and the first nuclease domain is operatively coupled to the Fc domain by the first linker domain.

[0007] In some embodiments, a hybrid nuclease molecule is a polypeptide, wherein the amino acid sequence of the first nuclease domain comprises a human, wild-type RNase amino acid sequence, wherein the first linker domain is (Gly4Ser)n (SEQ ID NO: 210)n, where n is 0, 1, 2, 3, 4 or 5, wherein the amino acid sequence of the Fc domain comprises a human, wild-type IgG1 Fc domain amino acid sequence, and wherein the first linker domain is coupled to the C-terminus of the first nuclease domain and the N-terminus of the Fc domain. In some embodiments, a hybrid nuclease molecule is a polypeptide comprising or consisting of a sequence shown in Table 2. In some embodiments, a hybrid nuclease molecule is a polypeptide comprising SEQ ID NO:149. In some embodiments, a hybrid nuclease molecule is a polypeptide comprising SEQ ID NO:145. In some embodiments, a hybrid nuclease molecule is a polypeptide comprising SEQ ID NO:161. In some embodiments, a hybrid nuclease molecule is a polypeptide comprising SEQ ID NO:162. In some embodiments, a hybrid nuclease molecule is a polypeptide comprising SEQ ID NO:163.

[0008] In some embodiments, a hybrid nuclease molecule comprises wild-type, human DNase1 linked to wild-type, human IgG1. In some embodiments, a hybrid nuclease molecule comprises human DNase1 G105R A114F linked to a wild-type, human IgG1 Fc domain by a (gly4ser)n linker domain (SEQ ID NO: 210)n where n=0, 1, 2, 3, 4, or 5. In some embodiments, a hybrid nuclease molecule comprises wild-type, human RNase1 linked to wild-type, human IgG1 linked to wild-type, human DNase1. In some embodiments, a hybrid nuclease molecule comprises wild-type, human RNase1 linked to wild-type, human IgG1 linked to human DNase1 G105R A114F. In some embodiments, a hybrid nuclease molecule is a polypeptide, wherein the amino acid sequence of the first nuclease domain comprises a RNase amino acid sequence, wherein the first linker domain is between 5 and 32 amino acids in length, wherein the amino acid sequence of the Fc domain comprises a human, Fc domain amino acid sequence, and wherein the first linker domain is coupled to the C-terminus of the first nuclease domain and the N-terminus of the Fc domain. In some embodiments, the linker domain includes (gly4ser)5 (SEQ ID NO: 137) and restriction sites BglII, AgeI, and XhoI. In some embodiments, a hybrid nuclease molecule is a polypeptide, wherein the amino acid sequence of the first nuclease domain comprises a human RNase amino acid sequence, wherein the first linker domain is a NLG peptide between 5 and 32 amino acids in length, wherein the amino acid sequence of the Fc domain comprises a human, wild-type Fc domain amino acid

to about 32 amino acids. In some embodiments, the first linker domain has a length of about 20 amino acids. In some embodiments, the first linker domain has a length of about 25 amino acids. In some embodiments, the first linker domain has a length of about 18 amino acids. In some embodiments, the first linker domain comprises a gly/ser peptide. In some embodiments, the gly/ser peptide is of the formula $(Gly_4Ser)n$ (SEQ ID NO: 210)n, wherein n is a positive integer selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 (SEQ ID NO: 208 if n = 10; SEQ ID NO: 211 if n = 6). In some embodiments, the gly/ser peptide includes $(Gly_4Ser)3$ (SEQ ID NO: 209). In some embodiments, the gly/ser peptide includes $(Gly_4Ser)4$ (SEQ ID NO: 136). In some embodiments, the gly/ser peptide includes $(Gly_4Ser)5$ (SEQ ID NO: 137). In some embodiments, the first linker domain includes at least one restriction site. In some embodiments, the first linker domain includes about 12 or greater nucleotides including at least one restriction site. In some embodiments, the first linker domain includes two or more restriction sites. In some embodiments, the first linker domain includes a plurality of restriction sites. In some embodiments, the first linker domain comprises an NLG peptide. In some embodiments, the first linker domain comprises an N-linked glycosylation site.

[0016] In some embodiments, the first nuclease domain is linked to the N-terminus of the Fc domain. In some embodiments, the first nuclease domain is linked to the C-terminus of the Fc domain.

[0017] In some embodiments, the hybrid nuclease molecule further includes a second nuclease domain. In some embodiments, the first and second nuclease domains are distinct nuclease domains. In some embodiments, the first and second nuclease domains are the same nuclease domains. In some embodiments, the second nuclease domain is linked to the C-terminus of the Fc domain. In some embodiments, the second nuclease domain is linked to the N-terminus of the Fc domain. In some embodiments, the second nuclease domain is linked to the C-terminus of the first nuclease domain. In some embodiments, the second nuclease domain is linked to the N-terminus of the first nuclease domain.

[0018] Also disclosed herein is a dimeric polypeptide comprising a first polypeptide and a second polypeptide, wherein the first polypeptide comprises a first nuclease domain, and an Fc domain, wherein the first nuclease domain is operatively coupled to the Fc domain. In some embodiments, the second polypeptide is a second hybrid nuclease molecule comprising a second nuclease domain, and a second Fc domain, wherein the second nuclease domain is operatively coupled to the second Fc domain.

[0019] Also disclosed herein is a pharmaceutical composition comprising at least one hybrid nuclease molecule and/or at least one dimeric polypeptide as described herein, and a pharmaceutically acceptable excipient.

[0020] Also disclosed herein is a nucleic acid molecule encoding a hybrid nuclease molecule disclosed herein. Also disclosed herein is a recombinant expression vector comprising a nucleic acid molecule disclosed herein. Also disclosed herein is a host cell transformed with a recombinant expression vector disclosed herein.

[0021] Also disclosed herein is a method of making a hybrid nuclease disclosed herein, comprising: providing a host cell comprising a nucleic acid sequence that encodes the hybrid nuclease molecule; and maintaining the host cell under conditions in which the hybrid nuclease molecule is expressed.

[0022] Also disclosed herein is a method for treating or preventing a condition associated with an abnormal immune response, comprising administering to a patient in need thereof an effective amount of an isolated hybrid nuclease molecule 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.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0023] These and other features, aspects, and advantages of the present invention will become better understood with regard to the following description, and accompanying drawings, where:

[0024] Figure (FIG.) 1 shows the nucleotide (SEQ ID NO: 213) and amino acid sequence (SEQ ID NO: 214) of the mRNase-mIgG2a with mutations at P238S, K322S, and P331S. This sequence is listed in the sequence listing as huVK3LP+mrib1+mIgG2A-C-2S (SEQ ID NO:114).

[0056] In certain aspects, the hybrid nuclease molecules 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 an Fc domain. Preferably, such polypeptide linkers can provide flexibility to the polypeptide molecule. In certain embodiments the polypeptide linker is used to connect (e.g., genetically fuse) one or more Fc domains and/or one or more nuclease domains. A hybrid nuclease molecule of the invention may comprise more than one linker domain or peptide linker.

[0057] 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 one embodiment, n=1. In one embodiment, n=2. In another embodiment, n=3, i.e., $(Gly_4Ser)3$. In another embodiment, n=4, i.e., $(Gly_4Ser)4$. In another embodiment, n=5. In yet another embodiment, n=6. In another embodiment, n=7. In yet another embodiment, n=8. In another embodiment, n=9. In yet another embodiment, n=10. Another exemplary gly/ser polypeptide linker comprises the amino acid sequence $(Gly_4Ser)n$. In one embodiment, n=1. In one embodiment, n=2. In a preferred embodiment, n=3. In another embodiment, n=4. In another embodiment, n=5. In yet another embodiment, n=6.

