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(54) Title: COMPOSITIONS FOR TREATING PATHOLOGICAL CALCIFICATION CONDITIONS, AND METHODS USING SAME

(57) Abstract: The present invention includes compositions and methods for treating diseases or disorders associated with pathological calcification or pathological ossification. In certain embodiments, the diseases or disorders are selected from the group consisting of Generalized Arterial Calcification of Infancy (GACI), Idiopathic Infantile Arterial Calcification (IIAC), Ossification of the Posterior Longitudinal Ligament (OPLL), hypophosphatemic rickets, osteoarthritis, calcification of atherosclerotic plaques, PXE, hereditary and non-hereditary forms of osteoarthritis, ankylosing spondylitis, hardening of the arteries occurring with aging, calci-phylaxis resulting from end stage renal disease and progeria.



## TITLE OF THE INVENTION

Compositions for Treating Pathological Calcification Conditions, and Methods Using Same

## CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Patent Application No. 62/163,500, filed May 19, 2015, which is hereby incorporated by reference in its entirety.

## BACKGROUND OF THE INVENTION

Generalized arterial calcification of infancy (GACI) is an ultra-rare neonatal disease characterized by infantile onset of widespread arterial calcifications in large and medium sized vessels resulting in cardiovascular collapse and death in the neonatal period. The disease presents clinically with heart failure, respiratory distress, hypertension, cyanosis, and cardiomegaly. The prognosis is grave, with older reports of a mortality rate of 85% at six months, while recently intensive treatment with bisphosphonates has lowered mortality to 55% at six months. Tempering this apparent progress is the severe skeletal toxicity associated with prolonged use of etridonate in patients with GACI, the observation that the limited available data makes it difficult to determine if bisphosphonate treatment is truly protective or reflects the natural history of the disease in less effected patients, and the ineffectiveness of bisphosphonates to prevent mortality in some patients even when instituted early.

The overall incidence of GACI is rare, with 200 reported cases in the medical literature and a disease frequency of one in 391,000. Although the disease was first described by Bryant and White in 1901, it was not until 2000 that Rutsch and colleagues noted that serum PPI levels and ENPP1 enzymatic activity was significantly impaired in GACI patients. ENPP1 (also known as NPP1 or PC-1) is a member of the ectonucleotide pyrophosphatase/ phosphodiesterase (also known as ENPP or NPP) family of enzymes, which are characterized by phosphodiesterase activity, and is a type II extracellular membrane bound glycoprotein located on the mineral-depositing matrix vesicles of osteoblasts and chondrocytes, as well as the vascular surface of cerebral capillaries. ENPP1 catabolizes the degradation of extracellular ATP into AMP and PPI. PPI inhibits ectopic tissue mineralization, presumably by occupying some of the Pi sites on the

surface of nascent or growing hydroxyapatite (HA) crystals, thereby creating irregularities that slow or terminate the propagation of crystal growth. Inactivating mutations in ENPP1 account for 75% of GACI patients, and a sizable fraction of the remaining patients result from inactivating mutations in the ATP dependent membrane transporter MRP6, encoded by the *abcc6* gene. Mutations in *abcc6* have been linked to decreased extracellular concentrations of nucleoside triphosphates, thereby limiting ENPP1's metabolism of ATP into extracellular PPi.

Kidneys are integral to maintenance of normal bone and mineral metabolism, including excretion of phosphate. Patients with kidney failure are unable to appropriately regulate serum mineral balance and tend to retain phosphate that is absorbed from the various dietary components. A high serum level of phosphate is associated with excessive secretion of parathyroid hormone and a tendency to calcification of the soft tissues including the blood vessels.

In patients with kidney failure, excess removal of phosphate and pyrophosphate anions can occur during hemodialysis or peritoneal dialysis. Depletion of these anions from tissues and plasma leads to disorders of bone and mineral metabolism, including osteomalacia and calcification of soft tissues and bone disease. Pyrophosphate deficiency may be a risk factor for deposition of calcium into the small vessels of the skin, causing an inflammatory vasculitis called calciphylaxis that can lead to gangrene of the skin and underlying tissues, resulting in severe, chronic pain. Calciphylaxis may necessitate amputation of the affected limb and is commonly fatal, with no effective treatment for this condition. Ectopic calcification, if left untreated, results in increased morbidity and death. It is important to regulate the amount of pyrophosphate in the system and reduce the occurrence of calciphylaxis in patients.

In 2003, 19.5 million U.S. adults have chronic kidney disease (CKD), and 13.6 million had stage 2-5 CKD, as defined by the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF/DOQI). Adverse outcomes of chronic kidney disease can often be prevented or delayed through early detection and treatment.

The prevalence of end-stage renal disease (ESRD) is increasing at an alarming rate. In 2000, end stage kidney disease developed in over 90,000 people in the U.S. The population of patients on dialysis therapy or needing transplantation was 380,000 in 2003, and became 651,000 patients in 2010. Care for patients with ESRD already consumes more than \$18 billion per year in the U.S., a substantial burden for the health care system.



Calcific uremic arteriopathy (also known as CUA) is a fatal disease seen in patients with chronic kidney disease (CKD) on dialysis. Calcification of small arteries leads to ischemia of the tissue and skin, infarction and thrombosis, with patient mortality close to 80%. Currently there are 450,000 patients on dialysis in the U.S. who are at risk of acquiring CUA, and there is no FDA approved treatments for the disease. CUA has hallmarks resembling GACI and other disorders of calcification with exhibiting low levels of PPi and high levels of fibroblast growth factor 23 (or FGF23). In ESRD patients requiring dialysis, this calcification process is further accelerated, with an average life-expectancy of 5-6 years.

Pseudoxanthoma elasticum (PXE) is a heritable disorder characterized by mineralization of elastic fibers in skin, arteries and the retina, that result in dermal lesions with associated laxity and loss of elasticity, arterial insufficiency, cardiovascular disease and retinal hemorrhages leading to macular degeneration. Mutations associated with PXE are also located in the *abcc6* gene. The skin manifestations are among the most common characteristics of PXE, but the ocular and cardiovascular symptoms are responsible for the morbidity of the disease. Characteristic skin lesions (yellowish papules and plaques and laxity with loss of elasticity, typically seen on the face, neck, axilla, antecubital fossa, popliteal fossa, groin and periumbilical areas) are generally an early sign of PXE and result from an accumulation of abnormal mineralized elastic fibers in the mid-dermis and. They are usually detected during childhood or adolescence and progress slowly and often unpredictably. A PXE diagnosis can be confirmed by a skin biopsy that shows calcification of fragmented elastic fibers in the mid- and lower dermis.

Common cardiovascular complications of PXE are due to the presence of abnormal calcified elastic fibers in the internal elastic lamina of medium-sized arteries. The broad spectrum of phenotypes includes premature atherosclerotic changes, intimal fibroplasia causing angina or intermittent claudication or both, early myocardial infarction and hypertension. Fibrous thickening of the endocardium and atrioventricular valves can also result in restrictive cardiomyopathy. Approximately 10% of PXE patients also develop gastrointestinal bleeding and central nervous system complications (such as stroke and dementia) as a consequence of systemic arterial wall mineralization. In addition, renovascular hypertension and atrial septal aneurysm can be seen in PXE patients.

Conditions in which serum phosphate levels are reduced or elevated are referred to as hypophosphatemia and hyperphosphatemia, respectively. Hypophosphatemia, which often

results from renal phosphate wasting, is caused by a number of genetic disorders including X-linked hypophosphatemic rickets (XLH), hereditary hypophosphatemic rickets with hypercalciuria (HHRH), hypophosphatemic bone disease (HBD), and autosomal dominant hypophosphatemic rickets (ADHR). The exact molecular mechanisms by which proper serum phosphate concentrations are maintained are poorly understood, but it is crucial to maintain serum phosphate levels in order to alleviate symptoms of aforesaid diseases.

There is thus a need in the art for novel compositions and methods for treating diseases and disorders associated with pathological calcification and/or pathological ossification. Such compositions and methods should not undesirably disturb other physiologic processes. The present invention fulfills this need.

#### BRIEF SUMMARY OF THE INVENTION

The invention provides a compound of formula (I), or a salt or solvate thereof. The invention further provides a method of treating or preventing a disease or disorder associated with pathological calcification or pathological ossification in a subject in need thereof. The invention further provides a method of reducing or preventing cardiac calcifications, arterial calcifications and/or elastic fiber mineralizations in an infant afflicted with at least one disease or disorder selected from the group consisting of GACI and PXE.

In a first aspect there is provided a compound of formula (I), or a salt thereof: PROTEIN-Z-DOMAIN-X-Y (I), wherein in (I): PROTEIN comprises residues 23-849 of SEQ ID NO:20, residues 23-852 of SEQ ID NO:18, or residues 93-925 of SEQ ID NO:16; DOMAIN is selected from the group consisting of a human IgG Fc domain (Fc) and human serum albumin protein (ALB); X and Z are independently absent or a polypeptide comprising 1-20 amino acids; Y is absent; and the compound lacks a negatively-charged bone-targeting sequence.

In a second aspect there is provided a method of treating and/or preventing a disease or disorder associated with pathological calcification and/or pathological ossification in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of at least one compound of the first aspect, wherein the disease or disorder comprises at least one selected from the group consisting of Generalized Arterial Calcification of Infancy (GACI), Idiopathic Infantile Arterial Calcification (IIAC), Ossification of the Posterior

Longitudinal Ligament (OPLL), Chronic Kidney Disease (CKD), hypophosphatemic rickets, osteoarthritis, and calcification of atherosclerotic plaques.

In a third aspect there is provided use of at least one compound of the first aspect in the preparation of a medicament for treating and/or preventing a disease or disorder in a subject, wherein the disease or disorder comprises at least one selected from the group consisting of Generalized Arterial Calcification of Infancy (GACI), Idiopathic Infantile Arterial Calcification (IIAC), Ossification of the Posterior Longitudinal Ligament (OPLL), Chronic Kidney Disease (CKD), hypophosphatemic rickets, osteoarthritis, and calcification of atherosclerotic plaques.

In a fourth aspect there is provided a method of treating and/or preventing a disease or disorder associated with pathological calcification and/or pathological ossification in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of at least one compound of the first aspect, wherein the disease or disorder comprises at least one selected from the group consisting of PXE, hereditary and non-hereditary forms of osteoarthritis, ankylosing spondylitis, hardening of the arteries occurring with aging, calciphylaxis resulting from end stage renal disease (ESRD) and progeria.

In a fifth aspect there is provided use of at least one compound of the first aspect in the preparation of a medicament for treating and/or preventing a disease or disorder in a subject, wherein the disease or disorder comprises at least one selected from the group consisting of PXE, hereditary and non-hereditary forms of osteoarthritis, ankylosing spondylitis, hardening of the arteries occurring with aging, calciphylaxis resulting from end stage renal disease (ESRD) and progeria.

In a sixth aspect there is provided a method of reducing and/or preventing cardiac calcifications, arterial calcifications and/or elastic fiber mineralizations in a subject afflicted with at least one disease or disorder selected from the group consisting of GACI and PXE, the method comprising administering to the subject a therapeutically effective amount of at least one compound of the first aspect, whereby the administering of the at least one compound increases extracellular pyrophosphate (PPi) concentrations in the subject, thus reducing or preventing cardiac calcifications, arterial calcifications and/or elastic fiber mineralization in the subject.

In a seventh aspect there is provided use of at least one compound of the first aspect in the preparation of a medicament for reducing and/or preventing cardiac calcifications,

arterial calcifications and/or elastic fiber mineralizations in a subject, whereby administering of the medicament to the subject increases extracellular pyrophosphate (PPi) concentrations in the subject, thus reducing or preventing cardiac calcifications, arterial calcifications and/or elastic fiber mineralization in the subject.

In an eighth aspect there is provided a method of reducing and/or preventing cardiac calcifications, arterial calcifications and/or elastic fiber mineralizations in a subject afflicted with at least one disease or disorder selected from the group consisting of GACI and PXE, the method comprising administering to the infant a therapeutically effective amount of a given polypeptide comprising a ENPP1 polypeptide and an ALB, wherein the given polypeptide lacks a polyaspartic acid domain, wherein the administering increases extracellular PPi concentrations in the subject, thus reducing or preventing cardiac calcifications, arterial calcifications and/or elastic fiber mineralization in the subject.

In a ninth aspect there is provided use of a polypeptide comprising a ENPP1 polypeptide and an ALB, wherein the given polypeptide lacks a polyaspartic acid domain in the preparation of a medicament for reducing and/or preventing cardiac calcifications, arterial calcifications and/or elastic fiber mineralizations in a subject afflicted with at least one disease or disorder selected from the group consisting of GACI and PXE, wherein the polypeptide lacks a polyaspartic acid domain, and wherein administering the medicament to the subject increases extracellular PPi concentrations in the subject, thus reducing or preventing cardiac calcifications, arterial calcifications and/or elastic fiber mineralization in the subject

In a tenth aspect there is provided a method of reducing and/or preventing cardiac calcifications, arterial calcifications and/or elastic fiber mineralizations in a subject afflicted with a disease characterized by having a loss of function mutation in ABCC6, the method comprising administering to the subject a therapeutically effective amount of at least one compound of the first aspect, thereby reducing and/or preventing cardiac calcifications, arterial calcifications and/or elastic fiber mineralizations in the subject.

In an eleventh aspect there is provided use of at least one compound of the first aspect in the preparation of a medicament for reducing and/or preventing cardiac calcifications, arterial calcifications and/or elastic fiber mineralizations in a subject afflicted with a disease characterized by having a loss of function mutation in ABCC6.

In a twelfth aspect there is provided a method of reducing and/ or preventing cardiac calcifications, arterial calcifications and/or elastic fiber mineralizations in a subject afflicted with a disease characterized by having a loss of function mutation in ENPP1, the method comprising administering to the subject a therapeutically effective amount of at least one compound of the first aspect, thereby reducing and/ or preventing cardiac calcifications, arterial calcifications and/or elastic fiber mineralizations in the subject.

In a thirteenth aspect there is provided use of at least one compound of the first aspect in the preparation of a medicament for reducing and/ or preventing cardiac calcifications, arterial calcifications and/or elastic fiber mineralizations in a subject afflicted with a disease characterized by having a loss of function mutation in ENPP1.

In certain embodiments, the compound of formula (I) is PROTEIN-Z-DOMAIN-X-Y (I), wherein in (I): PROTEIN is selected from the group consisting of SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, and SEQ ID NO:24; DOMAIN is selected from the group consisting of a human IgG Fc domain (also referred to as Fc), human serum albumin protein (also referred to as ALB) and fragment thereof; X and Z are independently absent or a polypeptide comprising 1-20 amino acids; and, Y is absent or is a sequence selected from the group consisting of: (DSS)<sub>n</sub> (SEQ ID NO:4), (ESS)<sub>n</sub> (SEQ ID NO:5), (RQQ)<sub>n</sub> (SEQ ID NO:6), (KR)<sub>n</sub> (SEQ ID NO:7), R<sub>m</sub> (SEQ ID NO:8), DSSSEKFLRRIGRFG (SEQ ID NO:9), EEEEEEPRGDT (SEQ ID NO:10), APWHLSSQYSRT (SEQ ID NO:11), STLPIPHEFSRE (SEQ ID NO:12), VTKHLNQISQSY (SEQ ID NO:13), and E<sub>m</sub> (SEQ ID NO:14), wherein m is an integer ranging from 1 to 15, and wherein n is an integer ranging from 1 to 10.

