacgccagcgtggaagatagcacc ctggaacccaagagcagcgacaagacccacacctgtc ccccctgccctgcccctgagctgctgggcggaagcag cgtgttcctgttcccccaaagcccaaggacaccctg atgatcagccggaccccgaagtgacctgcgtggtgg tggatgtgtcccacgaggaccccgaagtgaagttcaa ttggtacgtggacggcgtggaagtgcacaacgccaag accaagcccagagaggaacagtacaacagcacctacc gggtggtgtccgtgctgaccgtgctgcaccaggactg gctgaacggcaaagagtacaagtgcaaggtctccaac aaggccctgcccgccagcatcgagaaaaccatcagca aggccaagggccagcctcgcgagccccaggtgtacac actgccccccagccgggacgagctgaccaagaaccag gtgtccctgacctgcctggtgaaaggcttctacccca gcgatatcgccgtggaatgggagagcaacggccagcc cgagaacaactacaagaccacccccccctgtgctggac tccgacggctcattcttcctgtacagcaagctgaccg tggacaagagccggtggcagcagggcaacgtgttcag ctgcagcgtgatgcacgaggccctgcacaaccactac acccagaagtccctgagcctgagccccggcaaggtgg acggcgccagctcccctgtgaacgtgtccagccccag cgtgcaggacatcctgaagatcgccgccttcaacatc cagaccttcggcgagacaaagatgagcaacgccaccc tggtgtcctacatcgtgcagatcctgagcagatacga tatcgccctggtgcaagaagtgcgggacagccacctg accgccgtgggcaagctgctggacaacctgaaccagg acgcccccgacacctaccactacgtggtgtccgagcc tctgggccggaacagctacaaagaaagatacctgttc gtgtaccggcccgatcaggtgtccgccgtggacagct actactacgacgacggctgcgagccctgccggaacga caccttcaaccgcgagcccttcatcgtgcggttcttc agccggttcaccgaagtgcgcgagttcgccatcgtgc ccctgcatgctgcccctggcgacgccgtggccgagat cgatgccctgtacgacgtgtacctggatgtgcaagaa aagtggggcctggaagatgtgatgctgatgggcgact tcaacgccggctgcagctacgtgcggcccagccagtg gtccagcatcagactgtggacctcccccaccttccag tggctgatccccgacagcgccgataccaccgccaccc ccacccactgtgcctacgacagaatcgtggtggccgg catgctgctgagaggcgccgtggtgcctgacagcgcc ctgccattcaattttcaagccgcctacggcctgagcg atcagctggcccaggccatcagcgaccactaccccgt ggaagtgatgctgaagtga
103	RSLV132: wthRNase-SCC-mthIgG1 P238S P331S	kesrakkfqrqhmdsdsspsssstycnqmmrrrnmtq grckpvntfvheplvdvqnvcfqekvtckngqgncyk snssmhitdcrltngsrypncayrtspkerhiivace gspyvpvhfdasvedstlepkssdkthtcppcpapel lggssvflfppkpkdtlmisrtpevtcvvvdvshedp evkfnwyvdgvevhnaktkpreeqynstyrvvsvltv

		lhqdwlngkeykckvsnkalpasiektiskakgqpre pqvytlppsrdeltknqvsltclvkgfypsdiavewe sngqpennykttppvldsdgsfflyskltvdksrwqq gnvfscsvmhealhnhytqkslslspgk
104	RSLV132: wthRNase-SCC-mthIgG1 P238S P331S	aaagagtcccgggccaagaagttccagcggcagcaca tggactccgactccagcccttccagctcctccaccta ctgcaaccagatgatgcggcggagaaacatgacccag ggccggtgcaagcccgtgaacacctttgtgcacgagc ccctggtggacgtgcagaacgtgtgttttcaagagaa agtgacctgcaagaacggccagggcaactgctacaag tccaactcctccatgcacatcaccgactgccggctga ccaacggctccagataccccaactgcgcctaccggac ctcccccaaagaacggcacatcatcgtggcctgcgag ggctctccttacgtgcccgtgcacttcgacgcctccg tggaagattccaccctggaacccaagtcctccgacaa gacccacacctgtccccccttgtcctgcccctgaactg ctgggcggctcctccgtgttcctgttcccccccaaagc ccaaggacaccctgatgatctcccggacccccgaagt gacatgcgtggtggtggatgtgtcccacgaggaccct gaagtgaagttcaattggtacgtggacggggtggaag tgcacaacgccaagaccaagcccagagaggaacagta caacagcacctaccgggtggtgtccgtgctgaccgtg ctgcaccaggattggctgaacggaaaagagtacaagt gcaaggtgtccaacaaggccctgcccgcctccatcga aaagaccatctccaaggccaagggccagccccgggaa ccccaggtgtacacactgccccctagcagggacgagc tgaccaagaaccaggtgtccctgacctgcctcgtgaa gggcttctacccctccgatatcgccgtggaatgggag tccaacggccagcctgagaacaactacaagaccaccc cccctgtgctggacagcgacggctcattcttcctgta ctccaagctgacagtggacaagtcccggtggcagcag ggcaacgtgttctcctgctccgtgatgcacgaggctc tgcacaaccactacacccagaagtccctgtccctgag ccccggcaaatga
105	RSLV133: hRNAseWT-SCC-mthIgG1- P238S-P331S-NLG-hDNAse 105/114	kesrakkfqrqhmdsdsspsssstycnqmmrrrnmtq grckpvntfvheplvdvqnvcfqekvtckngqgncyk snssmhitdcrltngsrypncayrtspkerhiivace gspyvpvhfdasvedstlepkssdkthtcppcpapel lggssvflfppkpkdtlmisrtpevtcvvvdvshedp evkfnwyvdgvevhnaktkpreeqynstyrvvsvltv lhqdwlngkeykckvsnkalpasiektiskakgqpre pqvytlppsrdeltknqvsltclvkgfypsdiavewe sngqpennykttppvldsdgsfflyskltvdksrwqq gnvfscsvmhealhnhytqkslslspgkvdgasspvn vsspsvqdilkiaafniqtfgetkmsnatlvsyivqi lsrydialvqevrdshltavgklldnlnqdapdtyhy vvseplgrnsykerylfvyrpdqvsavdsyyyddgce pcrndtfnrepfivrffsrftevrefaivplhaapgd avaeidalydvyldvqekwgledvmlmgdfnagcsyv rpsqwssirlwtsptfqwlipdsadttatpthcaydr ivvagmllrgavvpdsalpfnfqaayglsdqlaqais dhypvevmlk
106	RSLV133: hRNAseWT-SCC-mthIgG1- P238S-P331S-NLG-hDNAse 105/114	aaagagagccgggccaagaagttccagcggcagcaca tggacagcgacagcagccccagcagctccagcaccta ctgcaaccagatgatgcggcggagaaacatgacccag ggccggtgcaagcccgtgaacaccttcgtgcacgagc