[0058] As used herein, the terms "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.

[0059] As used herein, the term "Fc region" shall be defined as the portion of a native immunoglobulin formed by the respective Fc domains (or Fc moieties) of its two heavy chains.

[0060] As used herein, the term "Fc domain" refers to a portion of a single immunoglobulin (Ig) heavy chain. As such, Fc domain can also be referred to as "Ig" or "IgG." 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

upper hinge domain (e.g., an IgG1, an IgG2, an IgG3, or IgG4 upper hinge domain). In another embodiment, a polypeptide linker comprises a middle hinge domain (e.g., an IgG1, an IgG2, an IgG3, or an IgG4 middle hinge domain). In another embodiment, a polypeptide linker comprises a lower hinge domain (e.g., an IgG1, an IgG2, an IgG3, or an IgG4 lower hinge domain).

[00138] In other embodiments, polypeptide linkers can be constructed which combine hinge elements derived from the same or different antibody isotypes. In one embodiment, the polypeptide linker comprises a chimeric hinge comprising at least a portion of an IgG1 hinge region and at least a portion of an IgG2 hinge region. In one embodiment, the polypeptide linker comprises a chimeric hinge comprising at least a portion of an IgG1 hinge region and at least a portion of an IgG3 hinge region. In another embodiment, a polypeptide linker comprises a chimeric hinge comprising at least a portion of an IgG1 hinge region and at least a portion of an IgG4 hinge region. In one embodiment, the polypeptide linker comprises a chimeric hinge comprising at least a portion of an IgG2 hinge region and at least a portion of an IgG3 hinge region. In one embodiment, the polypeptide linker comprises a chimeric hinge comprising at least a portion of an IgG2 hinge region and at least a portion of an IgG4 hinge region. In one embodiment, the polypeptide linker comprises a chimeric hinge comprising at least a portion of an IgG1 hinge region, at least a portion of an IgG2 hinge region, and at least a portion of an IgG4 hinge region. In another embodiment, a polypeptide linker can comprise an IgG1 upper and middle hinge and a single IgG3 middle hinge repeat motif. In another embodiment, a polypeptide linker can comprise an IgG4 upper hinge, an IgG1 middle hinge and a IgG2 lower hinge.

[00139] 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 $(Gly_4Ser)n$, (SEQ ID NO: 210)n wherein n is a positive integer (e.g., 1, 2, 3, 4, or 5). A preferred gly/ser linker is $(Gly_4Ser)4$ (SEQ ID NO: 136). Another preferred gly/ser linker is $(Gly_4Ser)3$ (SEQ ID NO: 209). Another preferred gly/ser linker is $(Gly_4Ser)5$ (SEQ ID NO: 137). 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 one embodiment, a polypeptide linker of the

invention comprises at least a portion of an upper hinge region (e.g., derived from an IgG1, IgG2, IgG3, or IgG4 molecule), at least a portion of a middle hinge region (e.g., derived from an IgG1, IgG2, IgG3, or IgG4 molecule) and a series of gly/ser amino acid residues (e.g., a gly/ser linker such as $(Gly_4Ser)n$ (SEQ ID NO: 210)n).

[00140] In one embodiment, a polypeptide linker of the invention comprises a non-naturally occurring immunoglobulin hinge region domain, e.g., a hinge region domain that is not naturally found in the polypeptide comprising the hinge region domain and/or a hinge region domain that has been altered so that it differs in amino acid sequence from a naturally occurring immunoglobulin hinge region domain. In one embodiment, mutations can be made to hinge region domains to make a polypeptide linker of the invention. In one embodiment, a polypeptide linker of the invention comprises a hinge domain which does not comprise a naturally occurring number of cysteines, i.e., the polypeptide linker comprises either fewer cysteines or a greater number of cysteines than a naturally occurring hinge molecule.

[00141] 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 one 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.

[00142] 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

to the manufacturer's instructions accompanying the kit. Four microgram (4 μg) of total RNA was used as template to prepare cDNA by reverse transcription. The RNA, 300 ng random primers, and 500 ng Oligo dT (12-18) (18mer Oligo dT SEQ ID NO: 212), and 1 μl 25 mM dNTPs were combined and denatured at 80°C. for 5 minutes prior to addition of enzyme. Superscript III reverse transcriptase (Invitrogen, Life Technologies) was added to the RNA plus primer mixture in a total volume of 25 μl in the presence of 5.times.second strand buffer and 0.1 M DTT provided with the enzyme. The reverse transcription reaction was allowed to proceed at 50°C for one hour.

[00211] The cDNA generated in the reverse transcriptase reaction was purified by QIAquick PCR purification kits (QIAGEN, Valencia CA) and tailed with a poly-G sequence using terminal transferase (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. Tailed cDNA was again purified by QIAquick PCR purification and eluted in 30 ul elution buffer (EB buffer) provided with the kits. Two microliters of tailed cDNA was used as template along with an anchor-tail 5' primer containing a poly-C domain, and constant region specific, degenerate 3' primers to amplify by PCR the variable regions for the light and heavy chain of the H564 antibody. The two variable chains were designed with restriction enzyme sites so that a scFv could be assembled by three way ligation of the two V regions to a linker sequence after amplification and restriction enzyme digestion.

[00212] A (gly4ser)4 peptide linker to be inserted between the two V regions was incorporated by amplification of this linker sequence by overlap extension PCR using overlapping primers encoding the two halves of the molecule. PCR fragments were isolated by agarose gel electrophoresis, fragments isolated by cutting the appropriate bands from the gel and purifying the amplified DNA using QIAquick gel extraction kits (QIAGEN, Valencia, CA). scFv derivatives from the H564 hybridoma were assembled as VH-linker-VL fusion genes that could be attached at either end of a larger –Ig fusion gene. The V.sub.H domain was amplified without a leader peptide, but included a 5' AgeI restriction site for fusion to the V.sub.L and a BglII restriction site at the 3' end for fusion to the linker domain.

[00213] The scFv-Ig was assembled by inserting the scFv HindIII-XhoI fragment into pDG containing the human IgG1 hinge, CH2, and CH3 regions, which was digested with restriction enzymes, HindIII and XhoI. After ligation, the ligation products were transformed into DH5-alpha bacteria. The scFv-Ig cDNA was subjected to cycle sequencing on a PE 9700 thermocycler using a 25-cycle program by denaturing at 96°C for 10 seconds, annealing at 50°C. for 30 seconds, and extending at 72°C for 4 minutes. The sequencing primers were pDG forward and reverse primers and an internal primer that annealed to the CH2 domain

human in the IgG constant region portion. Sequencing reactions were performed using the Big Dye Terminator Ready Sequencing Mix v3.1 (PE-Applied Biosystems, Foster City, Calif.) according to the manufacturer's instructions. Samples were subsequently purified using Autoseq G25 columns (GE Healthcare) and the eluates dried in a Savant vacuum dryer, denatured in Template Suppression Reagent (PE-ABI), and analyzed on an ABI 310 Genetic Analyzer (PE-Applied Biosystems). The sequence was edited, translated, and analyzed using Vector Nti version 10.0 (Informax/Invitrogen, North Bethesda, Md.).