In certain embodiments, DOMAIN is a Fc or fragment thereof. In other embodiments, DOMAIN is an ALB or fragment thereof.

In certain embodiments, Y is absent and the compound lacks a negatively-charged bone-targeting sequence.

In certain embodiments, the PROTEIN has a mutation in at least one position selected from the group consisting of Ser 532, Tyr 529, Tyr 451, Ile 450, Ser 381, Tyr 382, Ser 377, Phe 346, Gly 531, Ser 289, Ser 287, Ala 454, Gly 452, Gln 519, Glu 526, Lys 448, Glu 508, Arg 456, Asp 276, Tyr 434, Gln 519, Ser 525, Gly 342, Ser 343 and Gly 536, relative to SEQ ID NO:1. In other embodiments, the nuclease domain of the PROTEIN or mutant thereof is absent. In yet other embodiments, the nuclease domain from about residue 524 to about residue 885 relative to SEQ ID NO:1 is absent in the PROTEIN or mutant thereof. In yet other  
10   embodiments, a segment of the extracellular region of NNP2 containing a furin or signal peptide cleavage site is, or is not, substituted into the PROTEIN or mutant thereof.

In certain embodiments, DOMAIN is a Fc or fragment thereof, and wherein PROTEIN-Z-DOMAIN comprises (SEQ ID NO:15)-Z-(Fc or fragment thereof), (SEQ ID NO:17)-Z-(Fc or fragment thereof), (SEQ ID NO:19)-Z-(Fc or fragment thereof), (SEQ ID NO:24)-Z-(Fc or fragment thereof), or a mutant thereof comprising at least one mutation in at least one position selected from the group consisting of Ser 532, Tyr 529, Tyr 451, Ile 450, Ser 381, Tyr 382, Ser 377, Phe 346, Gly 531, Ser 289, Ser 287, Ala 454, Gly 452, Gln 519, Glu 526, Lys 448, Glu 508, Arg 456, Asp 276, Tyr 434, Gln 519, Ser 525, Gly 342, Ser 343 and Gly 536, relative to SEQ ID NO:1.  
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In certain embodiments, PROTEIN-Z-DOMAIN comprises SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, (SEQ ID NO:24)-Z-(SEQ ID NO:26), or a mutant thereof comprising at least one mutation in at least one position selected from the group consisting of Ser 532, Tyr 529, Tyr 451, Ile 450, Ser 381, Tyr 382, Ser 377, Phe 346, Gly 531, Ser 289, Ser 287, Ala 454, Gly 452, Gln 519, Glu 526, Lys 448, Glu 508, Arg 456, Asp 276, Tyr 434, Gln 519, Ser 525, Gly 342, Ser 343 and Gly 536, relative to SEQ ID NO:1.  
20   25

In certain embodiments, DOMAIN is an ALB or fragment thereof, and wherein PROTEIN-Z-DOMAIN comprises (SEQ ID NO:15)-Z-(ALB or fragment thereof), (SEQ ID NO:17)-Z-(ALB or fragment thereof), (SEQ ID NO:19)-Z-(ALB or fragment thereof), (SEQ ID NO:24)-Z-(ALB or fragment thereof), or a mutant thereof comprising at least one mutation in at least one position selected from the group consisting of Ser 532, Tyr 529, Tyr 451, Ile 450, Ser 381, Tyr 382, Ser 377, Phe 346, Gly 531, Ser 289, Ser 287, Ala 454, Gly 452, Gln 519, Glu 526,  
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Lys 448, Glu 508, Arg 456, Asp 276, Tyr 434, Gln 519, Ser 525, Gly 342, Ser 343 and Gly 536, relative to SEQ ID NO:1.

In certain embodiments, PROTEIN-Z-DOMAIN comprises SEQ ID NO:21, (SEQ ID N:17)-Z-(SEQ ID NO:27), SEQ ID NO:22, SEQ ID NO:25, or a mutant thereof comprising at least one mutation in at least one position selected from the group consisting of Ser 532, Tyr 529, Tyr 451, Ile 450, Ser 381, Tyr 382, Ser 377, Phe 346, Gly 531, Ser 289, Ser 287, Ala 454, Gly 452, Gln 519, Glu 526, Lys 448, Glu 508, Arg 456, Asp 276, Tyr 434, Gln 519, Ser 525, Gly 342, Ser 343 and Gly 536, relative to SEQ ID NO:1.

In certain embodiments, the compound has a  $k_{cat}$  value greater than or equal to about  $3.4 (\pm 0.4) \text{ s}^{-1} \text{ enzyme}^{-1}$ , wherein the  $k_{cat}$  is determined by measuring the compound's ATP hydrolysis rate.

In certain embodiments, the compound has a  $K_M$  value less than or equal to about  $2 \mu\text{M}$ , wherein the  $K_M$  is determined by measuring the compound's ATP hydrolysis rate.

In certain embodiments, the NPP1 polypeptide is a cleavage product of a precursor NPP1 polypeptide comprising an ecto-nucleotide pyrophosphate/phosphodiesterase-2 (NPP2) transmembrane domain.

In certain embodiments, the NPP2 transmembrane domain is residues 12-30 of NCBI accession no. NP\_001124335 (SEQ ID NO:2), which corresponds to SEQ ID NO:23.

In certain embodiments, the method comprises administering to the subject a therapeutically effective amount of at least one compound of the invention.

In certain embodiments, the disease comprises at least one selected from the group consisting of Generalized Arterial Calcification of Infancy (GACI), Idiopathic Infantile Arterial Calcification (IIAC), Ossification of the Posterior Longitudinal Ligament (OPLL), hypophosphatemic rickets, osteoarthritis, and calcification of atherosclerotic plaques.

In certain embodiments, the disease comprises at least one selected from the group consisting of PXE, hereditary and non-hereditary forms of osteoarthritis, ankylosing spondylitis, hardening of the arteries occurring with aging, calciphylaxis resulting from end stage renal disease and progeria.

In certain embodiments, Y is absent and the compound lacks a negatively-charged bone-targeting sequence.

In certain embodiments, the method comprises administering to the infant a

therapeutically effective amount of a given polypeptide comprising an ecto-nucleotide pyrophosphate/phosphodiesterase-1 (NPP1) polypeptide and an IgG Fc domain, wherein the given polypeptide lacks a polyaspartic acid domain, whereby the administering of the given polypeptide increases extracellular pyrophosphate (PPi) concentrations in the infant.

5 In certain embodiments, the method comprises administering to the infant a therapeutically effective amount of a given polypeptide comprising an ecto-nucleotide pyrophosphate/phosphodiesterase-1 (NPP1) polypeptide and an ALB, wherein the given polypeptide lacks a polyaspartic acid domain, whereby the administering of the given polypeptide increases extracellular pyrophosphate (PPi) concentrations in the infant.

10 In certain embodiments, the administering is at least one selected from the group consisting of inhalational, oral, nasal, rectal, parenteral, sublingual, transdermal, transmucosal (*e.g.*, sublingual, lingual, (trans)buccal, (trans)urethral, vaginal (*e.g.*, trans- and perivaginally), (intra)nasal, and (trans)rectal), intravesical, intrapulmonary, intraduodenal, intragastrical, intrathecal, subcutaneous, intramuscular, intradermal, intra-arterial, intravenous, intrabronchial, 15 inhalation, and topical. In other embodiments, the administering is subcutaneous.

In certain embodiments, the administering restores the infant's extracellular pyrophosphate concentrations to a level within the range found in an infant not afflicted with GACI and/or PXE.

20 In certain embodiments, the infant presents and/or is diagnosed with "failure to thrive" prior to the administering.

## BRIEF DESCRIPTION OF THE DRAWINGS

The following detailed description of illustrative embodiments of the invention will be better understood when read in conjunction with the appended drawings. For the purpose 25 of illustrating the invention, there are shown in the drawings specific embodiments. It should be understood, however, that the invention is not limited to the precise arrangements and instrumentalities of the embodiments shown in the drawings.

FIGs. 1A-1G comprise a set of images and graphs illustrating a natural history study. FIG. 1A: Average daily weights of ENPP1-asj/asj and ENPP1-WT sibling pairs on acceleration diet. Daily weights of ENPP1-WT (Cyan squares) and ENPP1-asj/asj mice (Green 30 circles) on the acceleration diet over a 70 day period. A failure to thrive point is noted in the



ENPP1-asj/asj cohort at day 26, when the weights diverge from ENPP1-WT. Death events are marked with red arrows. FIG. 1B: Survival Curves, Natural History Study. Mean survival of ENPP1-asj/asj was 58 days. No deaths were observed in the ENPP1-WT cohort. FIG. 1C: Representative Micro-CT and histology, Natural History Study. Some asj/asj animals displayed dramatic calcifications in heart and aorta visible on E). Aortas of ENPP1-asj/asj mouse all possessed near circumferential calcifications that were pervasive in the vascular walls, as illustrated by Alzarian red staining of the aortas. FIG. 1F: Histology of asj/asj mice, Left Ventricle (40x). Extensive calcifications surrounded by scar tissue revealing the presence of repeated, old, healed myocardial infarctions. FIG. 1G: Histology of asj/asj mice, Septum (100X). More typically, the asj mice displayed small foci of calcifications with surrounding scar tissue as seen here in the myocardial septum, also diagnostic of previous myocardial infarctions.

FIGs. 2A-2E comprise a set of images and graphs illustrating a metabolic pathway, as well as design, stability, and kinetic properties of a therapeutic protein of the invention. FIG. 2A: Schematic of the metabolic pathway of purinergic metabolism related to ectopic calcification. ENPP1 converts extracellular ATP into AMP and PPi, TNAP converts PPi into Pi, and CD73 converts AMP into adenosine and Pi. The *abcc6* gene encodes MRP6, a membrane transporter that increases the extracellular concentration of ATP. Loss of function mutations in TNAP result in familial hypophosphatasia. Loss of function mutations in ENPP1 result in GACI, loss of function mutations in MRP6 result in PXE, and loss of function mutations in CD73 results in a disease of arterial and joint calcification termed 'ACDC'. FIG. 2B: Design of ENPP1 protein therapeutic. To produce a soluble recombinant protein, a segment of the extracellular region of NPP2 containing a furin cleavage site was substituted into ENPP1 as previously described, and the protein was C-terminally fused with the Fc domain of human immunoglobulin 1 (IgG1). FIG. 2C: Stability of ENPP1 therapeutic. ENPP1-Fc Ap3A activity was seen to be stable to freeze-thaw cycle in PBS following storage at -80° C. FIGs. 2D-2E: Steady state kinetics of hENPP1-Fc. FIG. 2D: Time courses of AMP formation measured by HPLC analysis after addition of 10 nM hNPP1-Fc to (from bottom to top) 1.0, 2.0, 7.8, 125 and 250 μM ATP. The smooth curves though data are fits obtained by non-linear kinetic time course analysis. The insert shows the lower [ATP] time courses in Panel A, (from bottom to top) 1.0, 2.0, 7.8 μM ATP. The time course of 1.0 μM ATP shows the ATP was depleted completely after 1 min and therefore the rate was not able to be determined. FIG. 2E: ATP concentration

dependent initial ATP hydrolysis rate per enzyme. The initial rates after 7.8  $\mu\text{M}$  are essentially the same with  $k_{cat}$  (the average) =  $3.4 (\pm 0.4) \text{ s}^{-1} \text{ enzyme}^{-1}$ . The initial rate at 2.0  $\mu\text{M}$  ATP concentration is about a half of  $k_{cat}$  value, and therefore  $K_M \sim 2 \mu\text{M}$  is estimated for ATP hydrolysis by hNPP1-Fc protein.

5                    FIGs. 3A-3D comprise a set of images and graphs illustrating a proof of concept study. FIG. 3A: Daily animal weights. Average daily weights of ENPP1-WT and ENPP1-asj/asj sibling pairs dosed with vehicle (daily PBS injections supplemented with weekly GK 1.5) compared to ENPP1-asj/asj sibling pairs dosed with daily with mouse ENPP1-Fc (mENPP1-Fc) @ 500 au/Kg qD in PBS and weekly GK1.5 immunosuppression. Dosing and weighing  
10 commenced on day 14. Deaths in the ENPP1-asj/asj + vehicle cohort are denoted by red arrows on the day of death. No deaths were noted in the ENPP1-WT + vehicle or ENPP1-asj/asj + ENPP1-Fc cohort. FIG. 3B: Survival Curves, Proof of Concept Study. FIG. 3C: Left ventricle histology, (40x, H&E), untreated asj/asj mouse displaying large focus of calcifications and micro-infarctions in the free wall. FIG. 3D: Left ventricle histology, (40x, H&E), treated asj/asj  
15 mouse. None of the treated ENPP1-asj mice displayed abnormal Left Ventricular Histology.

FIGs. 4A-4G comprise a set of images illustrating representative histology and a proof of concept study. Figs 4A-4B: Aorta (40x, alzarian red). Untreated ENPP1-asj mice (FIG. 4A) displayed nearly circumferential aortic calcifications, while treated ENPP1-asj mice (FIG. 4B) did not. FIG. 4C: Untreated ENPP1-asj/asj mice, Right Ventricle (40x, H&E). Two  
20 untreated ENPP1-asj mice had large, confluent, myocardial infarctions in the free wall of the Right Ventricle. FIG. 4D: Treated ENPP1-asj/asj mice, Right Ventricle (40x, H&E). All treated ENPP1-asj mice displayed normal Right. ventricle myocardium. FIG. 4E: Untreated ENPP1-asj/asj mice, Coronary Arteries (100x, H&E). All untreated ENPP1-asj/asj mice had coronary calcifications, with most displaying circumferential calcifications in coronary arteries  
25 surrounded by scar tissue, diagnostic of ischemia and myocardial infarction. FIG. 4F: Untreated ENPP1-asj/asj mice, Myocardial Septum (100x, H&E). Nearly all animals (77%) displayed intracardiac calcifications surrounded by scar tissue, as demonstrated in this animal in the myocardial septum. FIG. 4G: Phenotypic comparison, treated and untreated ENPP1-asj/asj mice. There is a dramatic size difference in the treated and untreated animals, and a marked  
30 difference in the mobility and health of the animals, best seen in the movie submitted in the supplemental data.