		ccctggtggacgtgcagaacgtgtgttttcaagaaaa agtgacctgcaagaacggccagggcaactgctacaag agcaacagcagcatgcacatcaccgactgccggctga ccaacggcagcagataccccaactgcgcctaccggac cagccccaaagaacggcacatcatcgtggcctgcgag ggcagcccttacgtgcccgtgcactttgacgccagcg tggaagatagcaccctggaacccaagagcagcgacaa gacccacacctgtccccccctgccctgcccctgagctg ctgggcggaagcagcgtgttcctgttcccccccaaagc ccaaggacaccctgatgatcagccggacccccgaagt gacctgcgtggtggtggatgtgtcccacgaggacccc gaagtgaagttcaattggtacgtggacggcgtggaag tgcacaacgccaagaccaagcccagagaggaacagta caacagcacctaccgggtggtgtccgtgctgaccgtg ctgcaccaggactggctgaacggcaaagagtacaagt gcaaggtctccaacaaggccctgcccgccagcatcga gaaaaccatcagcaaggccaagggccagcctcgcgag ccccaggtgtacacactgcccccccagccgggacgagc tgaccaagaaccaggtgtccctgacctgcctggtgaa aggcttctaccccagcgatatcgccgtggaatgggag agcaacggccagcccgagaacaactacaagaccaccc cccctgtgctggactccgacggctcattcttcctgta cagcaagctgaccgtggacaagagccggtggcagcag ggcaacgtgttcagctgcagcgtgatgcacgaggccc tgcacaaccactacacccagaagtccctgagcctgag ccccggcaaggtggacggcgccagctcccctgtgaac gtgtccagccccagcgtgcaggacatcctgaagatcg ccgccttcaacatccagaccttcggcgagacaaagat gagcaacgccaccctggtgtcctacatcgtgcagatc ctgagcagatacgatatcgccctggtgcaagaagtgc gggacagccacctgaccgccgtgggcaagctgctgga caacctgaaccaggacgcccccgacacctaccactac gtggtgtccgagcctctgggccggaacagctacaaag aaagatacctgttcgtgtaccggcccgatcaggtgtc cgccgtggacagctactactacgacgacggctgcgag ccctgccggaacgacaccttcaaccgcgagcccttca tcgtgcggttcttcagccggttcaccgaagtgcgcga gttcgccatcgtgcccctgcatgctgcccctggcgac gccgtggccgagatcgatgccctgtacgacgtgtacc tggatgtgcaagaaaagtggggcctggaagatgtgat gctgatgggcgacttcaacgccggctgcagctacgtg cggcccagccagtggtccagcatcagactgtggacct cccccaccttccagtggctgatccccgacagcgccga taccaccgccacccccacccactgtgcctacgacaga atcgtggtggccggcatgctgctgagaggcgccgtgg tgcctgacagcgccctgccattcaattttcaagccgc ctacggcctgagcgatcagctggcccaggccatcagc gaccactaccccgtggaagtgatgctgaagtga
107	RSLV-329 (RNase-linker-HSA-linker-DNase E13R/N74K/A114F/T205K)	METPAQLLFLLLLWLPDTTGKESRAKKFQRQHMDSDS SPSSSSTYCNQMMRRRNMTQGRCKPVNTFVHEPLVDV QNVCFQEKVTCKNGQGNCYKSNSSMHITDCRLTNGSR YPNCAYRTSPKERHIIVACEGSPYVPVHFDASVEDST GGGGSGGGGSGGGGSDAHKSEVAHRFKDLGEENFKAL VLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADESA ENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEP ERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEET

		FLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQ AADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFG ERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTE CCHGDLLECADDRADLAKYICENQDSISSKLKECCEK PLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKN YAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYET TLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNC ELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRN LGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEK TPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFN AETFTFHADICTLSEKERQIKKQTALVELVKHKPKAT KEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVA ASQAALGLVDGASSPVNVSSPSVQDILKIAAFNIQTF GRTKMSNATLVSYIVQILSRYDIALVQEVRDSHLTAV GKLLDNLNQDAPDTYHYVVSEPLGRKSYKERYLFVYR PDQVSAVDSYYYDDGCEPCGNDTFNREPFIVRFFSRF TEVREFAIVPLHAAPGDAVAEIDALYDVYLDVQEKWG LEDVMLMGDFNAGCSYVRPSQWSSIRLWTSPTFQWLI PDSADTTAKPTHCAYDRIVVAGMLLRGAVVPDSALPF NFQAAYGLSDQLAQAISDHYPVEVMLK
108	RSLV-329 (RNase-linker-HSA-linker-DNase E13R/N74K/A114F/ T205K; w/o leader)	KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRNMTQ GRCKPVNTFVHEPLVDVQNVCFQEKVTCKNGQGNCYK SNSSMHITDCRLTNGSRYPNCAYRTSPKERHIIVACE GSPYVPVHFDASVEDSTGGGGSGGGGSGGGGSDAHKS EVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKL VNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVAT LRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLV RPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPE LLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGK ASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYI CENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPAD LPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRH PDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFD EFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYT KKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCA EDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRP CFSALEVDETYVPKEFNAETFTFHADICTLSEKERQI KKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCK ADDKETCFAEEGKKLVAASQAALGLVDGASSPVNVSS PSVQDILKIAAFNIQTFGRTKMSNATLVSYIVQILSR YDIALVQEVRDSHLTAVGKLLDNLNQDAPDTYHYVVS EPLGRKSYKERYLFVYRPDQVSAVDSYYYDDGCEPCG NDTFNREPFIVRFFSRFTEVREFAIVPLHAAPGDAVA EIDALYDVYLDVQEKWGLEDVMLMGDFNAGCSYVRPS QWSSIRLWTSPTFQWLIPDSADTTAKPTHCAYDRIVV AGMLLRGAVVPDSALPFNFQAAYGLSDQLAQAISDHY PVEVMLK
109	RSLV-329 nucleic acid	ATGGAAACCCCTGCCCAGCTGCTGTTCCTGCTGCTGC TGTGGCTGCCCGACACCACCGGCAAAGAGTCCCGGGC CAAGAAGTTCCAGCGGCAGCACATGGACTCCGACTCC AGCCCCTCCAGCTCCTCCACCTACTGCAACCAGATGA TGCGGCGGAGAAACATGACCCAGGGCCGGTGCAAGCC CGTGAACACCTTCGTGCACGAGCCCCTGGTGGACGTG CAGAACGTGTGTTTTCAAGAAAAAGTCACCTGCAAGA ACGGCCAGGGCAACTGCTACAAGTCCAACTCCTCCAT GCACATCACCGACTGCCGGCTGACCAACGGCTCCAGA TACCCCAACTGCGCCTACCGGACCTCCCCCAAAGAAC

		GGCACATCATCGTGGCCTGCGAGGGCTCCCCTTACGT GCCCGTGCACTTCGACGCCTCCGTGGAAGATTCTACC GGCGGAGGCGGATCTGGAGGCGGAGGAAGTGGCGGGG GAGGCTCTGATGCCCACAAGTCTGAGGTGGCCCACCG GTTCAAGGACCTGGGCGAGGAAAACTTCAAGGCCCTG GTGCTGATCGCCTTCGCCCAGTACCTGCAGCAGTGCC CCTTCGAGGACCACGTGAAGCTGGTGAACGAAGTGAC CGAGTTCGCCAAGACCTGCGTGGCCGACGAGTCCGCC GAGAACTGCGACAAGAGCCTGCACACCCTGTTCGGCG ACAAGCTGTGCACCGTGGCCACCCTGCGGGAAACCTA CGGCGAGATGGCCGACTGCTGCGCCAAGCAGGAACCC GAGCGGAACGAGTGCTTCCTGCAGCACAAGGACGACA ACCCCAACCTGCCCCGGCTGGTCCGACCCGAGGTGGA CGTGATGTGCACCGCCTTCCACGACAACGAGGAAACC TTCCTGAAGAAGTACCTGTACGAGATCGCCAGACGGC ACCCCTACTTCTACGCCCCCGAGCTGCTGTTTTTCGC CAAGCGGTACAAGGCCGCCTTCACCGAGTGCTGCCAG GCCGCCGATAAGGCCGCCTGCCTGCTGCCTAAGCTGG ACGAGCTGAGGGACGAGGGCAAGGCCTCCTCTGCCAA GCAGCGGCTGAAGTGCGCCTCCCTGCAGAAGTTCGGC GAGCGGGCCTTTAAGGCCTGGGCCGTGGCTCGGCTGT CCCAGAGATTCCCCAAGGCCGAGTTTGCCGAGGTGTC CAAGCTGGTGACAGACCTGACCAAGGTGCACACCGAG TGTTGTCACGGCGACCTGCTGGAATGCGCCGACGACA GAGCCGACCTGGCCAAGTACATCTGCGAGAACCAGGA CTCCATCTCCTCCAAGCTGAAAGAGTGCTGCGAGAAG CCCCTGCTGGAAAAGTCCCACTGTATCGCCGAGGTGG AAAACGACGAGATGCCCGCCGACCTGCCTTCCCTGGC CGCCGACTTCGTGGAATCCAAGGACGTGTGCAAGAAC TACGCCGAGGCCAAGGATGTGTTCCTGGGCATGTTCC TGTACGAGTACGCTCGGCGGCACCCCGACTACTCCGT GGTGCTGCTGCTGAGACTGGCCAAGACCTACGAGACA ACCCTGGAAAAGTGCTGCGCCGCTGCCGACCCCCACG AGTGCTACGCCAAGGTGTTCGACGAGTTCAAGCCTCT GGTGGAAGAACCCCAGAACCTGATCAAGCAGAACTGC GAGCTGTTCGAGCAGCTGGGCGAGTACAAGTTCCAGA ACGCCCTGCTGGTCCGATACACCAAGAAAGTGCCCCA GGTGTCCACCCCCACCCTGGTGGAAGTGTCCCGGAAC CTGGGCAAAGTGGGCTCCAAGTGCTGCAAGCACCCTG AGGCCAAGCGGATGCCCTGCGCCGAGGACTACCTGAG CGTGGTGCTGAACCAGCTGTGCGTGCTGCACGAAAAG ACCCCCGTGTCCGACAGAGTGACCAAGTGCTGTACCG AGTCCCTGGTGAACAGACGGCCCTGCTTCTCCGCCCT GGAAGTGGACGAGACATACGTGCCCAAAGAGTTCAAC GCCGAGACATTCACCTTCCACGCCGACATCTGCACCC TGTCCGAGAAAGAGCGGCAGATCAAGAAACAGACCGC ACTGGTGGAACTGGTGAAACACAAGCCCAAGGCCACC AAAGAACAGCTGAAGGCCGTGATGGACGACTTCGCCG CCTTTGTGGAAAAGTGTTGCAAGGCCGACGACAAAGA GACATGCTTCGCCGAAGAGGGCAAGAAACTGGTGGCC GCCTCTCAGGCCGCCCTGGGACTGGTGGATGGCGCCT CCTCTCCCGTGAACGTGTCCAGCCCTTCCGTGCAGGA CATCCTGAAGATCGCCGCCTTCAACATCCAGACCTTC GGCCGGACCAAGATGTCCAACGCTACCCTGGTGTCCT ACATCGTGCAGATCCTGTCCAGATACGATATCGCCCT GGTGCAGGAAGTGCGGGACTCCCACCTGACCGCCGTG GGCAAGCTGCTGGACAACCTGAACCAGGACGCCCCCG ACACCTACCACTACGTGGTGTCTGAGCCCCTGGGCCG GAAGTCCTACAAAGAAAGATACCTGTTCGTGTACCGG CCCGACCAGGTGTCCGCCGTGGACTCCTACTACTACG ACGACGGCTGCGAGCCCTGCGGCAACGACACCTTCAA CCGCGAGCCCTTCATCGTGCGGTTCTTCAGCCGGTTC ACCGAAGTGCGCGAGTTTGCCATCGTGCCCCTGCACG CTGCTCCAGGCGACGCCGTGGCTGAGATCGACGCCCT