[00214] Construction of a Human RNaseI-hIgG1 (SEQ ID NO:125-127) fusion gene

[00215] Human RNase1 (SEQ ID NO:113) was isolated by PCR amplification from human pancreas total RNA obtained from Ambion/Applied Biosystems (Austin, TX). Four microgram (4 μg) of total RNA was used as template to prepare cDNA by reverse transcription. The RNA, 300 ng random primers, and 500 ng Oligo dT (12-18) (18mer Oligo dT SEQ ID NO: 212), and 1 ul 25 mM dNTPs were combined and denatured at 80°C for 5 minutes prior to addition of enzyme. Superscript III reverse transcriptase (Invitrogen, Life Technologies) was added to the RNA plus primer mixture in a total volume of 25 μl in the presence of second strand buffer and 0.1 M DTT provided with the enzyme. The reverse transcription reaction was allowed to proceed at 50°C for one hour. Reactions were further purified by QIAquick PCR purification columns, and cDNA eluted in 40 microliters EB buffer prior to use in PCR reactions. Two microliters cDNA eluate were added to PCR reactions containing 50 pmol 5' and 3' primers specific for human RNase1, and 45 microliters of PCR high fidelity supermix (Invitrogen, Carlsbad, CA) was added to 0.2 ml PCR reaction tubes. PCR reactions were performed using a C1000 thermal cycler (BioRad, Hercules CA). Reactions included an initial denaturation step at 95C for 2 minutes, followed by 34 cycles with a 94°C, 30 sec denaturation, 50°C, 30 sec annealing, and 68°C, 1 minute extension step, followed by a final 4 minute extension at 72°C. Once wild type tails were isolated, the fragments were TOPO cloned into pCR2.1 vectors; DNA prepared using the QIAGEN spin plasmid miniprep kits according to manufacturer's instructions. Plasmid DNA was sequenced using ABI Dye Terminator v3.1 ready reaction mix according to manufacturer's instructions.

Example 3: Isolation of human and mouse –Fc domains and introduction of mutations into the coding sequence.

[00216] For isolation of mouse (SEQ ID NO:114) and human –Fc domains (SEQ ID NO:110), RNA was derived from mouse or human tissue as follows. A single cell suspension was generated from mouse spleen in RPMI culture media. Alternatively, human PBMCs were

isolated from fresh, whole blood using Lymphocyte Separation Media (LSM) Organon Teknika (Durham, NC), buffy coats harvested according to manufacturer's directions, and cells washed three times in PBS prior to use. Cells were pelleted by centrifugation from the culture medium, and $2x10^7$ cells were used to prepare RNA. RNA was isolated from the cells using the QIAGEN RNAeasy kit (Valencia, Calif.) total RNA isolation kit and QIAGEN QIAshredder columns according to the manufacturer's instructions accompanying the kits. One microgram (4 μg) of total RNA was used as template to prepare cDNA by reverse transcription. The RNA, 300 ng random primers, and 500 ng Oligo dT (12-18) (18mer Oligo dT SEQ ID NO: 212), and 1 μl 25 mM dNTPs were combined and denatured at 80°C for 5 minutes prior to addition of enzyme. Superscript III reverse transcriptase (Invitrogen, Life Technologies) was added to the RNA plus primer mixture in a total volume of 25 μl in the presence of .second strand buffer and 0.1 M DTT provided with the enzyme. The reverse transcription reaction was allowed to proceed at 50°C for one hour. cDNA was purified using QIAquick (QIAGEN) PCR purification columns according to manufacturer's directions, and eluted in 40 microliters EB buffer prior to use in PCR reactions.

[00217] Wild type mouse and human –Fc domains were isolated by PCR amplification using the cDNA described above as template. The following primers were used for initial amplification of wild type sequences, but incorporated the desired mutational changes in the hinge domain:

[00218] mahIgG1CH2M: 47 mer

[00219] 5'-tgtccaccgtgtccagcacctgaactcctgggtggatcgtcagtcttcc-3' (SEQ ID NO:6)

[00220] hIgG1-5scc: 49 mer

[00221] 5'-agatctcgagcccaaatcttctgacaaaactcacacatgtccaccgtgt-3' (SEQ ID NO:7)

[00222] mahIgG1S: 51 mer

[00223] 5'-tctagattatcatttacccggagacagagagaggctcttctgcgtgtagtg-3' (SEQ ID NO:8)

[00224] muIgG2aCH2: 58mer

[00225] 5'-cctccatgcaaatgcccagcacctaacctcttgggtggatcatccgtcttcatcttcc-3' (SEQ ID NO:9)

[00226] mIgG2a-5scc: 47mer

[00227] 5'-gaagatctcgagcccagaggtcccacaatcaagccctctcctcca-3' (SEQ ID NO:10)

[00228] mIgG2a3S: 48mer

[00229] 5'-gtttctagattatcatttacccggagtccgagagaagctcttagtcgt-3' (SEQ ID NO:11)

[00230] PCR reactions were performed using a C1000 thermal cycler (BioRad, Hercules CA) or an Eppendorf thermal cycler (ThermoFisher Scientific, Houston TX). Reactions

Primers are shown in Table 1. The nucleotide and amino acid sequences of representative hybrid nuclease molecules are shown in Table 2.

[00259] General Approach for Generation of Hybrid nuclease molecules

[00260] Human cDNAs were isolated from human pancreas RNA (Ambion) or human PBMC RNA from normal human peripheral blood lymphocytes (approximately 5x10e6) using QIAgen RNAeasy kits (Valencia, CA) and QIAshredder kits to homogenize cell lysates (Qiagen, Valencia, CA). Human PBMCs were isolated from heparinized human blood diluted 1:1 in D-PBS and layered over LSM Lymphocyte Separation Medium (MP Biomedicals, Irvine, CA) Ficoll gradients.

[00261] Mouse spleen RNA was isolated using QIAgen RNAeasy kits (Valencia, CA) from approximately 5x10e6 splenocytes. Cells were pelleted by centrifugation from the culture medium, and 5x10e6 cells were used to prepare RNA. RNA was isolated from the cells using the QIAGEN RNAeasy kit (Valencia, Calif.) total RNA isolation kit and QIAGEN QIAshredder according to the manufacturer's instructions accompanying the kit. One to two microgram (1-2 μg) of total RNA was used as template to prepare cDNA by reverse transcription. The RNA, 300 ng random primers, and 500 ng Oligo dT (12-18) (18mer Oligo dT SEQ ID NO: 212), and 1 μl 25 mM dNTPs were combined and denatured at 80°C for 5 minutes prior to addition of enzyme. Superscript III reverse transcriptase (Invitrogen, Life Technologies) was added to the RNA plus primer mixture in a total volume of 25 μl in the presence of 5 times second strand buffer and 0.1 M DTT provided with the enzyme. The reverse transcription reaction was allowed to proceed at 50°C for one hour.

[00262] Between 10-100ng cDNA was used in PCR amplification reactions using primers specific for the nuclease gene of interest (RNaseA, RNase1, DNase1, Trex1, DNase1L3, etc.) For initial cloning reactions, primers were designed to isolate the full length cDNA or truncation products encoding the gene of interest. Full length or shortened PCR fragments were isolated by agarose gel electrophoresis, and purified using Qiagen QIAquick columns to remove nucleotides, primers, and unwanted amplified products. Purified fragments were cloned into pCR2.1 TOPO cloning vectors (Invitrogen, Carlsbad, CA) and transformed into TOP10 competent bacteria. Isolated colonies were picked into Luria Broth media containing 50 ug/ml carbenicillin, and grown overnight to isolate plasmids. TOPO clones were screened for inserts of the correct size by digestion with EcoRI (NEB, Ipswich, MA) restriction enzyme and agarose gel electrophoresis of digested fragments. DNA sequence analysis of positive clones was performed with ABI Ready Reaction Mix v 3.1 and analyzed using an

of the wild type or mutant Fc containing molecules. Figure 14 shows the binding pattern of hRNase1-WT-hIgG1-WT (SEQ ID NO:161) to these two cell lines. Cells were incubated with 5 ug/ml purified fusion protein in PBS/2% FBS for 45 minutes on ice, washed three times in PBS/2% FBS, and incubated with FITC-goat anti-human IgG (Fc specific) (Jackson Immunoresearch, West Grove, PA) at 1:200 for 45 minutes on ice. Cells were washed two times in PBS/2% FBS and analyzed by flow cytometry using a FACS Canto (BD, Franklin Lakes, NJ) flow cytometer, and FlowJo software (TreeStar, Ashland, OR).