FIGs. 5A-5F comprise a set of images and graphs illustrating biomarkers of disease response. FIG. 5A: Postmortem high-resolution micro-CT scans revealed extensive calcifications in untreated ENPP1-asj/asj mice in the hearts, coronary arteries, and ascending and descending aortas, but absolutely no calcifications in these organs in the treated ENPP1-asj/asj cohort or in ENPP1-WT mice. FIG. 5B: Plasma [PPi] in ENPP1-WT and treated and untreated ENPP1-asj/asj animals revealed that treatment with ENPP1-FC increased [PPi] in ENPP1-asj/asj mice above WT levels, and well above the nearly undetectable levels present in untreated ENPP1-asj/asj mice. FIGs. 5C-5D: Percent uptake of injected <sup>99m</sup>PYP in heads of WT and asj/asj animals. The % uptake of <sup>99m</sup>PYP in heads of animals in the natural history study were recorded weekly in the WT and asj/asj animals on the acceleration diet, demonstrating that <sup>99m</sup>PYP uptake remains nearly constant over an 80 day period following birth, but differs markedly between the two experimental groups. FIG. 5D: In the natural history study, the average <sup>99m</sup>PYP uptake in heads of WT animals was around 15% of injected dose over the 80 day period, while the PYP uptake in asj/asj animals was around 20% (p<0.001, students 2-tailed T-test). FIGs. 5E-5F: Percent <sup>99m</sup>PYP uptake of injected dose in the heads of WT, and treated and untreated asj/asj mice. <sup>99m</sup>PYP uptake was recorded in the middle of the study (day 30-35, (FIG. 5e)) and at the end of the study (day 50-65, FIG. 5F) in the experimental groups. WT and treated asj/asj animals had percent uptake in the skulls around 15%, while the untreated ENPP1-asj/asj cohort was at or above 20%. The difference between treated and untreated ENPP1-asj mice was statistically significant (p<0.001, students 2-tailed T-test), while the difference between WT and treated ENPP1-asj mice was not.

FIG. 6, comprising panels a-h, illustrates certain non-limiting constructs of NPP1 fusion proteins. X and Y are optional peptides in some embodiments. Z is an optional linker that connects either Fc domain or HSA domain to the C terminus of NPP1 protein. The N and C terminal regions of NPP1 protein are depicted as N and C in FIG. 6. Panels a-d illustrate fusion proteins comprising transmembrane domain of NPP2 (marked as '\*') and NPP1 (marked as '\*\*') along with NPP1 enzymatic domain. The enzymatic domain of NPP1 begins as PSCAKE amino acid sequence and ends at QED amino acid sequence. Panels e-h illustrate fusion proteins comprising signal peptide of NPP2 (marked as '\*') and transmembrane domain of NPP1 (marked as '\*\*') along with NPP1 enzymatic domain.

FIG. 7 is a graph illustrating measured plasma PPi levels in mice treated with

ENPP1-Fc as described in Example 1.

FIG. 8 is a schematic illustration of a plasmid used to express SEQ ID NO:22.

FIG. 9 is a schematic illustration of a plasmid used to express SEQ ID NO:25.

FIG. 10 is an image illustrating a silver staining image from purified human and  
5 mouse NPP1-Fc constructs.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the discovery that certain NPP1-containing polypeptides, mutants, or mutant fragments thereof, are useful for the treatment of diseases and  
10 disorders involving plasma pyrophosphate imbalance, pathological calcification and/or pathological ossification. Diseases and disorders involving pathological calcification and/or pathological ossification treatable by the compositions and methods of the invention, include, but are not limited to Generalized Arterial Calcification of Infancy (GACI), Chronic Kidney Disease (CKD), End Stage Renal Disease (ESRD), Idiopathic Infantile Arterial Calcification (IIAC),  
15 Ossification of the Posterior Longitudinal Ligament (OPLL), hypophosphatemic rickets, calcification of atherosclerotic plaques, Pseudoxanthoma elasticum (PXE), hereditary and non-hereditary forms of osteoarthritis, ankylosing spondylitis, hardening of the arteries occurring with aging, calciphylaxis (such as resulting from end stage renal disease) and progeria.

Such diseases are a result of myriad causes: some are genetic mutations and some  
20 are complication as a result of diabetes, heart failure or extensive dialysis. Yet, in certain embodiments, they share in common the symptom of plasma pyrophosphate imbalance and/or extensive calcification.

### Definitions

25 Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, illustrative methods and materials are described.

30 As used herein, each of the following terms has the meaning associated with it in this section.

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

5 “About” as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of  $\pm 20\%$  or  $\pm 10\%$ , in certain embodiments  $\pm 5\%$ , in certain embodimentse  $\pm 1\%$ , in certain embodimentse  $\pm 0.1\%$  from the specified value, as such variations are appropriate to perform the disclosed methods.

10 The term “abnormal” when used in the context of organisms, tissues, cells or components thereof, refers to those organisms, tissues, cells or components thereof that differ in at least one observable or detectable characteristic (*e.g.*, age, treatment, time of day, etc.) from those organisms, tissues, cells or components thereof that display the “normal” (expected) respective characteristic. Characteristics which are normal or expected for one cell or tissue type, might be abnormal for a different cell or tissue type.

As used herein, the term “ALB” refers to a human serum albumin protein.

15 A disease or disorder is “alleviated” if the severity of a symptom of the disease or disorder, the frequency with which such a symptom is experienced by a patient, or both, is reduced.

As used herein the terms “alteration,” “defect,” “variation” or “mutation” refer to a mutation in a gene in a cell that affects the function, activity, expression (transcription or translation) or conformation of the polypeptide it encodes. Mutations encompassed by the present invention can be any mutation of a gene in a cell that results in the enhancement or disruption of the function, activity, expression or conformation of the encoded polypeptide, including the complete absence of expression of the encoded protein and can include, for example, missense and nonsense mutations, insertions, deletions, frameshifts and premature terminations. Without being so limited, mutations encompassed by the present invention may alter splicing the mRNA (splice site mutation) or cause a shift in the reading frame (frameshift).

25 The term “amino acid sequence variant” refers to polypeptides having amino acid sequences that differ to some extent from a native sequence polypeptide. Ordinarily, amino acid sequence variants possess at least about 70% homology, at least about 80% homology, at least about 90% homology, or at least about 95% homology to the native polypeptide. The amino acid sequence variants possess substitutions, deletions, and/or insertions at certain positions within

the amino acid sequence of the native amino acid sequence.

The term “antibody,” as used herein, refers to an immunoglobulin molecule which is able to specifically bind to a specific epitope on an antigen. Antibodies can be intact immunoglobulins derived from natural sources or from recombinant sources and can be immunoreactive portions of intact immunoglobulins. The antibodies in the present invention may exist in a variety of forms including, for example, polyclonal antibodies, monoclonal antibodies, intracellular antibodies (“intrabodies”), Fv, Fab and F(ab)<sub>2</sub>, as well as single chain antibodies (scFv), heavy chain antibodies, such as camelid antibodies, synthetic antibodies, chimeric antibodies, and a humanized antibodies (Harlow, *et al.*, 1999, Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, NY; Harlow, *et al.*, 1989, Antibodies: A Laboratory Manual, Cold Spring Harbor, New York; Houston, *et al.*, 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; Bird, *et al.*, 1988, Science 242:423-426).

As used herein, the term “Ap3P” refers to adenosine-(5′)-triphospho-(5′)-adenosine or a salt thereof.

As used herein, the terms “child” and “infant” are used interchangeably.

The term “coding sequence,” as used herein, means a sequence of a nucleic acid or its complement, or a part thereof, that can be transcribed and/or translated to produce the mRNA and/or the polypeptide or a fragment thereof. Coding sequences include exons in a genomic DNA or immature primary RNA transcripts, which are joined together by the cell’s biochemical machinery to provide a mature mRNA. The anti-sense strand is the complement of such a nucleic acid, and the coding sequence can be deduced therefrom. In contrast, the term “non-coding sequence,” as used herein, means a sequence of a nucleic acid or its complement, or a part thereof, that is not translated into amino acid in vivo, or where tRNA does not interact to place or attempt to place an amino acid. Non-coding sequences include both intron sequences in genomic DNA or immature primary RNA transcripts, and gene-associated sequences such as promoters, enhancers, silencers, and the like.

As used herein, the terms “complementary” or “complementarity” are used in reference to polynucleotides (*i.e.*, a sequence of nucleotides) related by the base-pairing rules. For example, the sequence “A-G-T,” is complementary to the sequence “T-C-A.”

Complementarity may be “partial,” in which only some of the nucleic acids’ bases are matched according to the base pairing rules. Or, there may be “complete” or “total” complementarity

between the nucleic acids. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. This is of particular importance in amplification reactions, as well as detection methods that depend upon binding between nucleic acids.

5 As used herein, the terms “conservative variation” or “conservative substitution” as used herein refers to the replacement of an amino acid residue by another, biologically similar residue. Conservative variations or substitutions are not likely to change the shape of the peptide chain. Examples of conservative variations, or substitutions, include the replacement of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the  
10 substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine.

A “disease” is a state of health of an animal wherein the animal cannot maintain homeostasis, and wherein if the disease is not ameliorated then the animal’s health continues to deteriorate.

15 A “disorder” in an animal is a state of health in which the animal is able to maintain homeostasis, but in which the animal’s state of health is less favorable than it would be in the absence of the disorder. Left untreated, a disorder does not necessarily cause a further decrease in the animal’s state of health.

As used herein, the term “domain” refers to a part of a molecule or structure that  
20 shares common physicochemical features, such as, but not limited to, hydrophobic, polar, globular and helical domains or properties. Specific examples of binding domains include, but are not limited to, DNA binding domains and ATP binding domains.

As used herein, the terms “effective amount,” “pharmaceutically effective amount” and “therapeutically effective amount” refer to a nontoxic but sufficient amount of an  
25 agent to provide the desired biological result. That result may be reduction and/or alleviation of the signs, symptoms, or causes of a disease, or any other desired alteration of a biological system. An appropriate therapeutic amount in any individual case may be determined by one of ordinary skill in the art using routine experimentation.

“Encoding” refers to the inherent property of specific sequences of nucleotides in  
30 a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of

nucleotides (*i.e.*, rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as encoding the protein or other product of that gene or cDNA.

As used herein, the term “Fc” refers to a human IgG Fc domain.

As used herein, the term “failure to thrive” refers to a child or infant whose current weight or rate of weight gain is lower than that of other children of similar age and gender. The situation where a child or infant “fails to thrive” can be identified by consultation with a medical specialist, and/or comparison of the child’s or infant’s weight or weight gain rate with known average age-specific weight or weight gain rate data.

As used herein, the term “fragment,” as applied to a nucleic acid, refers to a subsequence of a larger nucleic acid. A “fragment” of a nucleic acid can be at least about 15 nucleotides in length; for example, at least about 50 nucleotides to about 100 nucleotides; at least about 100 to about 500 nucleotides, at least about 500 to about 1000 nucleotides; at least about 1000 nucleotides to about 1500 nucleotides; about 1500 nucleotides to about 2500 nucleotides; or about 2500 nucleotides (and any integer value in between). As used herein, the term “fragment,” as applied to a protein or peptide, refers to a subsequence of a larger protein or peptide. A “fragment” of a protein or peptide can be at least about 20 amino acids in length; for example, at least about 50 amino acids in length; at least about 100 amino acids in length; at least about 200 amino acids in length; at least about 300 amino acids in length; or at least about 400 amino acids in length (and any integer value in between).

“Homologous” refers to the sequence similarity or sequence identity between two polypeptides or between two nucleic acid molecules. When a position in both of the two compared sequences is occupied by the same base or amino acid monomer subunit, *e.g.*, if a position in each of two DNA molecules is occupied by adenine, then the molecules are homologous at that position. The percent of homology between two sequences is a function of the number of matching or homologous positions shared by the two sequences divided by the number of positions compared X 100. For example, if 6 of 10 of the positions in two sequences



are matched or homologous then the two sequences are 60% homologous. By way of example, the DNA sequences ATTGCC and TATGGC share 50% homology. Generally, a comparison is made when two sequences are aligned to give maximum homology.

As used herein, an “immunoassay” refers to any binding assay that uses an antibody capable of binding specifically to a target molecule to detect and quantify the target molecule.

The term “immunoglobulin” or “Ig,” as used herein is defined as a class of proteins, which function as antibodies. Antibodies expressed by B cells are sometimes referred to as the BCR (B cell receptor) or antigen receptor. The five members included in this class of proteins are IgA, IgG, IgM, IgD, and IgE. IgA is the primary antibody that is present in body secretions, such as saliva, tears, breast milk, gastrointestinal secretions and mucus secretions of the respiratory and genitourinary tracts. IgG is the most common circulating antibody. IgM is the main immunoglobulin produced in the primary immune response in most subjects. It is the most efficient immunoglobulin in agglutination, complement fixation, and other antibody responses, and is important in defense against bacteria and viruses. IgD is the immunoglobulin that has no known antibody function, but may serve as an antigen receptor. IgE is the immunoglobulin that mediates immediate hypersensitivity by causing release of mediators from mast cells and basophils upon exposure to allergen.

“Instructional material,” as that term is used herein, includes a publication, a recording, a diagram, or any other medium of expression which can be used to communicate the usefulness of the nucleic acid, peptide, and/or compound of the invention in the kit for identifying or alleviating or treating the various diseases or disorders recited herein. Optionally, or alternately, the instructional material may describe one or more methods of identifying or alleviating the diseases or disorders in a cell or a tissue of a subject. The instructional material of the kit may, for example, be affixed to a container that contains the nucleic acid, polypeptide, and/or compound of the invention or be shipped together with a container that contains the nucleic acid, polypeptide, and/or compound. Alternatively, the instructional material may be shipped separately from the container with the intention that the recipient uses the instructional material and the compound cooperatively.

“Isolated” means altered or removed from the natural state. For example, a nucleic acid or a polypeptide naturally present in a living animal is not “isolated,” but the same

nucleic acid or polypeptide partially or completely separated from the coexisting materials of its natural state is "isolated." An isolated nucleic acid or protein can exist in substantially purified form, or can exist in a non-native environment such as, for example, a host cell.

An "isolated nucleic acid" refers to a nucleic acid segment or fragment which has  
5 been separated from sequences which flank it in a naturally occurring state, *e.g.*, a DNA fragment which has been removed from the sequences which are normally adjacent to the fragment, *e.g.*, the sequences adjacent to the fragment in a genome in which it naturally occurs. The term also applies to nucleic acids which have been substantially purified from other components which naturally accompany the nucleic acid, *e.g.*, RNA or DNA or proteins, which  
10 naturally accompany it in the cell. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (*e.g.*, as a cDNA or a genomic or cDNA fragment produced by PCR or restriction enzyme digestion) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid  
15 gene encoding additional polypeptide sequence.

As used herein, the term "NPP" or "ENPP" refers to ectonucleotide pyrophosphatase/ phosphodiesterase.