		GTACGACGTGTACCTGGATGTGCAGGAAAAGTGGGGC CTGGAAGATGTGATGCTGATGGGCGACTTCAACGCCG GCTGCTCCTACGTGCGGCCCTCCCAGTGGTCCTCCAT CCGGCTGTGGACCAGCCCCACCTTCCAGTGGCTGATC CCCGACTCCGCCGATACCACCGCCAAGCCCACCCACT GTGCCTACGACAGAATCGTGGTGGCCGGCATGCTGCT GAGGGGCGCTGTGGTGCCTGACTCCGCCCTGCCATTC AATTTTCAAGCCGCCTACGGCCTGTCCGACCAGCTGG CCCAGGCCATCTCCGACCACTACCCCGTGGAAGTGAT GCTGAAGTGACTCGAG

We claim:

1. A polypeptide comprising one or more nuclease domains with altered glycosylation operably coupled to a pharmacokinetic (PK) domain, or a variant or fragment thereof, wherein glycosylation of the PK moiety is optionally altered.

2. The polypeptide of claim 1, wherein the first nuclease domain is operably coupled to the N-terminus of the PK moiety, or variant or fragment thereof.

3. The polypeptide of claim 1, wherein the first nuclease domain is operably coupled to the C-terminus of the PK moiety, or variant or fragment thereof.

4. The polypeptide of claim 2 or 3, wherein the first nuclease domain is operably coupled to the PK moiety, or variant or fragment thereof, via a linker domain.

5. The polypeptide of any one of claims 2-4, wherein the first nuclease domain is an RNase.

6. The polypeptide of any one of claims 2-4, wherein the first nuclease domain is a DNase.

7. The polypeptide of claim 1, further comprising a second nuclease domain operably coupled to the PK moiety, or variant or fragment thereof.

8. The polypeptide of claim 7, wherein the first nuclease domain is operably coupled to the N-terminus of the PK moiety, or variant or fragment thereof, and the second nuclease domain is operably coupled to the C-terminus of the PK moiety, or variant or fragment thereof.

9. The polypeptide of claim 8, wherein the first and second nuclease domains are operably coupled to the N- and C-terminus, respectively, of the PK moiety, or variant or fragment thereof, via a linker.

10. The polypeptide of claim 7, wherein the first nuclease molecule is operably coupled to the second nuclease domain via a linker, and the second nuclease domain is operably coupled to the PK moiety, or variant or fragment thereof.

11. The polypeptide of claim 10, wherein the second nuclease domain is operably coupled to the N-terminus of the PK moiety, or variant or fragment thereof.

12. The polypeptide of claim 10, wherein the second nuclease domain is operably coupled to the C-terminus of the PK moiety, or variant or fragment thereof.

13. The polypeptide of any one of claims 7-9, wherein the first and second nuclease domains each comprise an RNase.

14. The polypeptide of any one of claims 7-12, wherein the first nuclease domain comprises a DNase and the second nuclease domain comprises an RNase.

15. The polypeptide of any one of claims 7-12, wherein the first nuclease domain comprises an RNase and the second nuclease domain comprises a DNase.

16. The polypeptide of any one of the preceding claims, wherein the polypeptide is aglycosylated.

17. The polypeptide of any one of claims 1-5 and 7-16, wherein the RNase is a wild type human RNase, such as a human pancreatic RNase1 (SEQ ID NO: 1), or a mutant RNase, such as an aglycosylated, underglycosylated, or deglycosylated RNase1, such as human RNase1 N34S/N76S/N88S (SEQ ID NO: 2).

18. The polypeptide of claim 17, wherein the RNase is wild type human RNase1 (SEQ ID NO: 1).

19. The polypeptide of claim 17, wherein the RNase is human RNase1 N34S/N76S/N88S (SEQ ID NO: 2).

20. The polypeptide of claim 17, which degrades circulating RNA and RNA in immune complexes, or inhibits interferon-α production, or both.

21. The polypeptide of claim 20, wherein the activity of the RNase is not less than about 2- to 10-fold less than the activity of a control RNase molecule.

22. The polypeptide of claim 21, wherein the activity of the RNase is about equal to the activity of a control RNase molecule.

23. The polypeptide of any one of claims 1-4, 12, and 14-16, wherein the DNase is a wild type human DNase, such as a human pancreatic DNase1 (SEQ ID NO: 3), or a mutant DNase, such as human DNase1 A114F (SEQ ID NO: 4) or an aglycosylated, underglycosylated, or deglycosylated human DNase, such as mutant, human DNase1 N18S/N106S/A114F (SEQ ID NO: 5).

24. The polypeptide of claim 23, wherein the activity of the DNase is not less than about 2- to 10-fold less than the activity of a control DNase molecule.

25. The polypeptide of claim 24, wherein the activity of the DNase is about equal to the activity of a control DNase molecule.

26. The polypeptide of any one of claims 4-6 or 9-25, wherein the linker domain is a polypeptide linker, such as a gly-ser linker.

27. The polypeptide of any of the preceding claims, wherein the PK moiety, or variant or fragment thereof, increases the serum half-life and/or activity of the polypeptide relative to a polypeptide that does not contain the PK moiety, or variant or fragment thereof.

28. The polypeptide of any of the preceding claims, wherein the PK moiety is selected from the group consisting of: albumin, transferrin, Fc, and PEG.

29. The polypeptide of claim 28, wherein the PK moiety is albumin, or a variant or fragment thereof.

30. The polypeptide of claim 29, wherein the albumin is human serum albumin (SEQ ID NO: 6), or a variant or fragment thereof.

31. The polypeptide of claim 28, wherein the PK moiety is a transferrin, or a variant or fragment thereof.

32. The polypeptide of claim 31, wherein the PK moiety is human serum transferrin (SEQ ID NO: 7).

33. The polypeptide of claim 32, wherein the human serum transferrin has amino acid substitutions at position 32 and/or 413 and/or 611.