Example 15. IVIg Blocking of hRNase1-hIgG1 binding to human monocytic lines.

[00277] THP-1 or U937 cells were pre-incubated with IVIg starting at 10 mg/ml and performing 10-fold serial dilutions across the wells of a 96 well plate. Cells (approximately 1x10e6 per well) were incubated on ice for 45 minutes. Pre-bound cells were washed twice and AF750 conjugated hRNase1-WT-hIgG1-WT (SEQ ID NO:161) at approximately 5 ug/ml was added to each well. Binding reactions were incubated 45 minutes on ice, washed twice in PBS/2% FBS, and analyzed by flow cytometry as described above. IVIg was able to partially block the binding of the labeled nuclease fusion protein, but even at 10 mg/ml, there was still residual binding detectable above background. FIG. 15 shows the blocking activity of human IVIg for binding to U937 and THP-1 cells by hRNase1-WT-hIgG1-WT (SEQ ID NO:161).

Example 16. Trex1-Ig activity assay.

[00278] Murine Trex1 was cloned from mouse cDNA using the primers listed below:

[00279] mTrex1-5'age: accggtatgggctcacagaccctgccccatggtcaca (SEQ ID NO:20)

[00280] mTrex1-3'bx: ctcgagatctgttgttccagtggtagccggagtgccgtacatg (SEQ ID NO:21)

[00281] PCR reactions were performed using 50 pmol each primer in a total volume of 50 ul, under an amplification profile of 94C 30 sec; 50C 60 sec; 68C 90 sec for 35 cycles of amplification. PCR products were cloned into the pCR2.1 vector and TOPO clones screened as previously described for prototype nuclease fusion gene cloning. Once sequence was verified, the cassettes were subcloned into the pDG expression vector fused to the mIgG tail or co-cloned with one of the (g4s)n (SEQ ID NO: 210)n linkers to construct Trex1-lnk molecules with different length linkers. Plasmid isolates were transiently transfected into COS cells as described and stable CHO transfectants generated as described for prototype nuclease fusion genes.

[00282] Fusion genes were constructed encoding Trex1Ig as follows: the genes incorporate the human VK3 leader peptide fused to murine Trex1 truncated at the COOH

prepared with distilled water. Poly-C (Sigma) was dissolved in distilled water at 3 mg/ml. the gel plate was prepared as follows: 1.5 ml reaction buffer (0.2M Tris-HCl pH7.0, 40mM EDTA and 0.1 mg/ml Ethidium bromide), 1 ml Poly-C and 0.5 ml water were place in the tube and maintained at 50C for 5 min. 3 ml of the agarose (kept at 50C) was added to the tube. The mixture was immediately poured onto glass plate. Sampling wells were punched in the gel. Approximately 2 ul of each sample was loaded and the gel was incubated at 37C for 4 hours in the moist chamber. Then the gel was incubated in a buffer (20 mM sodium acetate pH5.2, 20mg/ml Ethidium bromide) on ice for 30 min. Gels were photographed on a UV transilluminator using a Kodak digital camera DC290 system equipped with ethidium bromide filters and analyzd using Kodak Molecular Imaging software.

[00309] Figure 22 shows a composite figure displaying results of DNase nuclease activity assays performed on COS supernatants from transfected cells. Culture supernatants were harvested 72 hours after transfecting the following clones of DNase1 wild type and mutant –Ig fusion proteins.: (1) 090210-8=hDNAse1-WT-hIgG1 WT (SEQ ID NO:154); (2) 090210-9=hDNase1-G105R;A114F-hIgG1 WT (SEQ ID NO:159); (3) 091210-8=hRNase1-WT-hIgG1-(SCC)-DNase1-G105R;A114F (SEQ ID NO:151), and (4) 091210-14=hRNase-WT-hIgG1-(SCC)-DNase1-A114F (SEQ ID NO:152).

[00310] The pH of the supernatants was adjusted to .0 with bicarbonate buffer to facilitate binding of expressed –Ig fusion proteins to protein A agarose beads. Panel A. of Figure 23 shows gel electrophoresis analysis of plasmid DNA digestion : Protein A agarose slurry (50 ul per sample) was washed in PBS, and incubated overnight at 4°C with 100 ul culture supernatant to immunoprecipitate –Ig fusion proteins. Immunoprecipitates were washed 4-5 times in 750 ul PBS, centrifuging at approximately 3500 rpm followed by aspiration of PBS. Final protein A precipitates were resuspended in 50 ul reaction buffer containing 20 mM Tris ph7.5. 2 mM CaCl2 and 2 mM MgCl2 containing 1.5 ug plasmid DNA (pDG expression vector). Reactions were incubated for 30 minutes at 37°C, heated at 65°C for 5 min, and the DNA present in reactions analyzed by agarose gel electrophoresis on 1.5% TBE-agarose gels.

[00311] Panel B shows the results of a nuclease activity assay performed on the same culture supernatants using the DNase Alert Kit (IDT/Ambion). Reaction tubes containing lyophilized DNase Alert Substrate (50 pmoles) were resuspended with 5 ul nuclease free ddH2O supplied with the kit, 5 ul 10X DNase alert buffer, and 40 ul protein A slurry immunoprecipitated as follows: For these immunoprecipitations, 50 ul protein A agarose beads were incubated overnight with 50 ul culture supernatant. Samples were then washed 5

91	hRNaseG88D-S	agactgccgcctgacaaacgactccaggtaccc
92	hRNAseG88D-AS	gggtacctggagtcgtttgtcaggcggcagtct
93	g4s5-5-1	GGC TCA GGT GGT GGA GGA TCT GGA GGA GGT GGC TCA GGT GGT GGA GGA TCT G
94	g4s5-2s	GTT AGA TCT CTC CGG AGG AGG TGG CTC AGG TGG TGG AGG ATC TGG A
95	g4s5-asxho	CTC GAG ACT CCC ACC TCC TCC AGA TCC TCC ACC ACC TGA GCC ACC T
96	g4s4-5'	AAA GAT CTC TCC GGA GGA GGT GGC TCA GGT GGT GGA GGA TCT GGA GGA GG
97	g4s4-3'	CTC GAG ACC GGT AGA ACC ACC TCC ACC ACT CCC ACC TCC TCC AGA TCC TC
98	g4s5-5	GTT AGA TCT CTC CGG AGG AGG TGG CTC A
99	g4s5-3	ACC GGT CTC GAG ACT CCC ACC TCC TCC AGA TC