A "nucleic acid" refers to a polynucleotide and includes poly-ribonucleotides and poly-deoxyribonucleotides. Nucleic acids according to the present invention may include any  
20 polymer or oligomer of pyrimidine and purine bases, preferably cytosine, thymine, and uracil, and adenine and guanine, respectively. See Albert L. Lehninger, Principles of Biochemistry, at 793-800 (Worth Pub. 1982) which is herein incorporated in its entirety for all purposes. Indeed, the present invention contemplates any deoxyribonucleotide, ribonucleotide or peptide nucleic acid component, and any chemical variants thereof, such as methylated, hydroxymethylated or  
25 glucosylated forms of these bases, and the like. The polymers or oligomers may be heterogeneous or homogeneous in composition, and may be isolated from naturally occurring sources or may be artificially or synthetically produced. In addition, the nucleic acids may be DNA or RNA, or a mixture thereof, and may exist permanently or transitionally in single-stranded or double-stranded form, including homoduplex, heteroduplex, and hybrid states.

30 An "oligonucleotide" or "polynucleotide" is a nucleic acid ranging from at least 2, in certain embodiments at least 8, 15 or 25 nucleotides in length, but may be up to 50, 100, 1000,

or 5000 nucleotides long or a compound that specifically hybridizes to a polynucleotide.

Polynucleotides include sequences of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) or mimetics thereof which may be isolated from natural sources, recombinantly produced or artificially synthesized. A further example of a polynucleotide of the present invention may be a peptide nucleic acid (PNA). (See U.S. Patent No. 6,156,501 which is hereby incorporated by reference in its entirety) The invention also encompasses situations in which there is a nontraditional base pairing such as Hoogsteen base pairing which has been identified in certain tRNA molecules and postulated to exist in a triple helix. "Polynucleotide" and "oligonucleotide" are used interchangeably herein. It is understood that when a nucleotide sequence is represented herein by a DNA sequence (*e.g.*, A, T, G, and C), this also includes the corresponding RNA sequence (*e.g.*, A, U, G, C) in which "U" replaces "T."

As used herein, the term "patient," "individual" or "subject" refers to a human or a non-human mammal. Non-human mammals include, for example, livestock and pets, such as ovine, bovine, porcine, canine, feline and murine mammals. In certain embodiments, the patient, individual or subject is human.

As used herein, the term "prevent" or "prevention" means no disorder or disease development if none had occurred, or no further disorder or disease development if there had already been development of the disorder or disease. Also considered is the ability of one to prevent some or all of the symptoms associated with the disorder or disease.

As used herein, the term "pharmaceutical composition" or "composition" refers to a mixture of at least one compound useful within the invention with a pharmaceutically acceptable carrier. The pharmaceutical composition facilitates administration of the compound to a patient. Multiple techniques of administering a compound exist in the art including, but not limited to, intravenous, oral, aerosol, inhalational, rectal, vaginal, transdermal, intranasal, buccal, sublingual, parenteral, intrathecal, intragastrical, ophthalmic, pulmonary and topical administration.

As used herein, the term "pharmaceutically acceptable" refers to a material, such as a carrier or diluent, which does not abrogate the biological activity or properties of the compound, and is relatively non-toxic, *i.e.*, the material may be administered to an individual without causing undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained.

As used herein, the term “pharmaceutically acceptable carrier” means a pharmaceutically acceptable material, composition or carrier, such as a liquid or solid filler, stabilizer, dispersing agent, suspending agent, diluent, excipient, thickening agent, solvent or encapsulating material, involved in carrying or transporting a compound useful within the invention within or to the patient such that it may perform its intended function. Typically, such constructs are carried or transported from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be “acceptable” in the sense of being compatible with the other ingredients of the formulation, including the compound useful within the invention, and not injurious to the patient. Some examples of materials that may serve as pharmaceutically acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; surface active agents; alginic acid; pyrogen-free water; isotonic saline; Ringer’s solution; ethyl alcohol; phosphate buffer solutions; and other non-toxic compatible substances employed in pharmaceutical formulations. As used herein, “pharmaceutically acceptable carrier” also includes any and all coatings, antibacterial and antifungal agents, and absorption delaying agents, and the like that are compatible with the activity of the compound useful within the invention, and are physiologically acceptable to the patient. Supplementary active compounds may also be incorporated into the compositions. The “pharmaceutically acceptable carrier” may further include a pharmaceutically acceptable salt of the compound useful within the invention. Other additional ingredients that may be included in the pharmaceutical compositions used in the practice of the invention are known in the art and described, for example in Remington’s Pharmaceutical Sciences (Genaro, Ed., Mack Publishing Co., 1985, Easton, PA), which is incorporated herein by reference.

As used herein, the language “pharmaceutically acceptable salt” refers to a salt of the administered compound prepared from pharmaceutically acceptable non-toxic acids and bases, including inorganic acids, inorganic bases, organic acids, inorganic bases, solvates,

hydrates, and clathrates thereof. Suitable pharmaceutically acceptable acid addition salts may be prepared from an inorganic acid or from an organic acid. Examples of inorganic acids include sulfate, hydrogen sulfate, hydrochloric, hydrobromic, hydriodic, nitric, carbonic, sulfuric, and phosphoric acids (including hydrogen phosphate and dihydrogen phosphate). Appropriate organic acids may be selected from aliphatic, cycloaliphatic, aromatic, araliphatic, heterocyclic, carboxylic and sulfonic classes of organic acids, examples of which include formic, acetic, propionic, succinic, glycolic, gluconic, lactic, malic, tartaric, citric, ascorbic, glucuronic, maleic, fumaric, pyruvic, aspartic, glutamic, benzoic, anthranilic, 4-hydroxybenzoic, phenylacetic, mandelic, embonic (pamoic), methanesulfonic, ethanesulfonic, benzenesulfonic, pantothenic, trifluoromethanesulfonic, 2-hydroxyethanesulfonic, p-toluenesulfonic, sulfanilic, cyclohexylaminosulfonic, stearic, alginic,  $\beta$ -hydroxybutyric, salicylic, galactaric and galacturonic acid. Suitable pharmaceutically acceptable base addition salts of compounds of the invention include, for example, metallic salts including alkali metal, alkaline earth metal and transition metal salts such as, for example, calcium, magnesium, potassium, sodium and zinc salts. Pharmaceutically acceptable base addition salts also include organic salts made from basic amines such as, for example, N,N'-dibenzylethylene-diamine, chlorprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine) and procaine. All of these salts may be prepared from the corresponding compound by reacting, for example, the appropriate acid or base with the compound.

As used herein, "polynucleotide" includes cDNA, RNA, DNA/RNA hybrid, antisense RNA, ribozyme, genomic DNA, synthetic forms, and mixed polymers, both sense and antisense strands, and may be chemically or biochemically modified to contain non-natural or derivatized, synthetic, or semi-synthetic nucleotide bases. Also, contemplated are alterations of a wild type or synthetic gene, including but not limited to deletion, insertion, substitution of one or more nucleotides, or fusion to other polynucleotide sequences.

As used herein, the term "polypeptide" refers to a polymer composed of amino acid residues, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof linked via peptide bonds. Synthetic polypeptides may be synthesized, for example, using an automated polypeptide synthesizer. As used herein, the term "protein" typically refers to large polypeptides. As used herein, the term "peptide" typically refers to short polypeptides. Conventional notation is used herein to represent polypeptide sequences: the left-

hand end of a polypeptide sequence is the amino-terminus, and the right-hand end of a polypeptide sequence is the carboxyl-terminus.

As used herein, amino acids are represented by the full name thereof, by the three letter code corresponding thereto, or by the one-letter code corresponding thereto, as indicated below: Aspartic Acid (Asp / D); Glutamic Acid (Glu / E); Lysine (Lys / K); Arginine (Arg / R); Histidine (His / H); Tyrosine (Tyr / Y); Cysteine (Cys / C); Asparagine (Asn / N); Glutamine (Gln / Q); Serine (Ser / S); Threonine (Thr / T); Glycine (Gly / G); Alanine (Ala / A); Valine (Val / V); Leucine (Leu / L); Isoleucine (Ile / I); Methionine (Met / M); Proline (Pro / P); Phenylalanine (Phe / F); Tryptophan (Trp / W).

“Sample” or “biological sample” as used herein means a biological material isolated from a subject. The biological sample may contain any biological material suitable for detecting a mRNA, polypeptide or other marker of a physiologic or pathologic process in a subject, and may comprise fluid, tissue, cellular and/or non-cellular material obtained from the individual.

By the term “specifically binds,” as used herein with respect to an antibody, is meant an antibody which recognizes a specific antigen, but does not substantially recognize or bind other molecules in a sample. For example, an antibody that specifically binds to an antigen from one species may also bind to that antigen from one or more species. But, such cross-species reactivity does not itself alter the classification of an antibody as specific. In another example, an antibody that specifically binds to an antigen may also bind to different allelic forms of the antigen. However, such cross reactivity does not itself alter the classification of an antibody as specific. In some instances, the terms “specific binding” or “specifically binding,” can be used in reference to the interaction of an antibody, a protein, or a peptide with a second chemical species, to mean that the interaction is dependent upon the presence of a particular structure (*e.g.*, an antigenic determinant or epitope) on the chemical species; for example, an antibody recognizes and binds to a specific protein structure rather than to proteins generally. If an antibody is specific for epitope “A”, the presence of a molecule containing epitope A (or free, unlabeled A), in a reaction containing labeled “A” and the antibody, will reduce the amount of labeled A bound to the antibody.

As used herein, “substantially purified” refers to being essentially free of other components. For example, a substantially purified polypeptide is a polypeptide which has been

separated from other components with which it is normally associated in its naturally occurring state.

As used herein, the term “treatment” or “treating” is defined as the application or administration of a therapeutic agent, *i.e.*, a compound useful within the invention (alone or in combination with another pharmaceutical agent), to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient (*e.g.*, for diagnosis or *ex vivo* applications), who has a disease or disorder, a symptom of a disease or disorder or the potential to develop a disease or disorder, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease or disorder, the symptoms of the disease or disorder, or the potential to develop the disease or disorder. Such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics.

As used herein, the term “wild-type” refers to a gene or gene product isolated from a naturally occurring source. A wild-type gene is that which is most frequently observed in a population and is thus arbitrarily designed the “normal” or “wild-type” form of the gene. In contrast, the term “modified” or “mutant” refers to a gene or gene product that displays modifications in sequence and/or functional properties (*i.e.*, altered characteristics) when compared to the wild-type gene or gene product. Naturally occurring mutants can be isolated; these are identified by the fact that they have altered characteristics (including altered nucleic acid sequences) when compared to the wild-type gene or gene product.

Ranges: throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

## Description

ENPP1 is the primary source of extracellular PPi in the body. Despite the

multiple genetic etiologies and multifactorial nature of the expression, progression, and severity of GACI, the present results demonstrate that disruption of NPP1's extracellular purinergic metabolism accounts for the pathologic sequela and mortality associated with GACI, and enzyme replacement therapy with ENPP1 is a tractable therapeutic approach. This was demonstrated using the ENPP1-asj mouse model of GACI on the 'acceleration diet'.

Diseases of ectopic tissue calcification range from the ultra-rare diseases, such as GACI, to nearly ubiquitous maladies in the aging population such as hardening of the arteries and osteoarthritis. The genetic etiology of human GACI suggests that the lethal arterial calcifications result from impairment of extracellular purinergic metabolism, either through loss of function mutations in ENPP1 or upstream reductions in nucleotide triphosphates metabolized by ENPP1 into extracellular PPi. As demonstrated herein, subcutaneous supplementation with untargeted ENPP1 or untargeted ENPP1-Fc increases extracellular PPi concentrations sufficiently to eliminate the mortality, as well as the cardiac and arterial calcifications in animal models of GACI. These results indicate that untargeted enzyme replacement therapy can be efficacious in GACI and other diseases resulting in uncontrolled vascular calcification.

The present results are surprising in light of previous studies treating hereditary hypophosphatasia (HPP), which claimed the necessity of a bone-targeting motif for efficacy. HPP is a rickets-like disease of reduced/absent of bone mineralization, and treatment with recombinant TNAP invoked the necessity of bone targeting to achieve a clinical effect (Millan, *et al.*, 2008, J. Bone Mineral Res. 23:777-787; Whyte, *et al.*, 2012, New Engl. J. Med. 366:904-913). Clinical trials attempting to treat HPP with serum enriched with untargeted TNAP failed (Whyte, *et al.*, 1982, J. Pediatrics 101:379-386; Whyte, *et al.*, 1984, J. Pediatrics 105:926-933; Weninger, *et al.*, 1989, Acta Paediatrica Scandinavica Suppl. 360:154-160). Further, the literature at the time of the invention indicated that untargeted NPP1 showed no efficacy with *in vitro* calcification assays (WO 2012/125182 to Quinn, *et al.*, such as for example Figure 23 therein), thus indicating that bone targeting was essential for the biological activity of an NPP1 containing biologic *in vivo*. However, in certain embodiments, the present results indicate that bone-targeting is not necessary for therapeutic efficacy.

The arterial calcifications of GACI may be accompanied by extravascular calcifications in the skin and retina that typify a second rare disease, PXE. PXE is a closely related to GACI, but instead results in ectopic tissue mineralization of elastic fibers affecting the



skin, eyes, and cardiovascular system. PXE has a later onset, slower progression, and is relatively more common than GACI, with an incidence of 1/25,000 to 1/75,000. The clinical manifestations begin in the skin with the development of small yellowish papules that coalesce into larger plaques of leathery skin followed by angioid streaks in the eye leading to bleeding, scarring, neovascularization, progressive loss of visual acuity and blindness. The cardiovascular system may also be affected by progressive mineralization of the medium sized arterial blood vessels, resulting in hypertension, claudication, occasional bleeding of the intestinal arteries, and (rarely) premature myocardial infarction. The genetic basis of PXE is loss of function mutations in the *abcc6* gene, resulting in impaired function of the MRP6 protein, which reduces extracellular nucleotriphosphate (NTP) concentrations *in vitro* and *in vivo*. This reduces ENPP1 substrate concentrations and thereby limits extracellular production of PPi.

The NPP1-asj mouse model of GACI possesses both the genetic etiology and the pathologic features of human GACI, but the mice also develop periarticular calcifications not characteristic of GACI but reminiscent of human diseases of unregulated periarticular calcification such as osteoarthritis and ossification of the posterior longitudinal ligament (OPLL). Mice possessing a missense mutation in ENPP1 (V246D) were initially described as ‘asj’ mice for ‘associated with stiffened joints’, reflecting the development of progressive periarticular calcifications in the forepaws of the mice. ENPP1 mutations in mice are used to model paraspinal calcifications in *ttw/ttw* mice to provide insight into OPLL, but identification of ENPP1 mutations in GACI led to a reappraisal of the presence of vascular calcifications in these animals and Uitto and coworkers noted that NPP1-asj mice, when fed a special diet high in  $\text{Ca}^{+2}$  and low in  $\text{Mg}^{+2}$ , recapitulated many of the essential features of human GACI. ENPP1 protein levels correlate inversely with the severity of cartilage calcification and osteoarthritis in humans, and ENPP1 genetic variants account for a substantial fraction of hand osteoarthritis in patient populations predisposed to hereditary forms of the disease. In certain embodiments, ENPP1 enzyme replacement therapy is a viable treatment strategy for forms of osteoarthritis resulting from ENPP1 deficiency and/or a reduction of extracellular PPi concentration. Such conditions include, but are not limited to, PXE, hereditary and non-hereditary forms of osteoarthritis, ankylosing spondylitis, hardening of the arteries occurring with aging, and calciphylaxis resulting from end stage renal disease.