34. The polypeptide of claim 27, wherein the PK moiety is an Fc, or a variant or fragment thereof.

35. The polypeptide of claim 34, wherein the Fc is a human wild-type IgG1 Fc domain (SEQ ID NO: 10).

36. The polypeptide of claim 35, wherein the human wild-type IgG1 Fc domain has an amino acid substitution at position 83 (SEQ ID NO: 11).

37. The polypeptide of claim 28, wherein the PK moiety is PEG.

38. A polypeptide comprising an amino acid sequence set forth in SEQ ID NOs: 14-21, 47-54 and 75-82, or a polypeptide comprising an amino acid sequence at least 90% identical to the amino acid sequence set forth in SEQ ID NOs: 14-21 and 47-54 and 75-82.

39. A composition comprising the polypeptide of any of the preceding claims and a carrier.

40. A nucleic acid molecule encoding the polypeptide according to claim 1.

41. A recombinant expression vector comprising a nucleic acid molecule according to claim 40.

42. A host cell transformed with the recombinant expression vector according to claim 41.

43. A method of making the polypeptide of any one of claims 1-38, comprising: providing a host cell comprising a nucleic acid sequence that encodes the polypeptide; and maintaining the host cell under conditions in which the polypeptide is expressed.

44. A method for treating or preventing a condition associated with an abnormal immune response, comprising administering to a subject an effective amount of a polypeptide of any one of claims 1-38.

45. The method of claim 44, wherein the condition is an autoimmune disease.

46. The method of claim 45, wherein the autoimmune disease is selected from the group consisting of insulin-dependent diabetes mellitus, multiple sclerosis, experimental autoimmune encephalomyelitis, rheumatoid arthritis, experimental autoimmune arthritis, myasthenia gravis, thyroiditis, an experimental form of uveoretinitis, Hashimoto's thyroiditis, primary myxoedema, thyrotoxicosis, pernicious anaemia, autoimmune atrophic gastritis, Addison's disease, premature menopause, male infertility, juvenile diabetes, Goodpasture's syndrome, pemphigus vulgaris, pemphigoid, sympathetic ophthalmia, phacogenic uveitis, autoimmune haemolytic anaemia, idiopathic leucopenia, primary biliary cirrhosis, active chronic hepatitis Hbs-ve, cryptogenic cirrhosis, ulcerative colitis, Sjogren's syndrome, scleroderma, Wegener's granulomatosis, polymyositis, dermatomyositis, discoid LE, systemic lupus erythematosus (SLE), and connective tissue disease.

47. The method of claim 46, wherein the autoimmune disease is SLE.

48. The method of claim 46, wherein the autoimmune disease is Sjogren's syndrome

49. A method of treating SLE comprising administering to a subject a hybrid nuclease-PK molecule containing composition in an amount effective to degrade immune complexes containing RNA, DNA or both RNA and DNA, wherein the composition comprises a pharmaceutically acceptable carrier and a polypeptide of any one of claims 1-38.

50. A method of treating Sjogren's syndrome comprising administering to a subject a hybrid nuclease-PK molecule containing composition in an amount effective to degrade immune complexes containing RNA, DNA or both RNA and DNA, wherein the composition comprises a pharmaceutically acceptable carrier and a polypeptide of any one of claims 1-38.

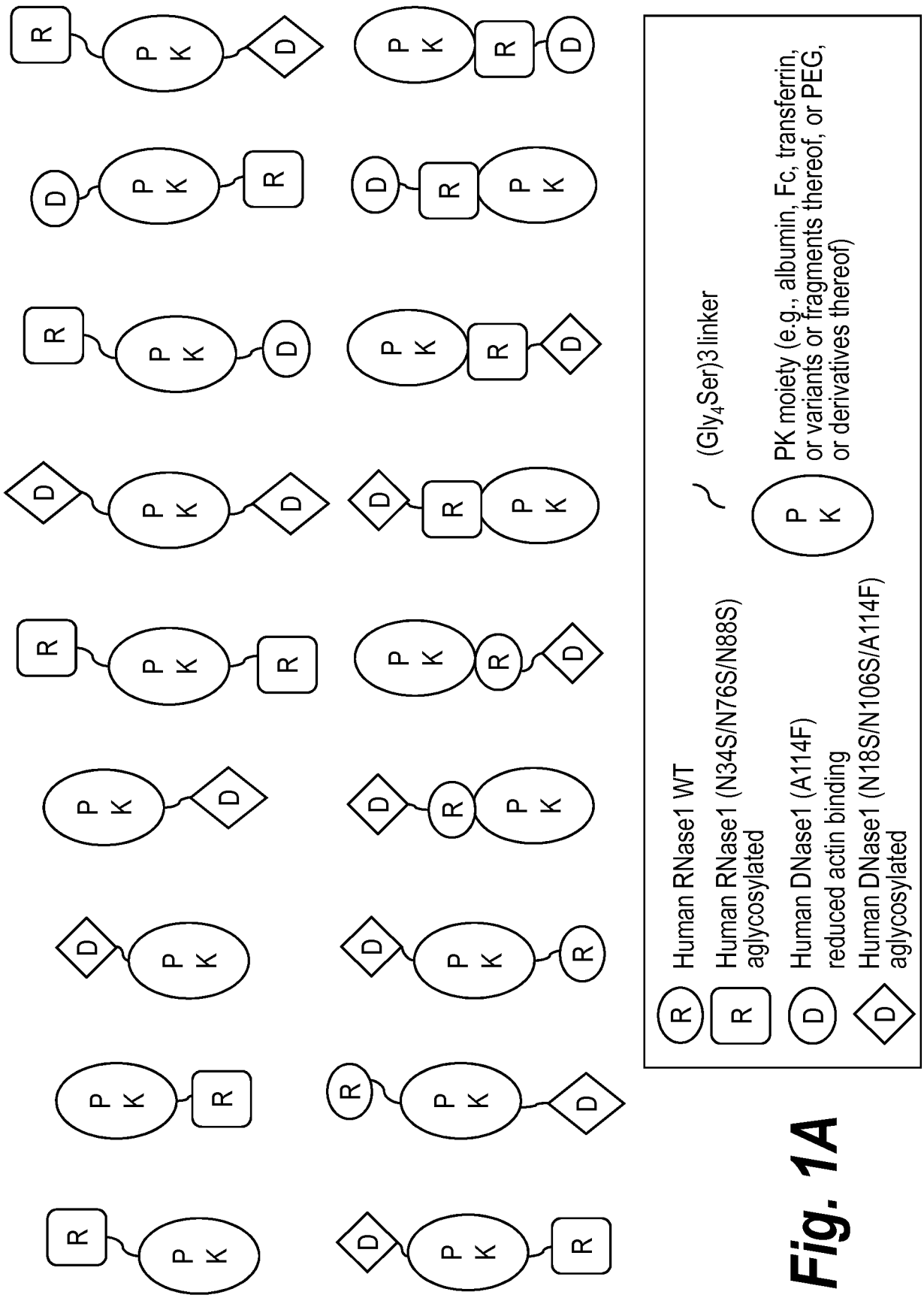

R
D
P K
Human RNase1 WT
Human RNase1 (N34S/N76S/N88S) aglycosylated
Human DNase1 (A114F) reduced actin binding
Human DNase1 (N18S/N106S/A114F) aglycosylated
(Gly4Ser)3 linker
PK moiety (e.g., albumin, Fc, transferrin, or variants or fragments thereof, or PEG, or derivatives thereof)