TABLE 2

SEQ ID NO.	DESCRIPTION	SEQUENCE (NUCLEOTIDE SEQUENCES ARE 5'-3')
100	g4s4lnk ("(G4S)4")	agatctctccggaggaggtggctcaggtggtggaggatctggaggaggtgggag tggtggaggtggttctaccggtctcgag
101	G4S5-1 ("(G4S)5")	agatctctccggaggaggtggctcaggtggtggaggatctggaggaggtggctc aggtggtggaggatctggaggaggtgggagtaccggtctcgag
102	G4S5-2 ("(G4S)5")	agatctctccggaggaggtggctcaggtggtggaggatctggaggaggtggctc aggtggtggaggatctggaggaggtgggagtctcgag
103	3'hRNase G88D	gtcgacggagctagcagccccgtgaacgtgagcagccccagcgtgcaggatatc ccttccctgggcaaggaatcccgggccaagaaattccagcggcagcatatggac tcagacagttcccccagcagcagctccacctactgtaaccaaatgatgaggcgc cggaatatgacacaggggcggtgcaaaccagtgaacacctttgtgcacgagccc ctggtagatgtccagaatgtctgtttccaggaaaaggtcacctgcaagaacggg cagggcaactgctacaagagcaactccagcatgcacatcacagactgccgcctg acaaacgactccaggtaccccaactgtgcataccggaccagcccgaaggagaga cacatcattgtggcctgtgaagggagcccatatgtgccagtccactttgatgct tctgtggaggactctacctaataatctaga
104	hDNase1-3'-G105R;A114F	gatatcctgaagatcgcagccttcaacatccagacatttggggagaccaagatg tccaatgccaccctcgtcagctacattgtgcagatcctgagccgctatgacatc gccctggtccaggaggtcagagacagccacctgactgccgtggggaagctgctg gacaacctcaatcaggatgcaccagacacctatcactacgtggtcagtgagcca ctgggacggaacagctataaggagcgctacctgttcgtgtacaggcctgaccag gtgtctgcggtggacagctactactacgatgatggctgcgagccctgcaggaac gacaccttcaaccgagagccattcattgtcaggttcttctcccggttcacagag gtcagggagtttgccattgttcccctgcatgcggccccgggggacgcagtagcc gagatcgacgctctctatgacgtctacctggatgtccaagagaaatggggcttg gaggacgtcatgttgatgggcgacttcaatgcgggctgcagctatgtgagaccc tcccagtggtcatccatccgcctgtggacaagccccaccttccagtggctgatc cccgacagcgctgacaccacagctacacccacgcactgtgcctatgacaggatc gtggttgcagggatgctgctccgaggcgccgttgttcccgactcggctcttccc tttaacttccaggctgcctatggcctgagtgaccaactggcccaagccatcagt gaccactatccagtggaggtgatgctgaagtgataatctaga
105	hDNase1-3'-WT	Gatatcctgaagatcgcagccttcaacatccagacatttggggagaccaagatg tccaatgccaccctcgtcagctacattgtgcagatcctgagccgctatgacatc gccctggtccaggaggtcagagacagccacctgactgccgtggggaagctgctg gacaacctcaatcaggatgcaccagacacctatcactacgtggtcagtgagcca ctgggacggaacagctataaggagcgctacctgttcgtgtacaggcctgaccag gtgtctgcggtggacagctactactacgatgatggctgcgagccctgcgggaac gacaccttcaaccgagagccagccattgtcaggttcttctcccggttcacagag gtcagggagtttgccattgttcccctgcatgcggccccgggggacgcagtagcc gagatcgacgctctctatgacgtctacctggatgtccaagagaaatggggcttg gaggacgtcatgttgatgggcgacttcaatgcgggctgcagctatgtgagaccc

TABLE 2		
SEQ ID NO.	DESCRIPTION	SEQUENCE (NUCLEOTIDE SEQUENCES ARE 5'-3')
		ccagactcgcacacggctgagggtgatgtcctggccctgctcagcatctgtcag tggagaccacaggccctgctgcggtgggtggatgctcacgccaggcctttcggc accatcaggcccatgtatggggtcacagcctctgctaggaccaaagatctctcc ggaggaggtggctcaggtggtggaggatctggaggaggtggctcaggtggtgga ggatctggaggaggtgggagtctcgagcccaaatcttctgacaaaactcacaca tgtccaccgtgcccagcacctgaactcctgggggaccgtcagtcttcctcttc ccccaaaacccaaggacaccctcatgatctcccggaccctgaggtcacatgc gtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtg gacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaac agcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaat ggcaaggagtacaagtgcaaggtctccaacaaagccctcccagccccatcgag aaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctg cccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtc aaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccg gagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttc ctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttc tcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctc tctctgtctccgggtaaatgataatctaga
136	g4s4lnk ("(G4S)4")	ggggsggggsggggsggggs
137	G4S5-1 ("(G4S)5")	ggggsggggsggggsggggsggggs
138	G4S5-2 ("(G4S)5")	ggggsggggsggggsggggsggggs
139	hDNase1-3'-G105R;A114F	dilkiaafniqtfgetkmsnatlvsyivqilsrydialvqevrdshltavgkll dnlnqdapdtyhyvvseplgrnsykerylfvyrpdqvsavdsyyyddgcepcrn dtfnrepfivrffsrftevrefaivplhaapgdavaeidalydvyldvqekwgl edvmlmgdfnagcsyvrpsqwssirlwtsptfqwlipdsadttatpthcaydri vvagmllrgavvpdsalpfnfqaayglsdqlaqaisdhypvevmlk*
140	hDNase1-3'-WT	dilkiaafniqtfgetkmsnatlvsyivqilsrydialvqevrdshltavgkll dnlnqdapdtyhyvvseplgrnsykerylfvyrpdqvsavdsyyyddgcepcgn dtfnrepaivrffsrftevrefaivplhaapgdavaeidalydvyldvqekwgl edvmlmgdfnagcsyvrpsqwssirlwtsptfqwlipdsadttatpthcaydri vvagmllrgavvpdsalpfnfqaayglsdqlaqaisdhypvevmlk*
141	hDNase1-3'A114F	dilkiaafniqtfgetkmsnatlvsyivqilsrydialvqevrdshltavgkll dnlnqdapdtyhyvvseplgrnsykerylfvyrpdqvsavdsyyyddgcepcgn dtfnrepfivrffsrftevrefaivplhaapgdavaeidalydvyldvqekwgl edvmlmgdfnagcsyvrpsqwssirlwtsptfqwlipdsadttatpthcaydri vvagmllrgavvpdsalpfnfqaayglsdqlaqaisdhypvevmlk*
142	hDNase1-5'-G105R;A114F	tglkiaafniqtfgetkmsnatlvsyivqilsrydialvqevrdshltavgkll dnlnqdapdtyhyvvseplgrnsykerylfvyrpdqvsavdsyyyddgcepcrn dtfnrepfivrffsrftevrefaivplhaapgdavaeidalydvyldvqekwgl edvmlmgdfnagcsyvrpsqwssirlwtsptfqwlipdsadttatpthcaydri vvagmllrgavvpdsalpfnfqaayglsdqlaqaisdhypvevmlkdle
143	hDNase1-5'-WT	tglkiaafniqtfgetkmsnatlvsyivqilsrydialvqevrdshltavgkll dnlnqdapdtyhyvvseplgrnsykerylfvyrpdqvsavdsyyyddgcepcgn dtfnrepaivrffsrftevrefaivplhaapgdavaeidalydvyldvqekwgl edvmlmgdfnagcsyvrpsqwssirlwtsptfqwlipdsadttatpthcaydri vvagmllrgavvpdsalpfnfqaayglsdqlaqaisdhypvevmlkdle
144	hDNase1-5'-A114F	tglkiaafniqtfgetkmsnatlvsyivqilsrydialvqevrdshltavgkll dnlnqdapdtyhyvvseplgrnsykerylfvyrpdqvsavdsyyyddgcepcgn dtfnrepfivrffsrftevrefaivplhaapgdavaeidalydvyldvqekwgl edvmlmgdfnagcsyvrpsqwssirlwtsptfqwlipdsadttatpthcaydri vvagmllrgavvpdsalpfnfqaayglsdqlaqaisdhypvevmlkdle
145	hIgG1WT	Dlepkssdkthtcppcpapellggpsvflfppkpkdtlmisrtpevtcvvvdvs hedpevkfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykc

TABLE 2		
SEQ ID NO.	DESCRIPTION	SEQUENCE (NUCLEOTIDE SEQUENCES ARE 5'-3')
		kvsnkalpapiektiskakgqprepqvytlppsrdeltknqvsltclvkgfyps diavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmh ealhnhytqkslslspgk
146	hRNase-G88D-3'	vdgasspvnvsspsvqdipslgkesrakkfqrqhmdsdsspsssstycnqmmrrrnmtqg rckpvntfvheplvdvqnvcfqekvtckngqgncyksnssmhitdcrltndsrypncayr tspkerhiivacegspyvpvhfdasvedst*
147	human DNase1+VK3 LP	metpaqllfllllwlpdttglkiaafniqtfgetkmsnatlvsyivqilsrydi alvqevrdshltavgklldnlnqdapdtyhyvvseplgrnsykerylfvyrpdq vsavdsyyyddgcepcgndtfnrepaivrffsrftevrefaivplhaapgdava eidalydvyldvqekwgledvmlmgdfnagcsyvrpsqwssirlwtsptfqwli pdsadttatpthcaydrivvagmllrgavvpdsalpfnfqaayglsdqlaqais dhypvevmlk*
148	DNase1L3	msrelapllllllsihsalamricsfnvrsfgeskqedknamdvivkvikrcdi ilvmeikdsnnricpilmeklnrnsrrgitynyvissrlgrntykeqyaflyke klvsvkrsyhyhdyqdgdadvfsrepfvvwfqsphtavkdfviiplhttpetsv keidelvevytdvkhrwkaenfifmgdfnagcsyvpkkawknirlrtdprfvwl igdqedttvkkstncaydrivlrgqeivssvvpksnsvfdfqkayklteeeald vsdhfpvefklqssraftnskksvtlrkktkskrs*
149	human pancreatic ribonuclease	Mglekslvrllllvlillvlgwvqpslgkesrakkfqrqhmdsdsspssssttyc nqmmrrrnmtqgrckpvntfvheplvdvqnvcfqekvtckngqgncyksnssmh itdcrltngsrypncayrtspkerhiivacegspyvpvhfdatv*
150	huVK3LP+mrib1+mIgG2A-C+2S	metpaqllfllllwlpdttgresaaqkfqrqhmdpdgssinsptycnqmmkrrd mtngsckpvntfvhepladvqavcsqenvtcknrksncyksssalhitdchlkg nskypncdykttqyqkhiivacegnpyvpvhfdatvleprgltikpsppckcpa pnllggssvfifppkikdvlmislspmvtcvvvdvseddpdvqiswfvnnvevh taqtqthredynstlrvvsalpiqhqdwmsgkefkcsvnnkdlpasiertiskp rgpvrapqvyvlpppaeemtkkefsltcmitgflpaeiavdwtsngrteqnykn tatvldsdgsyfmysklrvqkstwergslfacsvvheglhnhlttksfsrtpgk *
151	huVK3LP-hRNaseWT-hIgG1 (SCC)-NLG-hDNase1-G105R;A114F	metpaqllfllllwlpdttgkesrakkfqrqhmdsdsspsssstycnqmmrrrn mtqgrckpvntfvheplvdvqnvcfqekvtckngqgncyksnssmhitdcrltn gsrypncayrtspkerhiivacegspyvpvhfdasvedstdlepkssdkthtcp pcpapellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdg vevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiekt iskakgqprepqvytlppsrdeltknqvsltclvkgfypsdiavewesngqpen nykttppvldsdgsfflyskltvdksrwqqgnvfscsvmheglhnhytqkslsl spgkvdgasshvnvsspsvqdilkiaafniqtfgetkmsnatlvsyivqilsry dialvqevrdshltavgklldnlnqdapdtyhyvvseplgrnsykerylfvyrp dqvsavdsyyyddgcepcrndtfnrepfivrffsrftevrefaivplhaapgda vaeidalydvyldvqekwgsedvmlmgdfnagcsyvrpsqwssirlwtsptfqw lipdsadttatpthcaydrivvagmllrgavvpdsalpfnfqaayglsdqlaqa isdhypvevmlk**
152	huVK3LP-hRNaseWT-hIgG1 (SCC)-NLG-hDNase1-A114F	metpaqllfllllwlpdttgkesrakkfqrqhmdsdsspsssstycnqmmrrrn mtqgrckpvntfvheplvdvqnvcfqekvtckngqgncyksnssmhitdcrltn gsrypncayrtspkerhiivacegspyvpvhfdasvedstdlepkssdkthtcp pcpapellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdg vevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiekt iskakgqprepqvytlppsrdeltknqvsltclvkgfypsdiavewesngqpen nykttppvldsdgsfflyskltvdksrwqqgnvfscsvmheglhnhytqkslsl spgkvdgasshvnvsspsvqdilkiaafniqtfgetkmsnatlvsyivqilsry dialvqevrdshltavgklldnlnqdapdtyhyvvseplgrnsykerylfvyrp

TABLE 2		
SEQ ID NO.	DESCRIPTION	SEQUENCE (NUCLEOTIDE SEQUENCES ARE 5'-3')
		dqvsavdsyyyddgcepcgndtfnrepfivrffsrftevrefaivplhaapgda vaeidalydvyldvqekwgledvmlmgdfnagcsyvrpsqwssirlwtsptfqw lipdsadttatpthcaydrivvagmllrgavvpdsalpfnfqaayglsdqlaqa isdhypvevmlk*
153	huVK3LP-hRNaseWT-hIgG1 (SCC)-NLG-hDNAse1-WT	metpaqllfllllwlpdttgkesrakkfqrqhmdsdsspsssstycnqmmrrrnmtqgrc kpvntfvheplvdvqnvcfqekvtckngqgncyksnssmhitdcrltngsrypncayrts pkerhiivacegspyvpvhfdasvedstdlepkssdkthtcppcpapellggpsvflfpp kpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvvsv ltvlhqdwlngkeykckvsnkalpapiektiskakgqprepqvytlppsrdeltknqvsl tclvkgfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfsc svmheglhnhytqkslslspgkvdgasshvnvsspsvqdilkiaafniqtfgetkmsnat lvsyivqilsrydialvqevrdshltavgklldnlnqdapdtyhyvvseplgrnsykery lfvyrpdqvsavdsyyyddgcepcgndtfnrepaivrffsrftevrefaivplhaapgda vaeidalydvyldvqekwgledvmlmgdfnagcsyvrpsqwssirlwtsptfqwlipdsa dttatpthcaydrivvagmllrgavvpdsalpfnfqaayglsdqlaqaisdhypvevmlk *
154	hVK3LP-hDNase1(WT)-hIgG1WT	metpaqllfllllwlpdttglkiaafniqtfgetkmsnatlvsyivqilsrydialvqev rdshltavgklldnlnqdapdtyhyvvseplgrnsykerylfvyrpdqvsavdsyyyddg cepcgndtfnrepaivrffsrftevrefaivplhaapgdavaeidalydvyldvqekwgl edvmlmgdfnagcsyvrpsqwssirlwtsptfqwlipdsadttatpthcaydrivvagml lrgavvpdsalpfnfqaayglsdqlaqaisdhypvevmlkdlepkssdkthtcppcpape llggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpre eqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepqvytlpp srdeltknqvsltclvkgfypsdiavewesngqpennykttppvldsdgsfflyskltvd ksrwqqgnvfscsvmhealhnhytqkslslspgk*
155	hVK3LP-hDNase1-A114F-hIgG1WT	metpaqllfllllwlpdttglkiaafniqtfgetkmsnatlvsyivqilsrydialvqev rdshltavgklldnlnqdapdtyhyvvseplgrnsykerylfvyrpdqvsavdsyyyddg cepcgndtfnrepfivrffsrftevrefaivplhaapgdavaeidalydvyldvqekwgl edvmlmgdfnagcsyvrpsqwssirlwtsptfqwlipdsadttatpthcaydrivvagml lrgavvpdsalpfnfqaayglsdqlaqaisdhypvevmlkdlepkssdkthtcppcpape llggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpre eqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepqvytlpp srdeltknqvsltclvkgfypsdiavewesngqpennykttppvldsdgsfflyskltvd ksrwqqgnvfscsvmhealhnhytqkslslspgk*
156	hVK3LP-hDNase1-G105R;A114F-(G4S)4-hIgG1 WT	metpaqllfllllwlpdttglkiaafniqtfgetkmsnatlvsyivqilsrydialvqev rdshltavgklldnlnqdapdtyhyvvseplgrnsykerylfvyrpdqvsavdsyyyddg cepcrndtfnrepfivrffsrftevrefaivplhaapgdavaeidalydvyldvqekwgl edvmlmgdfnagcsyvrpsqwssirlwtsptfqwlipdsadttatpthcaydrivvagml lrgavvpdsalpfnfqaayglsdqlaqaisdhypvevmlkdlsggggsggggsggggsgg ggstglepkssdkthtcppcpapellggpsvflfppkpkdtlmisrtpevtcvvvdvshe dpevkfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalp apiektiskakgqprepqvytlppsrdeltknqvsltclvkgfypsdiavewesngqpen nykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk*
157	hVK3LP-hDNase1G105R;A114F(G4s)5-hIgG1	Metpaqllfllllwlpdttglkiaafniqtfgetkmsnatlvsyivqilsrydi alvqevrdshltavgklldnlnqdapdtyhyvvseplgrnsykerylfvyrpdq vsavdsyyyddgcepcrndtfnrepfivrffsrftevrefaivplhaapgdava eidalydvyldvqekwgledvmlmgdfnagcsyvrpsqwssirlwtsptfqwli pdsadttatpthcaydrivvagmllrgavvpdsalpfnfqaayglsdqlaqais