## Compositions

In certain embodiments, the compositions of the invention comprises at least one compound of formula (I), or a solvate or salt (such as a pharmaceutically acceptable salt) thereof:

PROTEIN-Z-DOMAIN-X-Y (I), wherein in (I)

5 PROTEIN is at least one selected from the group consisting of NPP121 (SEQ ID NO:15), NPP71 (SEQ ID NO:17), NPP71 lacking NPP1 N-terminus GLK (SEQ ID NO:19), and NPP51 (SEQ ID NO:24);

DOMAIN is at least one selected from the group consisting of a human IgG Fc domain (Fc), human serum albumin protein (ALB) and a fragment thereof;

10 X and Z are independently absent or a polypeptide comprising 1-20 amino acids; and,

Y is absent or a sequence selected from the group consisting of: (DSS)<sub>n</sub> (SEQ ID NO:4), (ESS)<sub>n</sub> (SEQ ID NO:5), (RQQ)<sub>n</sub> (SEQ ID NO:6), (KR)<sub>n</sub> (SEQ ID NO:7), R<sub>m</sub> (SEQ ID NO:8), DSSSEEKFLRRIGRFG (SEQ ID NO:9), EEEEEEEPRGDT (SEQ ID NO:10), APWHLSSQYSRT (SEQ ID NO:11), STLPHPHEFSRE (SEQ ID NO:12), VTKHLNQISQSY (SEQ ID NO:13), and E<sub>m</sub> (SEQ ID NO:14) wherein m is an integer ranging from 1 to 15, and  
15 wherein n is an integer ranging from 1 to 10.

In certain embodiments, the compositions of the invention comprises at least one compound of formula (II), or a pharmaceutical salt thereof:

PROTEIN-Z-DOMAIN-X-Y (II), wherein in (II)

20 PROTEIN is at least one selected from the group consisting of NPP121 (SEQ ID NO:15), NPP71 (SEQ ID NO:17), NPP71 lacking NPP1 N-terminus GLK (SEQ ID NO:19), and NPP51 (SEQ ID NO:24);

DOMAIN is at least one selected from the group consisting of a human IgG Fc domain (Fc), human serum albumin protein (ALB) and a fragment thereof ;

25 X and Z are independently absent or a polypeptide comprising 1-20 amino acids; and,

Y is a sequence selected from the group consisting of: (DSS)<sub>n</sub> (SEQ ID NO:4), (ESS)<sub>n</sub> (SEQ ID NO:5), (RQQ)<sub>n</sub> (SEQ ID NO:6), (KR)<sub>n</sub> (SEQ ID NO:7), R<sub>m</sub> (SEQ ID NO:8), DSSSEEKFLRRIGRFG (SEQ ID NO:9), EEEEEEEPRGDT (SEQ ID NO:10), APWHLSSQYSRT (SEQ ID NO:11), STLPHPHEFSRE (SEQ ID NO:12), VTKHLNQISQSY (SEQ ID NO:13), and E<sub>m</sub> (SEQ ID NO:14), wherein m is an integer ranging from 1 to 15, and  
30 wherein n is an integer ranging from 1 to 10.

In certain embodiments, DOMAIN comprises a human IgG Fc domain or fragment thereof. In other embodiments, DOMAIN consists essentially of a human IgG Fc domain or fragment thereof. In yet other embodiments, DOMAIN consists of a human IgG Fc domain or fragment thereof.

5 In certain embodiments, DOMAIN comprises a human serum albumin protein or a fragment thereof. In other embodiments, DOMAIN consists essentially of a human serum albumin protein or a fragment thereof. In yet other embodiments, DOMAIN consists of a human serum albumin protein or a fragment thereof.

10 In certain embodiments, Y is a negatively-charged bone-targeting sequence. In certain embodiments, Y is absent. In certain embodiments, Y is absent and the compound of formula (I) or (II) lacks a negatively-charged bone-targeting sequence. In yet other embodiments, a polyaspartic acid domain and SEQ ID NOs:4-14 are non-limiting examples of a negatively-charged bone-targeting sequence.

15 In certain embodiments, the PROTEIN has a mutation in at least one position selected from the group consisting of Ser 532, Tyr 529, Tyr 451, Ile 450, Ser 381, Tyr 382, Ser 377, Phe 346, Gly 531, Ser 289, Ser 287, Ala 454, Gly 452, Gln 519, Glu 526, Lys 448, Glu 508, Arg 456, Asp 276, Tyr 434, Gln 519, Ser 525, Gly 342, Ser 343 and Gly 536, relative to SEQ ID NO:1. In other embodiments, the PROTEIN or mutant thereof is truncated to remove the nuclease domain. In yet other embodiments, the PROTEIN or mutant thereof is truncated to  
20 remove the nuclease domain from about residue 524 to about residue 885 relative to SEQ ID NO:1, leaving only the catalytic domain from about residue 186 to about residue 586 relative to SEQ ID NO:1, which serves to preserve the catalytic activity of the protein.

25 In certain embodiments, in (I) or (II) PROTEIN-Z-DOMAIN comprises (SEQ ID NO:15)-Z-(Fc or fragment thereof), or a mutant thereof comprising at least one mutation in at least one position selected from the group consisting of Ser 532, Tyr 529, Tyr 451, Ile 450, Ser 381, Tyr 382, Ser 377, Phe 346, Gly 531, Ser 289, Ser 287, Ala 454, Gly 452, Gln 519, Glu 526, Lys 448, Glu 508, Arg 456, Asp 276, Tyr 434, Gln 519, Ser 525, Gly 342, Ser 343 and Gly 536, relative to SEQ ID NO:1. In other embodiments, Z is a tripeptide. In yet other embodiments, Z is L I N. In yet other embodiments, in (I) or (II) PROTEIN-Z-DOMAIN comprises SEQ ID  
30 NO:16, or a mutant thereof comprising at least one mutation in at least one position selected from the group consisting of Ser 532, Tyr 529, Tyr 451, Ile 450, Ser 381, Tyr 382, Ser 377, Phe

346, Gly 531, Ser 289, Ser 287, Ala 454, Gly 452, Gln 519, Glu 526, Lys 448, Glu 508, Arg 456, Asp 276, Tyr 434, Gln 519, Ser 525, Gly 342, Ser 343 and Gly 536, relative to SEQ ID NO:1.

In certain embodiments, in (I) or (II) PROTEIN-Z-DOMAIN comprises (SEQ ID NO:17)-Z-(Fc or fragment thereof), or a mutant thereof comprising at least one mutation in at least one position selected from the group consisting of Ser 532, Tyr 529, Tyr 451, Ile 450, Ser 381, Tyr 382, Ser 377, Phe 346, Gly 531, Ser 289, Ser 287, Ala 454, Gly 452, Gln 519, Glu 526, Lys 448, Glu 508, Arg 456, Asp 276, Tyr 434, Gln 519, Ser 525, Gly 342, Ser 343 and Gly 536, relative to SEQ ID NO:1. In other embodiments, Z is a tripeptide. In yet other embodiments, Z is L I N. In yet other embodiments, in (I) or (II) PROTEIN-Z-DOMAIN comprises SEQ ID NO:18, or a mutant thereof comprising at least one mutation in at least one position selected from the group consisting of Ser 532, Tyr 529, Tyr 451, Ile 450, Ser 381, Tyr 382, Ser 377, Phe 346, Gly 531, Ser 289, Ser 287, Ala 454, Gly 452, Gln 519, Glu 526, Lys 448, Glu 508, Arg 456, Asp 276, Tyr 434, Gln 519, Ser 525, Gly 342, Ser 343 and Gly 536, relative to SEQ ID NO:1.

In certain embodiments, in (I) or (II) PROTEIN-Z-DOMAIN comprises (SEQ ID NO:19)-Z-(Fc or fragment thereof), or a mutant thereof comprising at least one mutation in at least one position selected from the group consisting of Ser 532, Tyr 529, Tyr 451, Ile 450, Ser 381, Tyr 382, Ser 377, Phe 346, Gly 531, Ser 289, Ser 287, Ala 454, Gly 452, Gln 519, Glu 526, Lys 448, Glu 508, Arg 456, Asp 276, Tyr 434, Gln 519, Ser 525, Gly 342, Ser 343 and Gly 536, relative to SEQ ID NO:1. In other embodiments, Z is a tripeptide. In yet other embodiments, Z is L I N. In yet other embodiments, in (I) or (II) PROTEIN-Z-DOMAIN comprises SEQ ID NO:20, or a mutant thereof comprising at least one mutation in at least one position selected from the group consisting of Ser 532, Tyr 529, Tyr 451, Ile 450, Ser 381, Tyr 382, Ser 377, Phe 346, Gly 531, Ser 289, Ser 287, Ala 454, Gly 452, Gln 519, Glu 526, Lys 448, Glu 508, Arg 456, Asp 276, Tyr 434, Gln 519, Ser 525, Gly 342, Ser 343 and Gly 536, relative to SEQ ID NO:1.

In certain embodiments, in (I) or (II) PROTEIN-Z-DOMAIN comprises (SEQ ID NO:24)-Z-(Fc or fragment thereof), or a mutant thereof comprising at least one mutation in at least one position selected from the group consisting of Ser 532, Tyr 529, Tyr 451, Ile 450, Ser 381, Tyr 382, Ser 377, Phe 346, Gly 531, Ser 289, Ser 287, Ala 454, Gly 452, Gln 519, Glu 526, Lys 448, Glu 508, Arg 456, Asp 276, Tyr 434, Gln 519, Ser 525, Gly 342, Ser 343 and Gly 536, relative to SEQ ID NO:1. In other embodiments, Z is a tripeptide. In yet other embodiments, Z is L I N. In yet other embodiments, in (I) or (II) PROTEIN-Z-DOMAIN comprises (SEQ ID

NO:24)-Z-(SEQ ID NO:26), or a mutant thereof comprising at least one mutation in at least one position selected from the group consisting of Ser 532, Tyr 529, Tyr 451, Ile 450, Ser 381, Tyr 382, Ser 377, Phe 346, Gly 531, Ser 289, Ser 287, Ala 454, Gly 452, Gln 519, Glu 526, Lys 448, Glu 508, Arg 456, Asp 276, Tyr 434, Gln 519, Ser 525, Gly 342, Ser 343 and Gly 536, relative to SEQ ID NO:1.

In certain embodiments, in (I) or (II) PROTEIN-Z-DOMAIN comprises (SEQ ID NO:15)-Z-(ALB or fragment thereof), or a mutant thereof comprising at least one mutation in at least one position selected from the group consisting of Ser 532, Tyr 529, Tyr 451, Ile 450, Ser 381, Tyr 382, Ser 377, Phe 346, Gly 531, Ser 289, Ser 287, Ala 454, Gly 452, Gln 519, Glu 526, Lys 448, Glu 508, Arg 456, Asp 276, Tyr 434, Gln 519, Ser 525, Gly 342, Ser 343 and Gly 536, relative to SEQ ID NO:1. In other embodiments, Z is a tripeptide. In yet other embodiments, Z is one selected from the group consisting of SEQ ID NOs:28-30. In yet other embodiments, in (I) or (II) PROTEIN-Z-DOMAIN comprises SEQ ID NO:21, or a mutant thereof comprising at least one mutation in at least one position selected from the group consisting of Ser 532, Tyr 529, Tyr 451, Ile 450, Ser 381, Tyr 382, Ser 377, Phe 346, Gly 531, Ser 289, Ser 287, Ala 454, Gly 452, Gln 519, Glu 526, Lys 448, Glu 508, Arg 456, Asp 276, Tyr 434, Gln 519, Ser 525, Gly 342, Ser 343 and Gly 536, relative to SEQ ID NO:1.

In certain embodiments, in (I) or (II) PROTEIN-Z-DOMAIN comprises (SEQ ID NO:17)-Z-(ALB or fragment thereof), or a mutant thereof comprising at least one mutation in at least one position selected from the group consisting of Ser 532, Tyr 529, Tyr 451, Ile 450, Ser 381, Tyr 382, Ser 377, Phe 346, Gly 531, Ser 289, Ser 287, Ala 454, Gly 452, Gln 519, Glu 526, Lys 448, Glu 508, Arg 456, Asp 276, Tyr 434, Gln 519, Ser 525, Gly 342, Ser 343 and Gly 536, relative to SEQ ID NO:1. In other embodiments, Z is a tripeptide. In yet other embodiments, Z is one selected from the group consisting of SEQ ID NOs:28-30. In yet other embodiments, in (I) or (II) PROTEIN-Z-DOMAIN comprises (SEQ ID NO:17)-Z-(SEQ ID NO:27), or a mutant thereof comprising at least one mutation in at least one position selected from the group consisting of Ser 532, Tyr 529, Tyr 451, Ile 450, Ser 381, Tyr 382, Ser 377, Phe 346, Gly 531, Ser 289, Ser 287, Ala 454, Gly 452, Gln 519, Glu 526, Lys 448, Glu 508, Arg 456, Asp 276, Tyr 434, Gln 519, Ser 525, Gly 342, Ser 343 and Gly 536, relative to SEQ ID NO:1, wherein Z is one selected from the group consisting of SEQ ID NOs:28-30.

In certain embodiments, in (I) or (II) PROTEIN-Z-DOMAIN comprises (SEQ ID

NO:19)-Z-(ALB or fragment thereof), or a mutant thereof comprising at least one mutation in at least one position selected from the group consisting of Ser 532, Tyr 529, Tyr 451, Ile 450, Ser 381, Tyr 382, Ser 377, Phe 346, Gly 531, Ser 289, Ser 287, Ala 454, Gly 452, Gln 519, Glu 526, Lys 448, Glu 508, Arg 456, Asp 276, Tyr 434, Gln 519, Ser 525, Gly 342, Ser 343 and Gly 536, relative to SEQ ID NO:1. In other embodiments, Z is a tripeptide. In yet other embodiments, Z is one selected from the group consisting of SEQ ID NOs:28-30. In yet other embodiments, in (I) or (II) PROTEIN-Z-DOMAIN comprises SEQ ID NO:22, or a mutant thereof comprising at least one mutation in at least one position selected from the group consisting of Ser 532, Tyr 529, Tyr 451, Ile 450, Ser 381, Tyr 382, Ser 377, Phe 346, Gly 531, Ser 289, Ser 287, Ala 454, Gly 452, Gln 519, Glu 526, Lys 448, Glu 508, Arg 456, Asp 276, Tyr 434, Gln 519, Ser 525, Gly 342, Ser 343 and Gly 536, relative to SEQ ID NO:1.