Fig. 1A

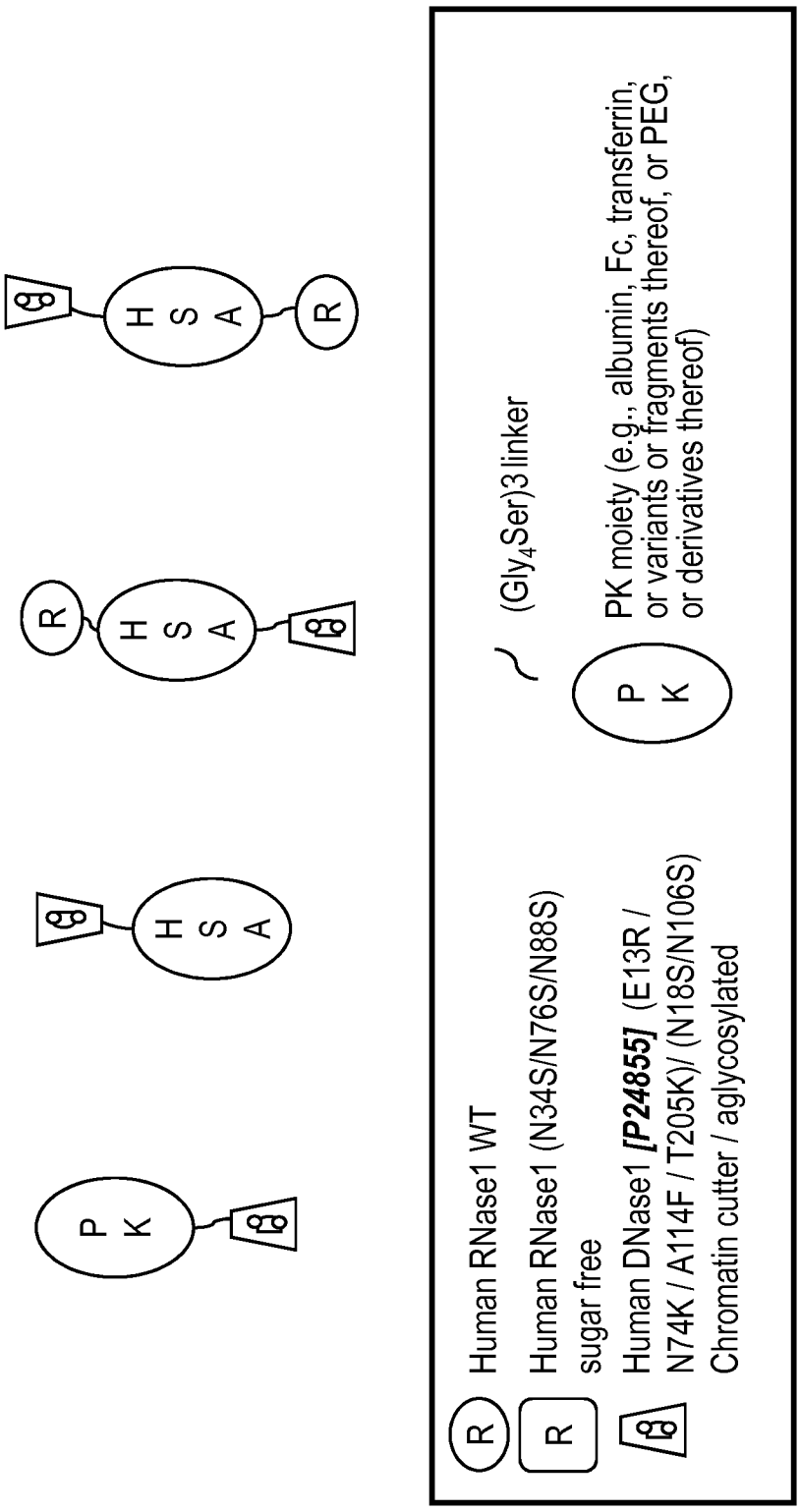

P K
H S A
R
H S A
R
H S A
R
Human RNase1 WT
Human RNase1 (N34S/N76S/N88S) sugar free
Human DNase1 [P24855] (E13R / N74K / A114F / T205K)/ (N18S/N106S) Chromatin cutter / aglycosylated
(Gly4Ser)3 linker
P K
PK moiety (e.g., albumin, Fc, transferrin, or variants or fragments thereof, or PEG, or derivatives thereof)

Fig. 1B

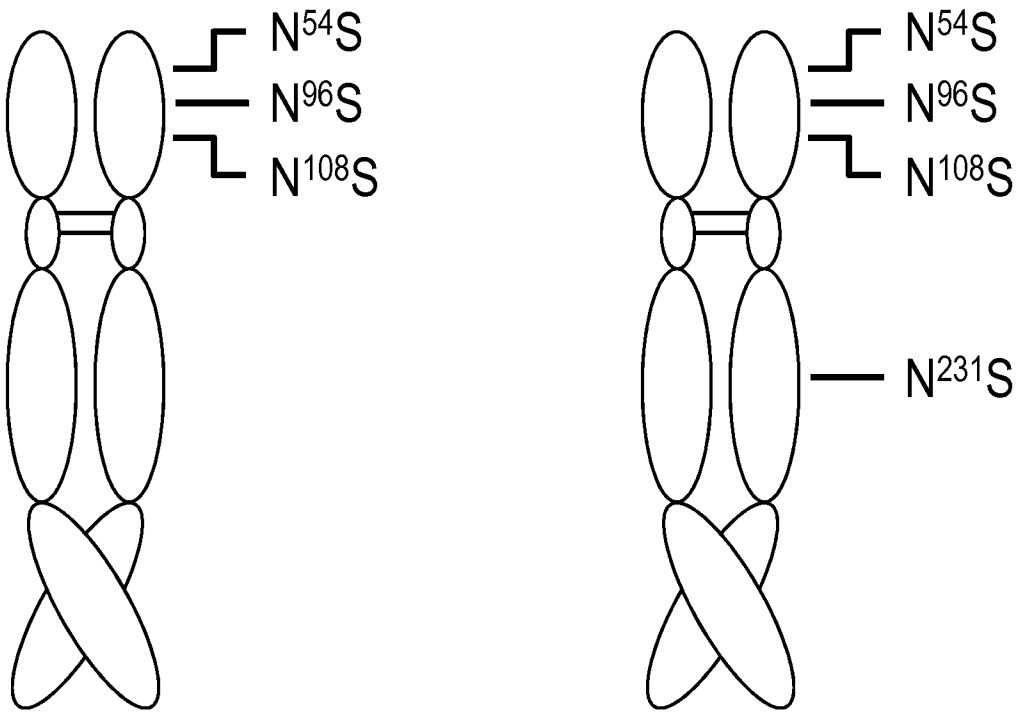
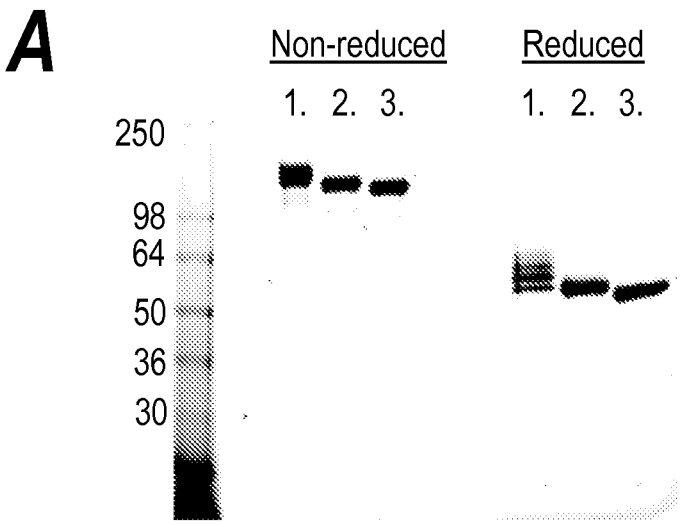