TABLE 2		
SEQ ID NO.	DESCRIPTION	SEQUENCE (NUCLEOTIDE SEQUENCES ARE 5'-3')
	WT-NLG-hRNase1-WT	ktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakg qprepqvytlppsrdeltknqvsltclvkgfypsdiavewesngqpennykttp pvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgkvd gasspvnvsspsvqdipslgkesrakkfqrqhmdsdsspsssstycnqmmrrrn mtqgrckpvntfvheplvdvqnvcfqekvtckngqgncyksnssmhitdcrltn gsrypncayrtspkerhiivacegspyvpvhfdasvedst*
174	huVK3LP-hRNAseG88D-hIgG1(SCC)-P238S;K322S;P331S	gttaagcttgccaccatggaaaccccagcgcagcttctcttcctcctgctactc tggctcccagataccaccggtccttccctgggcaaggaatcccgggccaagaaa ttccagcggcagcatatggactcagacagttcccccagcagcagctccacctac tgtaaccaaatgatgaggcgccggaatatgacacaggggcggtgcaaaccagtg aacacctttgtgcacgagcccctggtagatgtccagaatgtctgtttccaggaa aaggtcacctgcaagaacgggcagggcaactgctacaagagcaactccagcatg cacatcacagactgccgcctgacaaacgactccaggtaccccaactgtgcatac cggaccagcccgaaggagagacacatcattgtggcctgtgaagggagcccatat gtgccagtccactttgatgcttctgtggaggactctacagatctcgagcccaaa tcttctgacaaaactcacacatgtccaccgtgtccagcacctgaactcctgggt ggatcgtcagtcttcctcttcccccaaaacccaaggacactctcatgatctcc cggacccctgaggtcacgtgcgtggtggtggacgtgagccaggaagaccccgag gtccagttcaactggtacgtggacggcatggaggtgcataatgccaagacaaag ccacgggaggagcagttcaacagcacgttccgtgtggtcagcgtcctcaccgtc gtgcaccaggactggctgaacggcaaggagtacaagtgcaaggtctccaacaaa gccctcccagcctccatcgagaaaacaatctccaaaaccaaagggcagccccga gaaccacaggtgtacaccctgcccccatcccgggaggagatgaccaagaaccag gtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggag tgggagagcaatgggcagccggagaacaactacaacaccacgcctcccgtgctg gactccgacggctccttctccctctacagcaagctcaccgtggacaagagcagg tggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaac cactacacgcagaagagcctctctctgtctccgggtaaatgataatctaga
175	huVK3LP-hRNAseG88D-hIgG1(SCC)-P238S;K322S;P331S	metpaqllfllllwlpdttgpslgkesrakkfqrqhmdsdsspsssstycnqmm rrrnmtqgrckpvntfvheplvdvqnvcfqekvtckngqgncyksnssmhitdc rltndsrypncayrtspkerhiivacegspyvpvhfdasvedstdlepkssdkt htcppcpapellggssvflfppkpkdtlmisrtpevtcvvvdvsqedpevqfnw yvdgmevhnaktkpreeqfnstfrvvsvltvvhqdwlngkeykckvsnkalpas iektisktkgqprepqvytlppsreemtknqvsltclvkgfypsdiavewesng qpennynttppvldsdgsfslyskltvdksrwqqgnvfscsvmhealhnhytqk slslspgk*
176	huVK3LP-hDNase1-G105R;A114F-(G4S)5-1-hIgG1-WT-NLG-hRNase1-WT	gttaagcttgccaccatggaaaccccagcgcagcttctcttcctcctgctactc tggctcccagataccaccggtctgaagatcgcagccttcaacatccagacattt ggggagaccaagatgtccaatgccaccctcgtcagctacattgtgcagatcctg agccgctatgacatcgccctggtccaggaggtcagagacagccacctgactgcc gtggggaagctgctggacaacctcaatcaggatgcaccagacacctatcactac gtggtcagtgagccactgggacggaacagctataaggagcgctacctgttcgtg tacaggcctgaccaggtgtctgcggtggacagctactactacgatgatggctgc gagccctgcaggaacgacaccttcaaccgagagccattcattgtcaggttcttc tcccggttcacagaggtcagggagtttgccattgttcccctgcatgcggccccg ggggacgcagtagccgagatcgacgctctctatgacgtctacctggatgtccaa gagaaatggggcttggaggacgtcatgttgatgggcgacttcaatgcgggctgc agctatgtgagaccctcccagtggtcatccatccgcctgtggacaagccccacc ttccagtggctgatccccgacagcgctgacaccacagctacacccacgcactgt gcctatgacaggatcgtggttgcagggatgctgctccgaggcgccgttgttccc gactcggctcttcccctttaacttccaggctgcctatggcctgagtgaccaactg gcccaagccatcagtgaccactatccagtggaggtgatgctgaaagatctctcc ggaggaggtggctcaggtggtggaggatctggaggaggtggctcaggtggtgga ggatctggaggaggtgggagtaccggtctcgagcccaaatcttctgacaaaact cacacatgtccaccgtgcccagcacctgaactcctgggggggaccgtcagtcttc ctcttcccccaaaacccaaggacaccctcatgatctcccggacccctgaggtc acatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactgg tacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcag tacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactgg ctgaatggcaaggagtacaagtgcaaggtctccaacaaagccctcccagccccc