In certain embodiments, in (I) or (II) PROTEIN-Z-DOMAIN comprises (SEQ ID NO:24)-Z-(ALB or fragment thereof), or a mutant thereof comprising at least one mutation in at least one position selected from the group consisting of Ser 532, Tyr 529, Tyr 451, Ile 450, Ser 381, Tyr 382, Ser 377, Phe 346, Gly 531, Ser 289, Ser 287, Ala 454, Gly 452, Gln 519, Glu 526, Lys 448, Glu 508, Arg 456, Asp 276, Tyr 434, Gln 519, Ser 525, Gly 342, Ser 343 and Gly 536, relative to SEQ ID NO:1. In other embodiments, Z is a tripeptide. In yet other embodiments, Z is one selected from the group consisting of SEQ ID NOs:28-30. In yet other embodiments, in (I) or (II) PROTEIN-Z-DOMAIN comprises SEQ ID NO:25, or a mutant thereof comprising at least one mutation in at least one position selected from the group consisting of Ser 532, Tyr 529, Tyr 451, Ile 450, Ser 381, Tyr 382, Ser 377, Phe 346, Gly 531, Ser 289, Ser 287, Ala 454, Gly 452, Gln 519, Glu 526, Lys 448, Glu 508, Arg 456, Asp 276, Tyr 434, Gln 519, Ser 525, Gly 342, Ser 343 and Gly 536, relative to SEQ ID NO:1.

In certain embodiments, X and Z are independently absent or a polypeptide comprising 1-18 amino acids. In other embodiments, X and Z are independently absent or a polypeptide comprising 1-16 amino acids. In yet other embodiments, X and Z are independently absent or a polypeptide comprising 1-14 amino acids. In yet other embodiments, X and Z are independently absent or a polypeptide comprising 1-12 amino acids. In yet other embodiments, X and Z are independently absent or a polypeptide comprising 1-10 amino acids. In yet other embodiments, X and Z are independently absent or a polypeptide comprising 1-8 amino acids. In yet other embodiments, X and Z are independently absent or a polypeptide comprising 1-6

amino acids. In yet other embodiments, X and Z are independently absent or a polypeptide comprising 1-5 amino acids. In yet other embodiments, X and Z are independently absent or a polypeptide comprising 1-4 amino acids. In yet other embodiments, X and Z are independently absent or a polypeptide comprising 1-3 amino acids. In yet other embodiments, X and Z are independently absent or a polypeptide comprising 1-2 amino acids. In yet other embodiments, X and Z are independently absent or a single amino acid.

In certain embodiments, m is 1. In other embodiments, m is 2. In yet other embodiments, m is 3. In yet other embodiments, m is 4. In yet other embodiments, m is 5. In yet other embodiments, m is 6. In yet other embodiments, m is 7. In yet other embodiments, m is 8. In yet other embodiments, m is 9. In yet other embodiments, m is 10. In yet other embodiments, m is 11. In yet other embodiments, m is 12. In yet other embodiments, m is 13. In yet other embodiments, m is 14. In yet other embodiments, m is 15. In yet other embodiments, each occurrence of m is independently selected from the group consisting of an integer ranging from 1 to 15, from 2 to 15, from 3 to 15, from 4 to 15, from 5 to 15, from 6 to 15, from 7 to 15, from 8 to 15, from 9 to 15, from 10 to 15, from 11 to 15, from 12 to 15, from 13 to 15, from 14 to 15, from 1 to 14, from 2 to 14, from 3 to 14, from 4 to 14, from 5 to 14, from 6 to 14, from 7 to 14, from 8 to 14, from 9 to 14, from 10 to 14, from 11 to 14, from 12 to 14, from 13 to 14, from 1 to 13, from 2 to 13, from 3 to 13, from 4 to 13, from 5 to 13, from 6 to 13, from 7 to 13, from 8 to 13, from 9 to 13, from 10 to 13, from 11 to 13, from 12 to 13, from 1 to 12, from 2 to 12, from 3 to 12, from 4 to 12, from 5 to 12, from 6 to 12, from 7 to 12, from 8 to 12, from 9 to 12, from 10 to 12, from 11 to 12, from 1 to 11, from 2 to 11, from 3 to 11, from 4 to 11, from 5 to 11, from 6 to 11, from 7 to 11, from 8 to 11, from 9 to 11, from 10 to 11, from 1 to 10, from 2 to 10, from 3 to 10, from 4 to 10, from 5 to 10, from 6 to 10, from 7 to 10, from 8 to 10, from 9 to 10, from 1 to 9, from 2 to 9, from 3 to 9, from 4 to 9, from 5 to 9, from 6 to 9, from 7 to 9, from 8 to 9, from 1 to 8, from 2 to 8, from 3 to 8, from 4 to 8, from 5 to 8, from 6 to 8, from 7 to 8, from 1 to 7, from 2 to 7, from 3 to 7, from 4 to 7, from 5 to 7, from 6 to 7, from 1 to 6, from 2 to 6, from 3 to 6, from 4 to 6, from 5 to 6, from 1 to 5, from 2 to 5, from 3 to 5, from 4 to 5, from 1 to 4, from 2 to 4, from 3 to 4, from 1 to 3, from 2 to 3, and from 1 to 2.

In certain embodiments, n is 1. In other embodiments, n is 2. In yet other embodiments, n is 3. In yet other embodiments, n is 4. In yet other embodiments, n is 5. In yet other embodiments, n is 6. In yet other embodiments, n is 7. In yet other embodiments, n is 8.

In yet other embodiments, n is 9. In yet other embodiments, n is 10. In yet other embodiments, each occurrence of n is independently selected from the group consisting of an integer ranging from 1 to 10, from 2 to 10, from 3 to 10, from 4 to 10, from 5 to 10, from 6 to 10, from 7 to 10, from 8 to 10, from 9 to 10, from 1 to 9, from 2 to 9, from 3 to 9, from 4 to 9, from 5 to 9, from 6 to 9, from 7 to 9, from 8 to 9, from 1 to 8, from 2 to 8, from 3 to 8, from 4 to 8, from 5 to 8, from 6 to 8, from 7 to 8, from 1 to 7, from 2 to 7, from 3 to 7, from 4 to 7, from 5 to 7, from 6 to 7, from 1 to 6, from 2 to 6, from 3 to 6, from 4 to 6, from 5 to 6, from 1 to 5, from 2 to 5, from 3 to 5, from 4 to 5, from 1 to 4, from 2 to 4, from 3 to 4, from 1 to 3, from 2 to 3, and from 1 to 2.

In certain embodiments, the PROTEIN or mutant thereof is modified with a segment of the extracellular region of NPP2 containing a furin cleavage site, as compared to SEQ ID NO:1. In other embodiments, the PROTEIN or mutant thereof is not modified with a segment of the extracellular region of NPP2 containing a furin cleavage site, as compared to SEQ ID NO:1.

In certain embodiments, the PROTEIN or mutant thereof is modified with a segment of the extracellular region of NPP2 containing a signal peptidase cleavage site, as compared to SEQ ID NO: 1. In other embodiments, the PROTEIN or mutant thereof is not modified with a segment of the extracellular region of NPP2 containing a signal peptidase cleavage site, as compared to SEQ ID NO: 1

In certain embodiments, the compound of formula (I) or (II) is soluble. In other embodiments, the compound of formula (I) or (II) is a recombinant polypeptide. In yet other embodiments, the compound of formula (I) or (II) includes an NPP1 polypeptide or mutant thereof that lacks the NPP1 transmembrane domain. In yet other embodiments, the compound of formula (I) or (II) includes an NPP1 polypeptide or mutant thereof, wherein the NPP1 transmembrane domain or mutant thereof has been removed (and/or truncated) and replaced with the transmembrane domain of another polypeptide, such as, by way of non-limiting example, NPP2.

In certain embodiments, the compound of formula (I) or (II) comprises an NPP1 polypeptide or mutant thereof further comprising more than one transmembrane domain.

In certain embodiments, NPP1 is C-terminally fused to the Fc domain of human immunoglobulin 1 (IgG1).

In certain embodiments, NPP1 is C-terminally fused to human serum albumin.



In certain embodiments, a fragment and/or variant of NPP1 is fused with human serum albumin or variants and/or fragments thereof. Human serum albumin may be conjugated to NPP1 protein through a chemical linker, including but not limited to naturally occurring or engineered disulfide bonds, or by genetic fusion to NPP1, or a fragment and/or variant thereof.

5 In certain embodiment, the compound of formula (I) or (II) comprises an NPP1 polypeptide or mutant thereof comprising transmembrane domains of NPP1 and another polypeptide, such as, by way of non-limiting example, NPP2.

In certain embodiments, the compound of the formula (I) has a sequence selected from the group consisting of SEQ ID NOs:21, 22 and 25.

10 In certain embodiments, the compound of the formula (I) has a sequence selected from the group consisting of SEQ ID NOs:21, 22, 25 and (SEQ ID NO:17)-Z-(SEQ ID NO:27).

In certain embodiments, the compound of the formula (I) has a sequence selected from the group consisting of SEQ ID NOs:16, 18, 20 and (SEQ ID NO:24)-Z-(SEQ ID NO:26).

15 In certain embodiments, the compounds of the invention have more than one transmembrane domain. In other embodiments, the compounds of the invention are further pegylated. In yet other embodiments, the compounds of the invention have more than one transmembrane domain and are further pegylated.

20 In certain embodiments, the compound of formula (I) or (II) has a  $k_{cat}$  value greater than or equal to about  $3.4 (\pm 0.4) \text{ s}^{-1} \text{ enzyme}^{-1}$ , wherein the  $k_{cat}$  is determined by measuring the rate of hydrolysis of ATP for the compound.

In certain embodiments, the compound of formula (I) or (II) has a  $K_M$  value less than or equal to about  $2 \mu\text{M}$ , wherein the  $K_M$  is determined by measuring the rate of hydrolysis of ATP for the compound.

25 In certain embodiments, the compound of formula (I) or (II) is formulated as a liquid formulation.

The invention further provides a dry product form of a pharmaceutical composition comprising a therapeutic amount of a compound of formula (I) or (II), whereby the dry product is reconstitutable to a solution of the compound in liquid form.

## 30 **Methods**

The invention provides methods of treating or preventing disorders and diseases

in a subject where an increased activity or level of NPP1 polypeptide, fragment, derivative, mutant, or mutant fragment thereof is desirable. In certain embodiments, the subject is administered a therapeutically effective amount of at least one compound of the invention.

The invention further provides a method of treating or preventing a disease or disorder associated with pathological calcification or pathological ossification in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of at least one compound of formula (I) or (II), wherein the disease comprises GACI, IIAC, OPLL, hypophosphatemic rickets, osteoarthritis, and calcification of atherosclerotic plaques.

The invention further provides a method of treating or preventing a disease or disorder associated with pathological calcification or pathological ossification in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of at least one compound of formula (I) or (II), wherein the disease comprises PXE, hereditary and non-hereditary forms of osteoarthritis, ankylosing spondylitis, hardening of the arteries occurring with aging, or calciphylaxis resulting from end stage renal disease.

The invention further provides a method of reducing or preventing cardiac and/or arterial calcifications in an infant afflicted with generalized arterial calcification of infancy (GACI). In certain embodiments, the method comprises administering to the infant a therapeutically effective amount of a compound comprising (or consisting of) an ecto-nucleotide pyrophosphate/phosphodiesterase-1 (NPP1) polypeptide comprising (or fused to) an IgG Fc domain, wherein the compound lacks a polyaspartic acid domain, whereby the administering of the compound increases extracellular pyrophosphate (PPi) concentrations, thus reducing or preventing cardiac and/or arterial calcifications in the infant.

The invention further provides a method of reducing or preventing cardiac and/or arterial calcifications in an infant afflicted with generalized arterial calcification of infancy (GACI). In certain embodiments, the method comprises administering to the infant a therapeutically effective amount of a compound comprising (or consisting of) an ecto-nucleotide pyrophosphate/phosphodiesterase-1 (NPP1) polypeptide comprising (or fused to) a human serum albumin domain or fragment thereof, wherein the compound lacks a polyaspartic acid domain, whereby the administering increases extracellular pyrophosphate (PPi) concentrations, thus reducing or preventing cardiac and/or arterial calcifications in the infant.

In certain embodiments, the disorders and diseases comprise at least one selected from the group consisting of GACI, IIAC, OPLL, hypophosphatemic rickets, osteoarthritis, progeria, and calcification of atherosclerotic plaques. In other embodiments, the disorders or diseases comprise at least one selected from the group consisting of PXE, hereditary and non-hereditary forms of osteoarthritis, ankylosing spondylitis, hardening of the arteries occurring with aging, progeria, and calciphylaxis resulting from end stage renal disease.

In certain embodiments, the compound is administered acutely or chronically to the subject. In other embodiments, the compound is administered locally, regionally or systemically to the subject. In yet other embodiments, the administration is subcutaneous. In yet other embodiments, the subject is a mammal. In yet other embodiments, the mammal is human.

In certain embodiments, the compound of formula (I) or (II), fragment or mutant thereof has lower Ap3A hydrolytic activity as compared to the corresponding wild-type NPP1 polypeptide or fragment thereof. In other embodiments, the compound of formula (I) or (II), fragment or mutant thereof has substantially the same ATP hydrolytic activity as compared to the corresponding wild-type NPP1 polypeptide or fragment thereof. In yet other embodiments, the compound of formula (I) or (II), fragment or mutant thereof has lower Ap3A hydrolytic activity and substantially the same ATP hydrolytic activity as compared to the corresponding wild-type NPP1 polypeptide or fragment thereof.

In certain embodiments, the NPP1 polypeptide comprises a cleavage product of a precursor NPP1 polypeptide comprising an NPP2 transmembrane domain.

In certain embodiments, the NPP2 transmembrane domain comprises residues 12-30 of NCBI accession no. NP\_001124335 (SEQ ID NO:2), which corresponds to IISLFTFAVGVNICTLGFTA (SEQ ID NO:23).

In certain embodiments, administration of therapeutically effective amount comprises about 3-15 mg/kg qd of the NPP1-Fc polypeptide.

In certain embodiments, the administration results in reducing the infant's extracellular pyrophosphate concentrations to a level that is within the range that is found in an infant not afflicted with GACI. In certain embodiments, the infant presents and/or is diagnosed with "failure to thrive" prior to the administration.

One skilled in the art, based upon the disclosure provided herein, would understand that the invention is useful in subjects who, in whole (*e.g.*, systemically) or in part

(e.g., locally, tissue, organ), are being, or will be, treated for pathological calcification or ossification. In certain embodiments, the invention is useful in treating or preventing pathological calcification or ossification. The skilled artisan will appreciate, based upon the teachings provided herein, that the diseases and disorders treatable by the compositions and methods described herein encompass any disease or disorder where a decrease in calcification or ossification will promote a positive therapeutic outcome.