N54S
N96S
N108S
N54S
N96S
N108S
N231S
AG1-RSLV-132
AG2-RSLV-132

Fig. 2


A
Non-reduced
Reduced
1. 2. 3.
1. 2. 3.
250
98
64
50
36
30

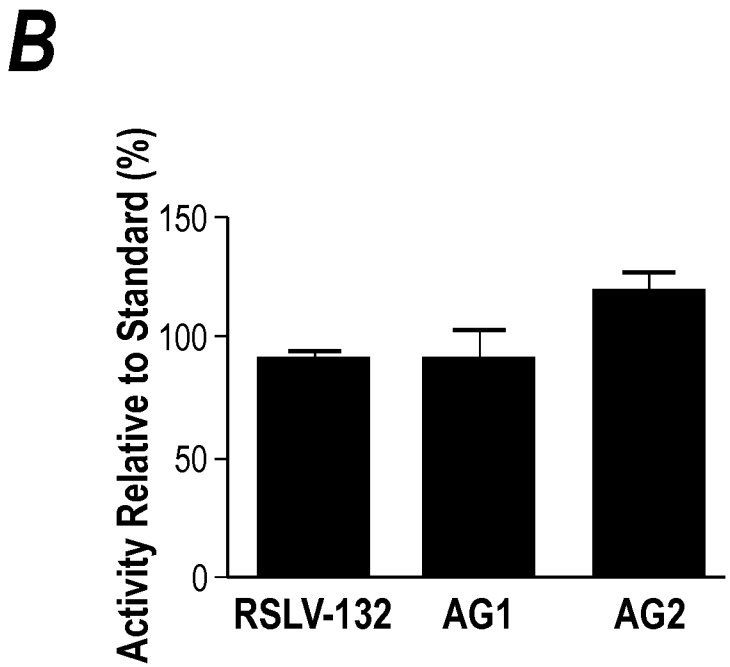

B
Activity Relative to Standard (%)
150
100
50
0
RSLV-132
AG1
AG2

Fig. 3

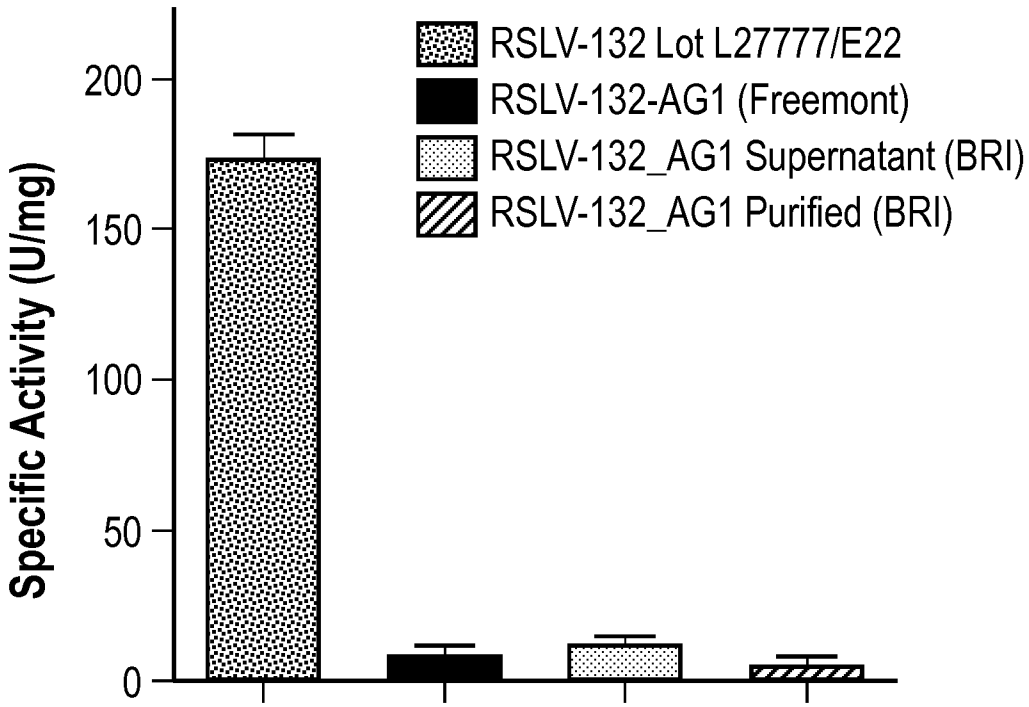

RSLV-132 Lot L27777/E22
RSLV-132-AG1 (Freemont)
RSLV-132_AG1 Supernatant (BRI)
RSLV-132_AG1 Purified (BRI)
Specific Activity (U/mg)
200
150
100
50
0

Fig. 4

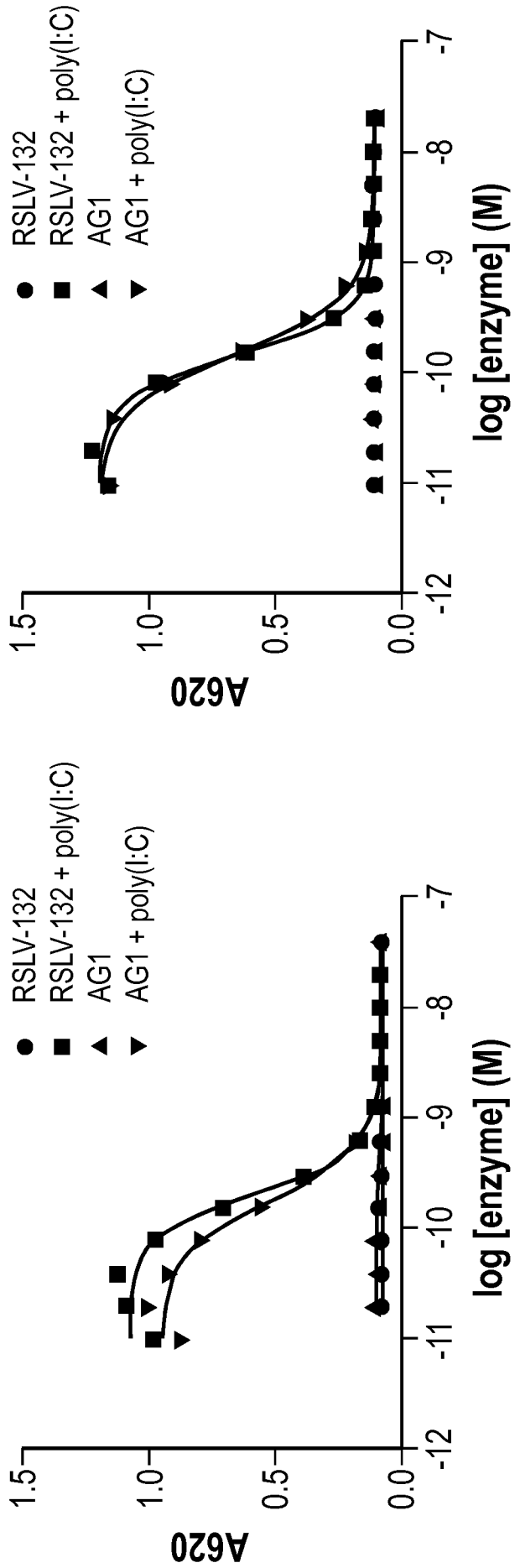

RSLV-132
RSLV-132 + poly(I:C)
AG1
AG1 + poly(I:C)
A620
log [enzyme] (M)
1.5
1.0
0.5
0.0
-12
-11
-10
-9
-8
-7