TABLE 2		
SEQ ID NO.	DESCRIPTION	SEQUENCE (NUCLEOTIDE SEQUENCES ARE 5'-3')
		tctccgggtaaagtcgacggagctagcagccccgtgaacgtgagcagccccagc gtgcaggatatcatgggccctggagctcgcagacagggcaggattgtgcaggga aggcctgagatgtgcttctgcccacccccctaccccactccctcccccttcggatc ttaacactgggcactcacacacccaccccatgctcctctccaggctcagcagca ggtacgtacccaaccatgggctcgcaggccctgcccccggggcccatgcagacc ctcatctttttcgacatggaggccactggcttgcccttctcccagcccaaggtc acggagctgtgcctgctggctgtccacagatgtgccctggagagccccccccacc tctcaggggccacctcccacagttcctccaccaccgcgtgtggtagacaagctc tccctgtgtgtggctccggggaaggcctgcagccctgcagccagcgagatcaca ggtctgagcacagctgtgctggcagcgcatgggcgtcaatgttttgatgacaac ctggccaacctgctcctagccttcctgcggcgccagccacagccctggtgcctg gtggcacacaatggtgaccgctacgacttccccctgctccaagcagagctggct atgctgggcctcaccagtgctctggatggtgccttctgtgtggatagcatcact gcgctgaaggccctggagcgagcaagcagcccctcagaacacggcccaaggaag agctacagcctaggcagcatctacactcgcctgtatgggcagtcccctccagac tcgcacacggctgagggtgatgtcctggccctgctcagcatctgtcagtggaga ccacaggccctgctgcggtgggtggatgctcacgccaggcctttcggcaccatc aggcccatgtatggggtcacagcctctgctaggaccaaatgataatctaga
207	huVK3LP-hDNase1L3-hIgG1-WT-NLG-hTREX1-72AA	metpaqllfllllwlpdttgmricsfnvrsfgeskqedknamdvivkvikrcdi ilvmeikdsnnricpilmeklnrnsrrgitynyvissrlgrntykeqyaflyke klvsvkrsyhyhdyqdgdadvfsrepfvvwfqsphtavkdfviiplhttpetsv keidelvevytdvkhrwkaenfifmgdfnagcsyvpkkawknirlrtdprfvwl igdqedttvkkstncaydrivlrgqeivssvvpksnsvfdfqkayklteeeald vsdhfpvefklqssraftnskksvtlrkktkskrsdlepkssdkthtcppcpap ellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgvevhn aktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskak gqprepqvytlppsrdeltknqvsltclvkgfypsdiavewesngqpennyktt ppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgkv dgasspvnvsspsvqdimgpgarrqgrivqgrpemcfcppptplpplriltlgt htptpcsspgsaagtyptmgsqalppgpmqtliffdmeatglpfsqpkvtelcl lavhrcalespptsqgppptvpppprvvdklslcvapgkacspaaseitglsta vlaahgrqcfddnlanlllaflrrqpqpwclvahngdrydfpllqaelamlglt saldgafcvdsitalkalerasspsehgprksyslgsiytrlygqsppdshtae gdvlallsicqwrpqallrwvdaharpfgtirpmygvtasartk*
208	(G4S)10	ggggsggggsggggsggggsggggsggggsggggsggggsggggsggggs
209	(G4S)3	ggggsggggsggggs
210	(G4S)	ggggs
211	(G4S)6	ggggsggggsggggsggggsggggsggggs
212	OligodT 18mer	ttttttttttttttttt
213		gttaagcttg ccacc atg ggt ctg gag aag tcc ctc att ctg ttt cca ttg ttt ttc ctg ctg ctt gga tgg gtc cag cct tcc ccg ggc agg gaa tct gca gca cag aag ttt cag cgg cag cac atg gat cca gat ggt tcc tcc atc aac agc ccc acc tac tgc aac caa atg atg aaa cgc cgg gat atg aca aat ggg tca tgc aag ccc gtg aac acc ttc gtg cat gag ccc ttg gca gat gtc cag gcc gtc tgc tcc cag gaa aat gtc acc tgc aag aac agg aag agc aac tgc tac aag agc agc tct gcc ctg cac atc act gac tgc cac ctg aag ggc aac tcc aag tat ccc aac tgt gac tac aag acc act caa tac cag aag cac atc att gtg gcc tgt gaa ggg aac ccc tac gta cca gtc cac ttt gat gct act gtg ctc

TABLE 2

SEQ ID NO.	DESCRIPTION	SEQUENCE (NUCLEOTIDE SEQUENCES ARE 5'-3')
		gag ccc aga ggt ctc aca atc aag ccc tct cct cca tgc aaa tgc cca gca cct aac ctc ttg ggt gga tca tcc gtc ttc atc ttc cct cca aag atc aag gat gta ctc atg atc tcc ctg agc ccc atg gtc aca tgt gtg gtg gtg gat gtg agc gag gat gac cca gac gtc cag atc agc tgg ttt gtg aac aac gtg gaa gta cac aca gct cag aca caa acc cat aga gag gat tac aac agt act ctc cgg gtg gtc agt gcc ctc ccc atc cag cac cag gac tgg atg agt ggc aag gag ttc aaa tgc tcg gtc aac aac aaa gac ctc cca gcg tcc atc gag aga acc atc tca aaa ccc aga ggg cca gta aga gct cca cag gta tat gtc ttg cct cca cca gca gaa gag atg act aag aaa gag ttc agt ctg acc tgc atg atc aca ggc ttc tta cct gcc gaa att gct gtg gac tgg acc agc aat ggg cgt aca gag caa aac tac aag aac acc gca aca gtc ctg gac tct gat ggt tct tac ttc atg tac agc aag ctc aga gta caa aag agc act tgg gaa aga gga agt ctt ttc gcc tgc tca gtg gtc cac gag ggt ctg cac aat cac ctt acg act aag agc ttc tct cgg act ccg ggt aaa tgataatcta gaa
214		Met Gly Leu Glu Lys Ser Leu Ile Leu Phe Pro Leu Phe Phe Leu Leu Leu Gly Trp Val Gln Pro Ser Pro Gly Arg Glu Ser Ala Ala Gln Lys Phe Gln Arg Gln His Met Asp Pro Asp Gly Ser Ser Ile Asn Ser Pro Thr Tyr Cys Asn Gln Met Met Lys Arg Arg Asp Met Thr Asn Gly Ser Cys Lys Pro Val Asn Thr Phe Val His Glu Pro Leu Ala Asp Val Gln Ala Val Cys Ser Gln Glu Asn Val Thr Cys Lys Asn Arg Lys Ser Asn Cys Tyr Lys Ser Ser Ser Ala Leu His Ile Thr Asp Cys His Leu Lys Gly Asn Ser Lys Tyr Pro Asn Cys Asp Tyr Lys Thr Thr Gln Tyr Gln Lys His Ile Ile Val Ala Cys Glu Gly Asn Pro Tyr Val Pro Val His Phe Asp Ala Thr Val Leu Glu Pro Arg Gly Leu Thr Ile Lys Pro Ser Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Leu Gly Gly Ser Ser Val Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu Met Ile Ser Leu Ser Pro Met Val Thr Cys Val Val Val Asp Val Ser Glu Asp Asp Pro Asp Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val His Thr Ala Gln Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Leu Arg Val Val Ser Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys Glu Phe Lys Cys Ser Val Asn Asn Lys Asp Leu Pro Ala Ser Ile Glu Arg Thr Ile Ser Lys Pro Arg Gly Pro Val Arg Ala Pro Gln Val Tyr Val

TABLE 2		
SEQ ID NO.	DESCRIPTION	SEQUENCE (NUCLEOTIDE SEQUENCES ARE 5'-3')
		Leu Pro Pro Pro Ala Glu Glu Met Thr Lys Lys Glu Phe Ser Leu Thr Cys Met Ile Thr Gly Phe Leu Pro Ala Glu Ile Ala Val Asp Trp Thr Ser Asn Gly Arg Thr Glu Gln Asn Tyr Lys Asn Thr Ala Thr Val Leu Asp Ser Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg Val Gln Lys Ser Thr Trp Glu Arg Gly Ser Leu Phe Ala Cys Ser Val Val His Glu Gly Leu His Asn His Leu Thr Thr Lys Ser Phe Ser Arg Thr Pro Gly Lys