It will be appreciated by one of skill in the art, when armed with the present disclosure including the methods detailed herein, that the invention is not limited to treatment of a disease or disorder once is established. Particularly, the symptoms of the disease or disorder need not have manifested to the point of detriment to the subject; indeed, the disease or disorder need not be detected in a subject before treatment is administered. That is, significant pathology from disease or disorder does not have to occur before the present invention may provide benefit. Therefore, the present invention, as described more fully herein, includes a method for preventing diseases and disorders in a subject, in that a compound of formula (I) or (II), or a mutant thereof, as discussed elsewhere herein, can be administered to a subject prior to the onset of the disease or disorder, thereby preventing the disease or disorder from developing.

One of skill in the art, when armed with the disclosure herein, would appreciate that the prevention of a disease or disorder in a subject encompasses administering to a subject a compound of formula (I) or (II), or a mutant thereof as a preventative measure against a disease or disorder.

The invention encompasses administration of a compound of formula (I) or (II), or a mutant thereof to practice the methods of the invention; the skilled artisan would understand, based on the disclosure provided herein, how to formulate and administer the compound of formula (I) or (II), or a mutant thereof to a subject. However, the present invention is not limited to any particular method of administration or treatment regimen. This is especially true where it would be appreciated by one skilled in the art, equipped with the disclosure provided herein, including the reduction to practice using an art-recognized model of pathological calcification or ossification, that methods of administering a compound of the invention can be determined by one of skill in the pharmacological arts.

### Pharmaceutical Compositions and Formulations

The invention provides pharmaceutical compositions comprising a compound of formula (I) or (II) within the methods of the invention.

Such a pharmaceutical composition is in a form suitable for administration to a subject, or the pharmaceutical composition may further comprise one or more pharmaceutically acceptable carriers, one or more additional ingredients, or some combination of these. The various components of the pharmaceutical composition may be present in the form of a physiologically acceptable salt, such as in combination with a physiologically acceptable cation or anion, as is well known in the art.

In an embodiment, the pharmaceutical compositions useful for practicing the method of the invention may be administered to deliver a dose of between 1 ng/kg/day and 100 mg/kg/day. In other embodiments, the pharmaceutical compositions useful for practicing the invention may be administered to deliver a dose of between 1 ng/kg/day and 500 mg/kg/day.

The relative amounts of the active ingredient, the pharmaceutically acceptable carrier, and any additional ingredients in a pharmaceutical composition of the invention will vary, depending upon the identity, size, and condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between about 0.1% and about 100% (w/w) active ingredient.

Pharmaceutical compositions that are useful in the methods of the invention may be suitably developed for inhalational, oral, rectal, vaginal, parenteral, topical, transdermal, pulmonary, intranasal, buccal, ophthalmic, intrathecal, intravenous or another route of administration. Other contemplated formulations include projected nanoparticles, liposomal preparations, resealed erythrocytes containing the active ingredient, and immunologically-based formulations. The route(s) of administration is readily apparent to the skilled artisan and depends upon any number of factors including the type and severity of the disease being treated, the type and age of the veterinary or human patient being treated, and the like.

The formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with a carrier or one or more other accessory ingredients, and then, if necessary or desirable, shaping or packaging the product into a desired single- or multi-dose unit.

As used herein, a “unit dose” is a discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient that would be administered to a subject or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage. The unit dosage form may be for a single daily dose or one of multiple daily doses (*e.g.*, about 1 to 4 or more times per day). When multiple daily doses are used, the unit dosage form may be the same or different for each dose.

Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions suitable for ethical administration to humans, it is understood by the skilled artisan that such compositions are generally suitable for administration to animals of all sorts. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and perform such modification with merely ordinary, if any, experimentation. Subjects to which administration of the pharmaceutical compositions of the invention is contemplated include, but are not limited to, humans and other primates, mammals including commercially relevant mammals such as cattle, pigs, horses, sheep, cats, and dogs.

In certain embodiments, the compositions are formulated using one or more pharmaceutically acceptable excipients or carriers. In certain embodiments, the pharmaceutical compositions comprise a therapeutically effective amount of the active agent and a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers, which are useful, include, but are not limited to, glycerol, water, saline, ethanol and other pharmaceutically acceptable salt solutions such as phosphates and salts of organic acids. Examples of these and other pharmaceutically acceptable carriers are described in Remington's Pharmaceutical Sciences, 1991, Mack Publication Co., New Jersey.

The carrier may be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity may be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms may be achieved by various antibacterial and antifungal agents, for

example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, isotonic agents, for example, sugars, sodium chloride, or polyalcohols such as mannitol and sorbitol, are used in the composition. Prolonged absorption of the injectable compositions may be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate or gelatin.

Formulations may be employed in admixtures with conventional excipients, *i.e.*, pharmaceutically acceptable organic or inorganic carrier substances suitable for oral, parenteral, nasal, intravenous, subcutaneous, enteral, or any other suitable mode of administration, known to the art. The pharmaceutical preparations may be sterilized and if desired mixed with auxiliary agents, *e.g.*, lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure buffers, coloring, flavoring and/or aromatic substances and the like. They may also be combined where desired with other active agents, *e.g.*, other analgesic agents.

As used herein, "additional ingredients" include, but are not limited to, one or more of the following: excipients; surface active agents; dispersing agents; inert diluents; granulating and disintegrating agents; binding agents; lubricating agents; sweetening agents; flavoring agents; coloring agents; preservatives; physiologically degradable compositions such as gelatin; aqueous vehicles and solvents; oily vehicles and solvents; suspending agents; dispersing or wetting agents; emulsifying agents, demulcents; buffers; salts; thickening agents; fillers; emulsifying agents; antioxidants; antibiotics; antifungal agents; stabilizing agents; and pharmaceutically acceptable polymeric or hydrophobic materials. Other "additional ingredients" that may be included in the pharmaceutical compositions of the invention are known in the art and described, for example in Genaro, ed., 1985, Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA, which is incorporated herein by reference.

The composition of the invention may comprise a preservative from about 0.005% to 2.0% by total weight of the composition. The preservative is used to prevent spoilage in the case of exposure to contaminants in the environment. Examples of preservatives useful in accordance with the invention included but are not limited to those selected from the group consisting of benzyl alcohol, sorbic acid, parabens, imidurea and combinations thereof. A particular preservative is a combination of about 0.5% to 2.0% benzyl alcohol and 0.05% to 0.5% sorbic acid.

The composition may include an antioxidant and a chelating agent, which inhibit

the degradation of the compound. Illustrative antioxidants for some compounds are BHT, BHA, alpha-tocopherol and ascorbic acid in the illustrative range of about 0.01% to 0.3%, for example BHT in the range of 0.03% to 0.1% by weight by total weight of the composition. The chelating agent may be present in an amount ranging from 0.01% to 0.5% by weight by total weight of the composition. Illustrative chelating agents include edetate salts (*e.g.* disodium edetate) and citric acid in the weight range of about 0.01% to 0.20%, for example in the range of 0.02% to 0.10% by weight by total weight of the composition. The chelating agent is useful for chelating metal ions in the composition, which may be detrimental to the shelf life of the formulation. While BHT and disodium edetate are illustrative antioxidant and chelating agent respectively for some compounds, other suitable and equivalent antioxidants and chelating agents may be substituted therefore as would be known to those skilled in the art.

Liquid suspensions may be prepared using conventional methods to achieve suspension of the active ingredient in an aqueous or oily vehicle. Aqueous vehicles include, for example, water, and isotonic saline. Oily vehicles include, for example, almond oil, oily esters, ethyl alcohol, vegetable oils such as arachis, olive, sesame, or coconut oil, fractionated vegetable oils, and mineral oils such as liquid paraffin. Liquid suspensions may further comprise one or more additional ingredients including, but not limited to, suspending agents, dispersing or wetting agents, emulsifying agents, demulcents, preservatives, buffers, salts, flavorings, coloring agents, and sweetening agents. Oily suspensions may further comprise a thickening agent. Known suspending agents include, but are not limited to, sorbitol syrup, hydrogenated edible fats, sodium alginate, polyvinylpyrrolidone, gum tragacanth, gum acacia, and cellulose derivatives (*e.g.*, sodium carboxymethylcellulose, hydroxypropylmethylcellulose, methylcellulose). Known dispersing or wetting agents include, but are not limited to, naturally-occurring phosphatides such as lecithin, condensation products of an alkylene oxide with a fatty acid, with a long chain aliphatic alcohol, with a partial ester derived from a fatty acid and a hexitol, or with a partial ester derived from a fatty acid and a hexitol anhydride (*e.g.*, polyoxyethylene stearate, heptadecaethyleneoxycetanol, polyoxyethylene sorbitol monooleate, and polyoxyethylene sorbitan monooleate, respectively). Known emulsifying agents include, but are not limited to, lecithin, and acacia. Known preservatives include, but are not limited to, methyl, ethyl, or n-propyl para- hydroxybenzoates, ascorbic acid, and sorbic acid. Known sweetening agents include, for example, glycerol, propylene glycol, sorbitol, sucrose, and



saccharin. Known thickening agents for oily suspensions include, for example, beeswax, hard paraffin, and cetyl alcohol.

Liquid solutions of the active ingredient in aqueous or oily solvents may be prepared in substantially the same manner as liquid suspensions, the primary difference being that the active ingredient is dissolved, rather than suspended in the solvent. As used herein, an “oily” liquid is one that comprises a carbon-containing liquid molecule and which exhibits a less polar character than water. Liquid solutions of the pharmaceutical composition of the invention may comprise each of the components described with regard to liquid suspensions, it being understood that suspending agents will not necessarily aid dissolution of the active ingredient in the solvent. Aqueous solvents include, for example, water, and isotonic saline. Oily solvents include, for example, almond oil, oily esters, ethyl alcohol, vegetable oils such as arachis, olive, sesame, or coconut oil, fractionated vegetable oils, and mineral oils such as liquid paraffin.

Powdered and granular formulations of a pharmaceutical preparation of the invention may be prepared using known methods. Such formulations may be administered directly to a subject, used, for example, to form tablets, to fill capsules, or to prepare an aqueous or oily suspension or solution by addition of an aqueous or oily vehicle thereto. Each of these formulations may further comprise one or more of dispersing or wetting agent, a suspending agent, and a preservative. Additional excipients, such as fillers and sweetening, flavoring, or coloring agents, may also be included in these formulations.

A pharmaceutical composition of the invention may also be prepared, packaged, or sold in the form of oil-in-water emulsion or a water-in-oil emulsion. The oily phase may be a vegetable oil such as olive or arachis oil, a mineral oil such as liquid paraffin, or a combination of these. Such compositions may further comprise one or more emulsifying agents such as naturally occurring gums such as gum acacia or gum tragacanth, naturally-occurring phosphatides such as soybean or lecithin phosphatide, esters or partial esters derived from combinations of fatty acids and hexitol anhydrides such as sorbitan monooleate, and condensation products of such partial esters with ethylene oxide such as polyoxyethylene sorbitan monooleate. These emulsions may also contain additional ingredients including, for example, sweetening or flavoring agents.

Methods for impregnating or coating a material with a chemical composition are known in the art, and include, but are not limited to methods of depositing or binding a chemical

composition onto a surface, methods of incorporating a chemical composition into the structure of a material during the synthesis of the material (*i.e.*, such as with a physiologically degradable material), and methods of absorbing an aqueous or oily solution or suspension into an absorbent material, with or without subsequent drying.

## 5 *Administration/Dosing*

The regimen of administration may affect what constitutes an effective amount. For example, several divided dosages, as well as staggered dosages may be administered daily or sequentially, or the dose may be continuously infused, or may be a bolus injection. Further, the dosages of the therapeutic formulations may be proportionally increased or decreased as  
10 indicated by the exigencies of the therapeutic or prophylactic situation.

Administration of the compositions of the present invention to a patient, psucha s a mammal, such as a human, may be carried out using known procedures, at dosages and for periods of time effective to treat a disease or disorder in the patient. An effective amount of the therapeutic compound necessary to achieve a therapeutic effect may vary according to factors  
15 such as the activity of the particular compound employed; the time of administration; the rate of excretion of the compound; the duration of the treatment; other drugs, compounds or materials used in combination with the compound; the state of the disease or disorder, age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well-known in the medical arts. Dosage regimens may be adjusted to provide the optimum  
20 therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation. A non-limiting example of an effective dose range for a therapeutic compound of the invention is from about 0.01 and 50 mg/kg of body weight/per day. One of ordinary skill in the art would be able to study the relevant factors and make the determination regarding the effective amount of the  
25 therapeutic compound without undue experimentation.

The compound can be administered to an animal as frequently as several times daily, or it may be administered less frequently, such as once a day, once a week, once every two weeks, once a month, or even less frequently, such as once every several months or even once a year or less. It is understood that the amount of compound dosed per day may be administered,  
30 in non-limiting examples, every day, every other day, every 2 days, every 3 days, every 4 days, or every 5 days. For example, with every other day administration, a 5 mg per day dose may be

initiated on Monday with a first subsequent 5 mg per day dose administered on Wednesday, a second subsequent 5 mg per day dose administered on Friday, and so on. The frequency of the dose is readily apparent to the skilled artisan and depends upon any number of factors, such as, but not limited to, the type and severity of the disease being treated, and the type and age of the animal.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active ingredient that is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

A medical doctor, *e.g.*, physician or veterinarian, having ordinary skill in the art may readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the compounds of the invention employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

In particular embodiments, it is especially advantageous to formulate the compound in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the patients to be treated; each unit containing a predetermined quantity of therapeutic compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical vehicle. The dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the therapeutic compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding/formulating such a therapeutic compound for the treatment of a disease or disorder in a patient.

In certain embodiments, the compositions of the invention are administered to the patient in dosages that range from one to five times per day or more. In other embodiments, the compositions of the invention are administered to the patient in range of dosages that include, but are not limited to, once every day, every two, days, every three days to once a week, and once every two weeks. It is readily apparent to one skilled in the art that the frequency of administration of the various combination compositions of the invention varies from subject to subject depending on many factors including, but not limited to, age, disease or disorder to be

treated, gender, overall health, and other factors. Thus, the invention should not be construed to be limited to any particular dosage regime and the precise dosage and composition to be administered to any patient will be determined by the attending physical taking all other factors about the patient into account.

5               Compounds of the invention for administration may be in the range of from about 1 µg to about 7,500 mg, about 20 µg to about 7,000 mg, about 40 µg to about 6,500 mg, about 80 µg to about 6,000 mg, about 100 µg to about 5,500 mg, about 200 µg to about 5,000 mg, about 400 µg to about 4,000 mg, about 800 µg to about 3,000 mg, about 1 mg to about 2,500 mg, about 2 mg to about 2,000 mg, about 5 mg to about 1,000 mg, about 10 mg to about 750 mg, about 20  
10 mg to about 600 mg, about 30 mg to about 500 mg, about 40 mg to about 400 mg, about 50 mg to about 300 mg, about 60 mg to about 250 mg, about 70 mg to about 200 mg, about 80 mg to about 150 mg, and any and all whole or partial increments therebetween.