Fig. 5

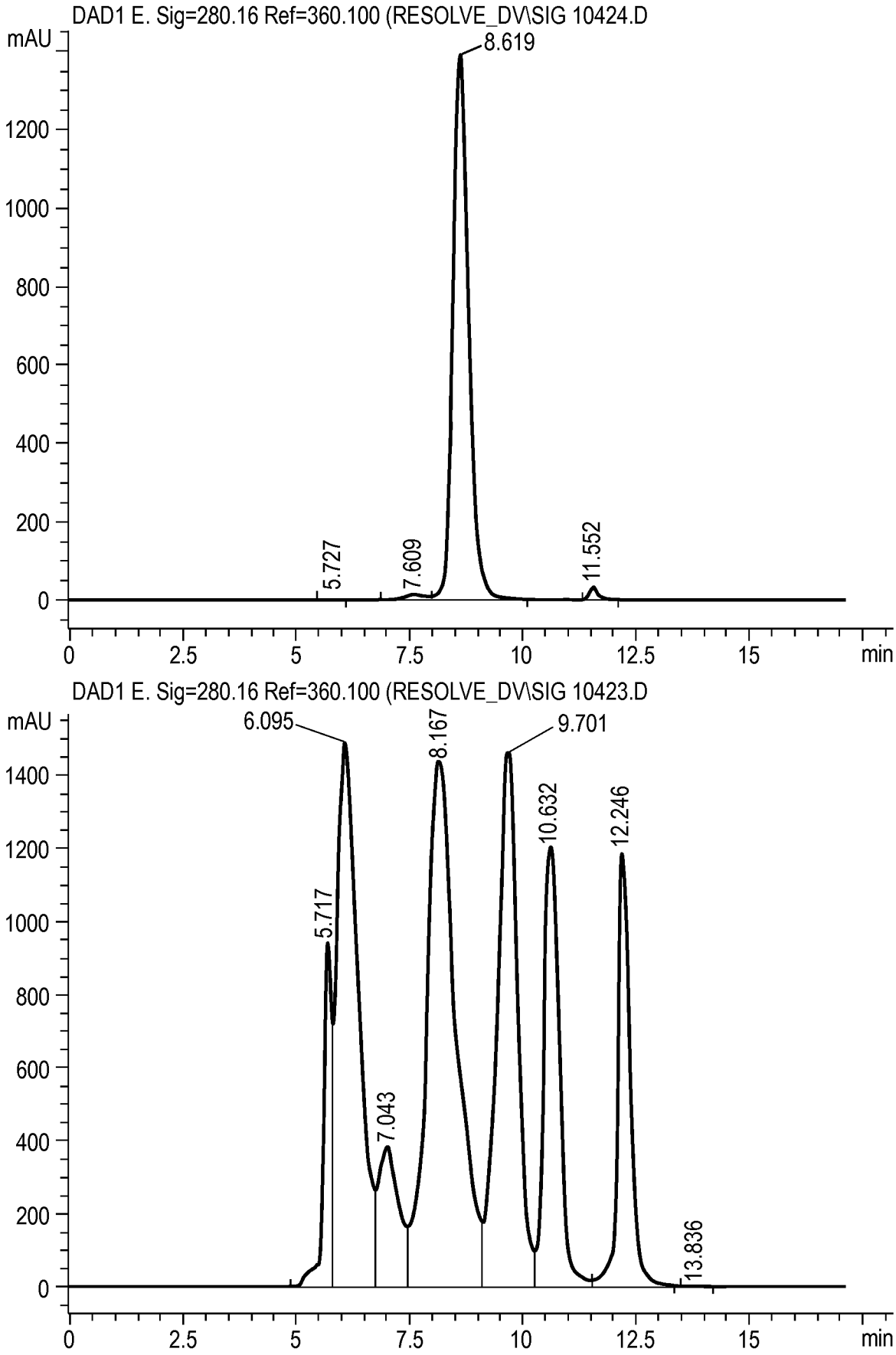
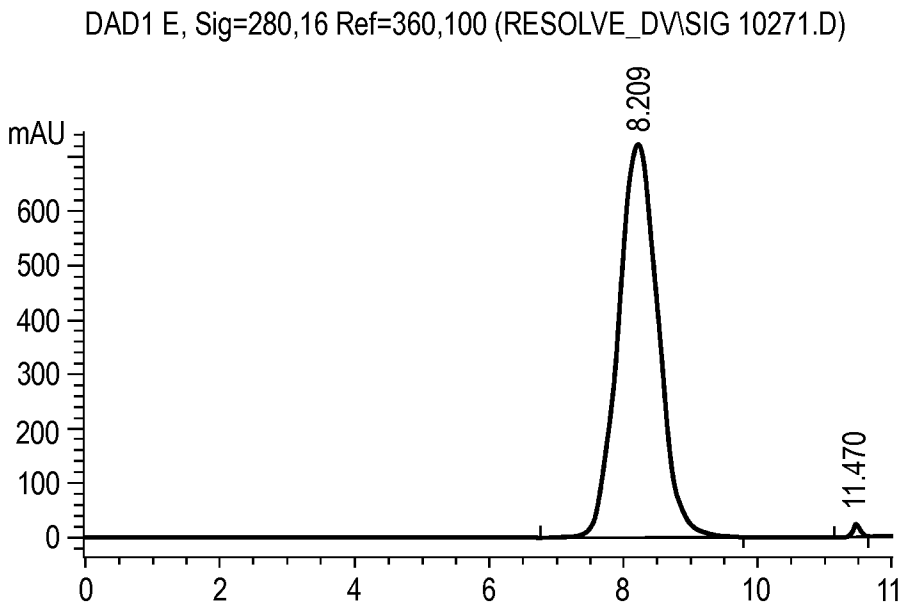

DAD1 E. Sig=280.16 Ref=360.100 (RESOLVE_DV\SIG 10424.D
mAU
8.619
5.727
7.609
11.552
min
DAD1 E. Sig=280.16 Ref=360.100 (RESOLVE_DV\SIG 10423.D
mAU
6.095
8.167
9.701
10.632
12.246
5.717
7.043
13.836
min

Fig. 6A

Pilot 2
500ug load



AG1
750ug load

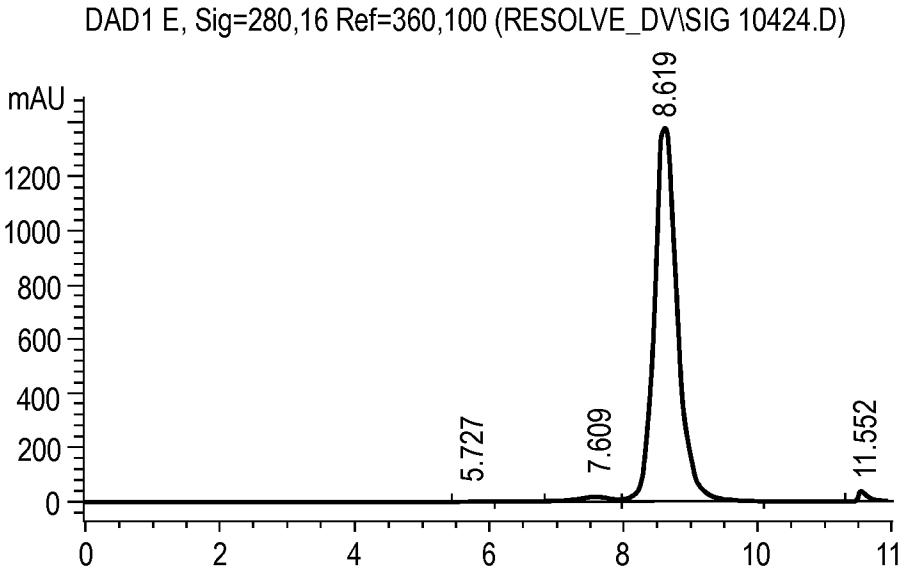


Fig. 6B

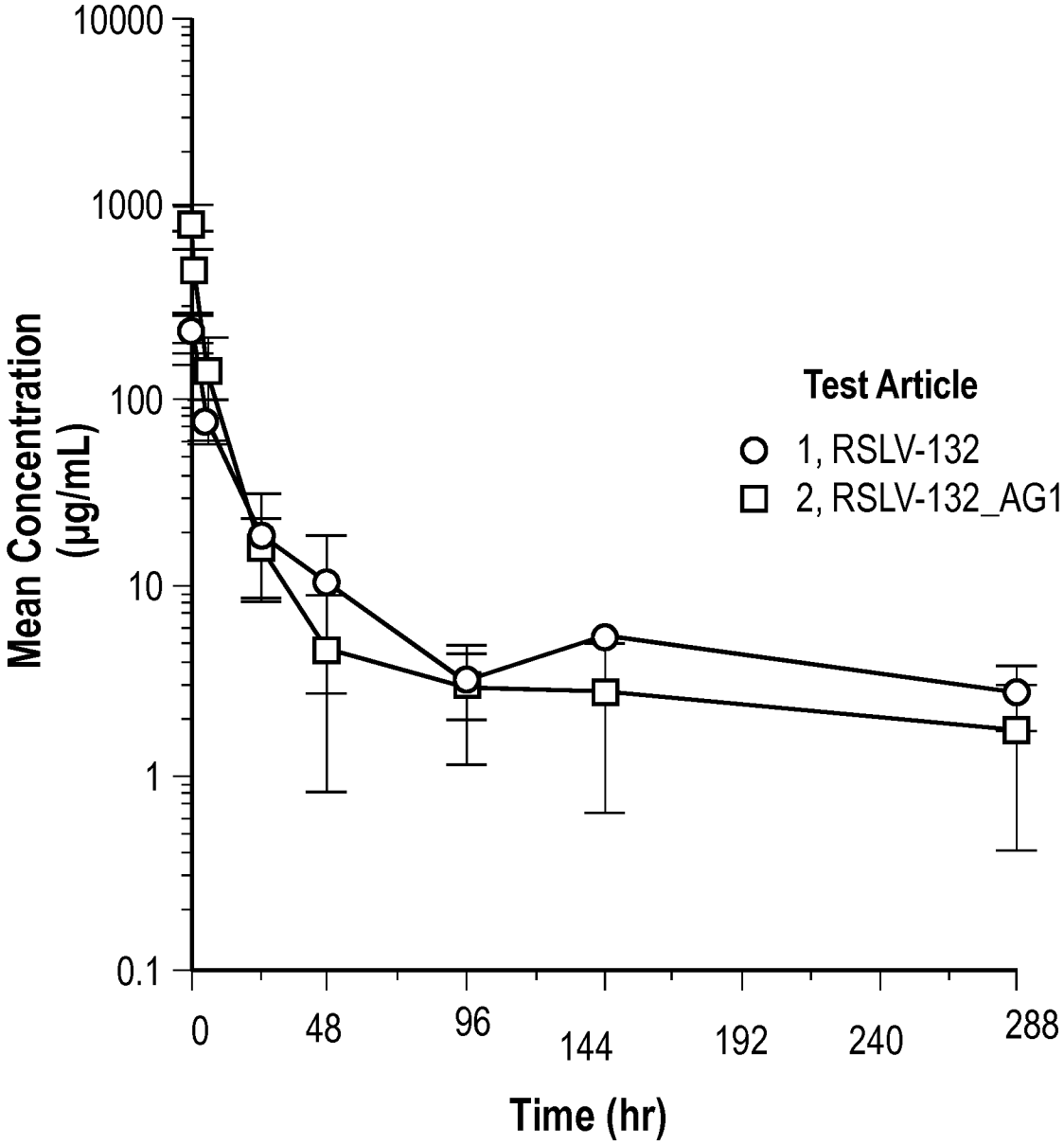

10000
1000
100
10
1
0.1
Mean Concentration (µg/mL)
0
48
96
144
192
240
288
Time (hr)
Test Article
1, RSLV-132
2, RSLV-132_AG1

Fig. 7

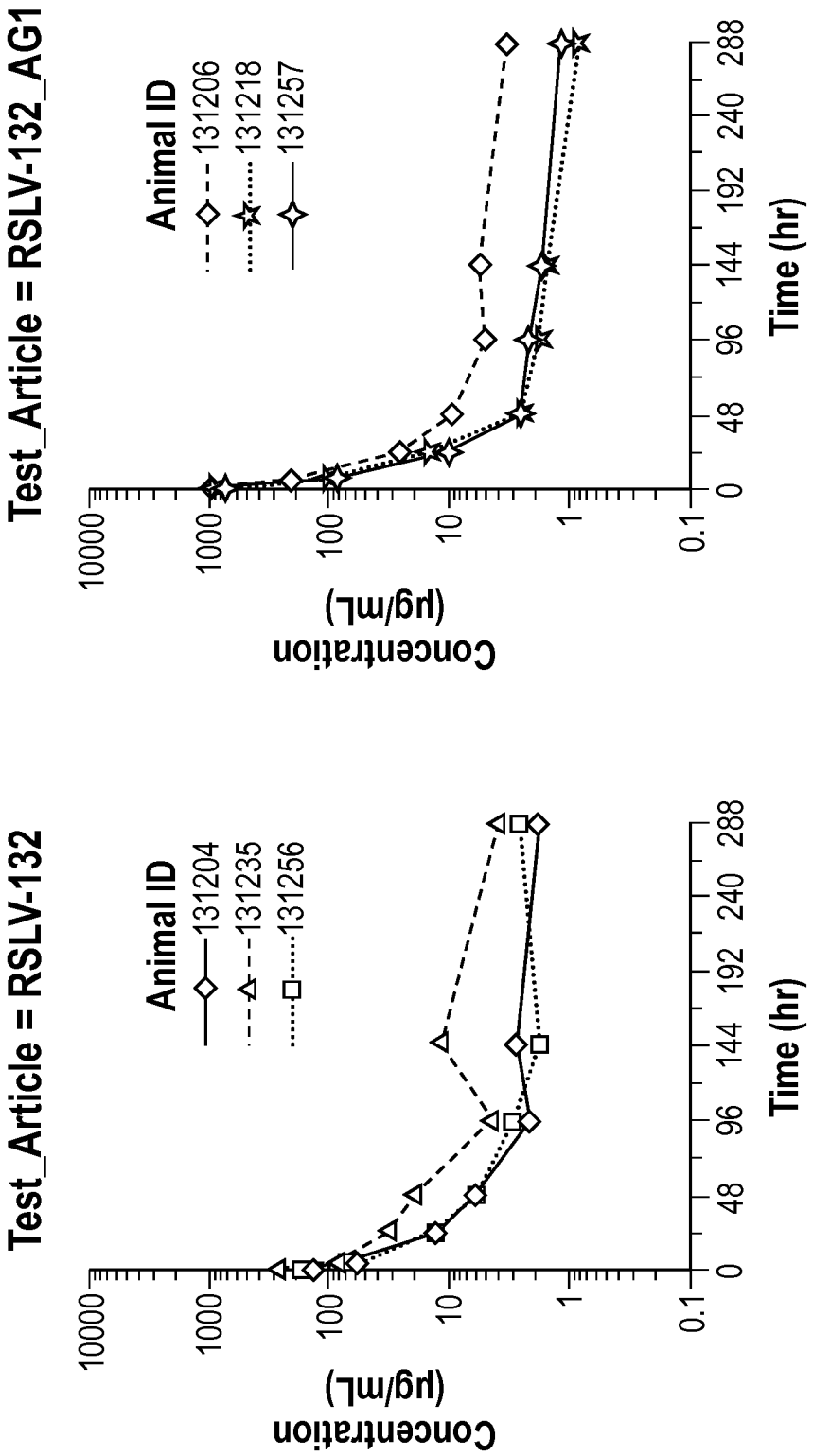

Test_Article = RSLV-132_AG1
Animal ID
131206
131218
131257
Concentration (µg/mL)
10000
1000
100
10
1
0.1
0
48
96
144
192
240
288
Time (hr)
Test_Article = RSLV-132
Animal ID
131204
131235
131256
Concentration (µg/mL)
Time (hr)

Fig. 8

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/063587

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N9/22 A61K38/46
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO 2012/149440 A2 (UNIV WASHINGTON [US]; LEDBETTER JEFFREY A [US]; HAYDEN-LEDBETTER MARTH) 1 November 2012 (2012-11-01) cited in the application paragraphs [0100], [0122], [0153], [0162], [0174], [0178], [0180] - [0184]; claims 8,13,15,34 -----	1-18, 20-28, 34,35, 39-50 31-33, 36,38
X Y	WO 90/07572 A1 (GENENTECH INC [US]) 12 July 1990 (1990-07-12) page 5, line 47 - page 6, line 13 page 8, line 32 page 10, line 30 - line 41 page 11, line 26 - page 12, line 29; claim 17 -----	1-6,16, 23-25, 27,28, 37,39-43 19, 31-33,38

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 February 2015	27/02/2015

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	van Heusden, Miranda

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96/26279 A1 (GENENTECH INC [US]) 29 August 1996 (1996-08-29)	1-3,6, 16, 23-25, 27-30, 37,39-49
Y	page 7, line 18 - line 37 page 8, line 8 - line 10 page 11, line 6 - line 10 page 13, line 29 - line 36 page 14, line 24 - line 28; example 1 -----	19, 31-33,38
X	C.-H. CHANG ET AL: "Ranpirnase (Frog RNase) Targeted with a Humanized, Internalizing, Anti-Trop-2 Antibody Has Potent Cytotoxicity against Diverse Epithelial Cancer Cells", MOLECULAR CANCER THERAPEUTICS, vol. 9, no. 8, 1 July 2010 (2010-07-01), pages 2276-2286, XP055057463, ISSN: 1535-7163, DOI: 10.1158/1535-7163.MCT-10-0338 abstract page 2277, column 2, paragraph 2 page 2278, column 1, paragraph 2 - paragraph 3; figures 1,3 page 2282, column 2, paragraph 2 -----	1,2,4,5, 16,17, 20-22, 26,27, 39-43
X	KRAUSS J ET AL: "Efficient killing of CD22<+> tumor cells by a humanized diabody-RNase fusion protein", BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, ACADEMIC PRESS INC. ORLANDO, FL, US, vol. 331, no. 2, 3 June 2005 (2005-06-03), pages 595-602, XP004867598, ISSN: 0006-291X, DOI: 10.1016/J.BBRC.2005.03.215 abstract page 596, column 2, paragraph 1-3; figures 3,4 page 598, column 1, paragraph 4 -----	1,2,4,5, 7,13,16, 20-22, 26,27, 39-43
X	US 2005/261232 A1 (STRONG LAURA E [US] ET AL) 24 November 2005 (2005-11-24) paragraphs [0086], [0101], [0131]; table 1 ----- -/--	1,2,4,5, 16,17, 20,26, 27,39-43

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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