              In some embodiments, the dose of a compound of the invention is from about 0.5 µg and about 5,000 mg. In some embodiments, a dose of a compound of the invention used in  
15 compositions described herein is less than about 5,000 mg, or less than about 4,000 mg, or less than about 3,000 mg, or less than about 2,000 mg, or less than about 1,000 mg, or less than about 800 mg, or less than about 600 mg, or less than about 500 mg, or less than about 200 mg, or less than about 50 mg. Similarly, in some embodiments, a dose of a second compound as described  
20 herein is less than about 1,000 mg, or less than about 800 mg, or less than about 600 mg, or less than about 500 mg, or less than about 400 mg, or less than about 300 mg, or less than about 200 mg, or less than about 100 mg, or less than about 50 mg, or less than about 40 mg, or less than about 30 mg, or less than about 25 mg, or less than about 20 mg, or less than about 15 mg, or less than about 10 mg, or less than about 5 mg, or less than about 2 mg, or less than about 1 mg, or less than about 0.5 mg, and any and all whole or partial increments thereof.

25               In certain embodiments, the present invention is directed to a packaged pharmaceutical composition comprising a container holding a therapeutically effective amount of a compound of the invention, alone or in combination with a second pharmaceutical agent; and instructions for using the compound to treat, prevent, or reduce one or more symptoms of a disease or disorder in a patient.

30               The term “container” includes any receptacle for holding the pharmaceutical composition. For example, In certain embodiments, the container is the packaging that contains

the pharmaceutical composition. In other embodiments, the container is not the packaging that contains the pharmaceutical composition, *i.e.*, the container is a receptacle, such as a box or vial that contains the packaged pharmaceutical composition or unpackaged pharmaceutical composition and the instructions for use of the pharmaceutical composition. Moreover, packaging techniques are well known in the art. It should be understood that the instructions for use of the pharmaceutical composition may be contained on the packaging containing the pharmaceutical composition, and as such the instructions form an increased functional relationship to the packaged product. However, it should be understood that the instructions may contain information pertaining to the compound's ability to perform its intended function, *e.g.*, treating, preventing, or reducing a disease or disorder in a patient.

#### ***Routes of Administration***

Routes of administration of any of the compositions of the invention include inhalational, oral, nasal, rectal, parenteral, sublingual, transdermal, transmucosal (*e.g.*, sublingual, lingual, (trans)buccal, (trans)urethral, vaginal (*e.g.*, trans- and perivaginally), (intra)nasal, and (trans)rectal), intravesical, intrapulmonary, intraduodenal, intragastrical, intrathecal, subcutaneous, intramuscular, intradermal, intra-arterial, intravenous, intrabronchial, inhalation, and topical administration.

Suitable compositions and dosage forms include, for example, tablets, capsules, caplets, pills, gel caps, troches, dispersions, suspensions, solutions, syrups, granules, beads, transdermal patches, gels, powders, pellets, magmas, lozenges, creams, pastes, plasters, lotions, discs, suppositories, liquid sprays for nasal or oral administration, dry powder or aerosolized formulations for inhalation, compositions and formulations for intravesical administration and the like. It should be understood that the formulations and compositions that would be useful in the present invention are not limited to the particular formulations and compositions that are described herein.

#### ***Oral Administration***

For oral application, particularly suitable are tablets, dragees, liquids, drops, suppositories, or capsules, caplets and gelcaps. Other formulations suitable for oral administration include, but are not limited to, a powdered or granular formulation, an aqueous or oily suspension, an aqueous or oily solution, a paste, a gel, toothpaste, a mouthwash, a coating, an oral rinse, or an emulsion. The compositions intended for oral use may be prepared according

to any method known in the art and such compositions may contain one or more agents selected from the group consisting of inert, non-toxic pharmaceutically excipients that are suitable for the manufacture of tablets. Such excipients include, for example an inert diluent such as lactose; granulating and disintegrating agents such as cornstarch; binding agents such as starch; and  
5 lubricating agents such as magnesium stearate.

Tablets may be non-coated or they may be coated using known methods to achieve delayed disintegration in the gastrointestinal tract of a subject, thereby providing sustained release and absorption of the active ingredient. By way of example, a material such as glyceryl monostearate or glyceryl distearate may be used to coat tablets. Further by way of  
10 example, tablets may be coated using methods described in U.S. Patents Nos. 4,256,108; 4,160,452; and 4,265,874 to form osmotically controlled release tablets. Tablets may further comprise a sweetening agent, a flavoring agent, a coloring agent, a preservative, or some combination of these in order to provide for pharmaceutically elegant and palatable preparation.

Hard capsules comprising the active ingredient may be made using a  
15 physiologically degradable composition, such as gelatin. Such hard capsules comprise the active ingredient, and may further comprise additional ingredients including, for example, an inert solid diluent such as calcium carbonate, calcium phosphate, or kaolin.

Soft gelatin capsules comprising the active ingredient may be made using a physiologically degradable composition, such as gelatin. Such soft capsules comprise the active  
20 ingredient, which may be mixed with water or an oil medium such as peanut oil, liquid paraffin, or olive oil.

For oral administration, the compounds of the invention may be in the form of tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents; fillers; lubricants; disintegrates; or wetting agents. If desired, the tablets  
25 may be coated using suitable methods and coating materials such as OPADRY™ film coating systems available from Colorcon, West Point, Pa. (*e.g.*, OPADRY™ OY Type, OYC Type, Organic Enteric OY-P Type, Aqueous Enteric OY-A Type, OY-PM Type and OPADRY™ White, 32K18400).

Liquid preparation for oral administration may be in the form of solutions, syrups  
30 or suspensions. The liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, methyl

cellulose or hydrogenated edible fats); emulsifying agent (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters or ethyl alcohol); and preservatives (*e.g.*, methyl or propyl para-hydroxy benzoates or sorbic acid). Liquid formulations of a pharmaceutical composition of the invention which are suitable for oral administration may be prepared, packaged, and sold either in liquid form or in the form of a dry product intended for reconstitution with water or another suitable vehicle prior to use.

A tablet comprising the active ingredient may, for example, be made by compressing or molding the active ingredient, optionally with one or more additional ingredients. Compressed tablets may be prepared by compressing, in a suitable device, the active ingredient in a free-flowing form such as a powder or granular preparation, optionally mixed with one or more of a binder, a lubricant, an excipient, a surface active agent, and a dispersing agent. Molded tablets may be made by molding, in a suitable device, a mixture of the active ingredient, a pharmaceutically acceptable carrier, and at least sufficient liquid to moisten the mixture. Pharmaceutically acceptable excipients used in the manufacture of tablets include, but are not limited to, inert diluents, granulating and disintegrating agents, binding agents, and lubricating agents. Known dispersing agents include, but are not limited to, potato starch and sodium starch glycolate. Known surface-active agents include, but are not limited to, sodium lauryl sulphate. Known diluents include, but are not limited to, calcium carbonate, sodium carbonate, lactose, microcrystalline cellulose, calcium phosphate, calcium hydrogen phosphate, and sodium phosphate. Known granulating and disintegrating agents include, but are not limited to, corn starch and alginic acid. Known binding agents include, but are not limited to, gelatin, acacia, pre-gelatinized maize starch, polyvinylpyrrolidone, and hydroxypropyl methylcellulose. Known lubricating agents include, but are not limited to, magnesium stearate, stearic acid, silica, and talc.

Granulating techniques are well known in the pharmaceutical art for modifying starting powders or other particulate materials of an active ingredient. The powders are typically mixed with a binder material into larger permanent free-flowing agglomerates or granules referred to as a "granulation." For example, solvent-using "wet" granulation processes are generally characterized in that the powders are combined with a binder material and moistened with water or an organic solvent under conditions resulting in the formation of a wet granulated mass from which the solvent must then be evaporated.

Melt granulation generally consists in the use of materials that are solid or semi-solid at room temperature (i.e. having a relatively low softening or melting point range) to promote granulation of powdered or other materials, essentially in the absence of added water or other liquid solvents. The low melting solids, when heated to a temperature in the melting point range, liquefy to act as a binder or granulating medium. The liquefied solid spreads itself over the surface of powdered materials with which it is contacted, and on cooling, forms a solid granulated mass in which the initial materials are bound together. The resulting melt granulation may then be provided to a tablet press or be encapsulated for preparing the oral dosage form. Melt granulation improves the dissolution rate and bioavailability of an active (i.e. drug) by forming a solid dispersion or solid solution.

U.S. Patent No. 5,169,645 discloses directly compressible wax-containing granules having improved flow properties. The granules are obtained when waxes are admixed in the melt with certain flow improving additives, followed by cooling and granulation of the admixture. In certain embodiments, only the wax itself melts in the melt combination of the wax(es) and additives(s), and in other cases both the wax(es) and the additives(s) will melt.

The present invention also includes a multi-layer tablet comprising a layer providing for the delayed release of one or more compounds useful within the methods of the invention, and a further layer providing for the immediate release of one or more compounds useful within the methods of the invention. Using a wax/pH-sensitive polymer mix, a gastric insoluble composition may be obtained in which the active ingredient is entrapped, ensuring its delayed release.

#### ***Parenteral Administration***

As used herein, "parenteral administration" of a pharmaceutical composition includes any route of administration characterized by physical breaching of a tissue of a subject and administration of the pharmaceutical composition through the breach in the tissue. Parenteral administration thus includes, but is not limited to, administration of a pharmaceutical composition by injection of the composition, by application of the composition through a surgical incision, by application of the composition through a tissue-penetrating non-surgical wound, and the like. In particular, parenteral administration is contemplated to include, but is not limited to, subcutaneous, intravenous, intraperitoneal, intramuscular, intrasternal injection, and kidney dialytic infusion techniques.



Formulations of a pharmaceutical composition suitable for parenteral administration comprise the active ingredient combined with a pharmaceutically acceptable carrier, such as sterile water or sterile isotonic saline. Such formulations may be prepared, packaged, or sold in a form suitable for bolus administration or for continuous administration.

5 Injectable formulations may be prepared, packaged, or sold in unit dosage form, such as in ampules or in multi-dose containers containing a preservative. Formulations for parenteral administration include, but are not limited to, suspensions, solutions, emulsions in oily or aqueous vehicles, pastes, and implantable sustained-release or biodegradable formulations. Such formulations may further comprise one or more additional ingredients including, but not limited to, suspending, stabilizing, or dispersing agents. In one embodiment of a formulation for  
10 parenteral administration, the active ingredient is provided in dry (*i.e.*, powder or granular) form for reconstitution with a suitable vehicle (*e.g.*, sterile pyrogen-free water) prior to parenteral administration of the reconstituted composition.

The pharmaceutical compositions may be prepared, packaged, or sold in the form  
15 of a sterile injectable aqueous or oily suspension or solution. This suspension or solution may be formulated according to the known art, and may comprise, in addition to the active ingredient, additional ingredients such as the dispersing agents, wetting agents, or suspending agents described herein. Such sterile injectable formulations may be prepared using a non-toxic parenterally-acceptable diluent or solvent, such as water or 1,3-butanediol, for example. Other  
20 acceptable diluents and solvents include, but are not limited to, Ringer's solution, isotonic sodium chloride solution, and fixed oils such as synthetic mono- or di-glycerides. Other parentally-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form, in a liposomal preparation, or as a component of a biodegradable polymer system. Compositions for sustained release or implantation may  
25 comprise pharmaceutically acceptable polymeric or hydrophobic materials such as an emulsion, an ion exchange resin, a sparingly soluble polymer, or a sparingly soluble salt.

#### ***Additional Administration Forms***

Additional dosage forms of this invention include dosage forms as described in U.S. Patents Nos. 6,340,475, 6,488,962, 6,451,808, 5,972,389, 5,582,837, and 5,007,790.  
30 Additional dosage forms of this invention also include dosage forms as described in U.S. Patent Applications Nos. 20030147952, 20030104062, 20030104053, 20030044466, 20030039688, and

20020051820. Additional dosage forms of this invention also include dosage forms as described in PCT Applications Nos. WO 03/35041, WO 03/35040, WO 03/35029, WO 03/35177, WO 03/35039, WO 02/96404, WO 02/32416, WO 01/97783, WO 01/56544, WO 01/32217, WO 98/55107, WO 98/11879, WO 97/47285, WO 93/18755, and WO 90/11757.

## 5 *Controlled Release Formulations and Drug Delivery Systems*

Controlled- or sustained-release formulations of a pharmaceutical composition of the invention may be made using conventional technology. In some cases, the dosage forms to be used can be provided as slow or controlled-release of one or more active ingredients therein using, for example, hydropropylmethyl cellulose, other polymer matrices, gels, permeable  
10 membranes, osmotic systems, multilayer coatings, microparticles, liposomes, or microspheres or a combination thereof to provide the desired release profile in varying proportions. Suitable controlled-release formulations known to those of ordinary skill in the art, including those described herein, can be readily selected for use with the pharmaceutical compositions of the invention. Thus, single unit dosage forms suitable for oral administration, such as tablets,  
15 capsules, gelcaps, and caplets, which are adapted for controlled-release are encompassed by the present invention.

Most controlled-release pharmaceutical products have a common goal of improving drug therapy over that achieved by their non-controlled counterparts. Ideally, the use of an optimally designed controlled-release preparation in medical treatment is characterized by  
20 a minimum of drug substance being employed to cure or control the condition in a minimum amount of time. Advantages of controlled-release formulations include extended activity of the drug, reduced dosage frequency, and increased patient compliance. In addition, controlled-release formulations can be used to affect the time of onset of action or other characteristics, such as blood level of the drug, and thus can affect the occurrence of side effects.

25 Most controlled-release formulations are designed to initially release an amount of drug that promptly produces the desired therapeutic effect, and gradually and continually release of other amounts of drug to maintain this level of therapeutic effect over an extended period of time. In order to maintain this constant level of drug in the body, the drug must be released from the dosage form at a rate that will replace the amount of drug being metabolized  
30 and excreted from the body.

Controlled-release of an active ingredient can be stimulated by various inducers,

for example pH, temperature, enzymes, water, or other physiological conditions or compounds. The term “controlled-release component” in the context of the present invention is defined herein as a compound or compounds, including, but not limited to, polymers, polymer matrices, gels, permeable membranes, liposomes, or microspheres or a combination thereof that facilitates the controlled-release of the active ingredient.

In certain embodiments, the formulations of the present invention may be, but are not limited to, short-term, rapid-offset, as well as controlled, for example, sustained release, delayed release and pulsatile release formulations.

The term sustained release is used in its conventional sense to refer to a drug formulation that provides for gradual release of a drug over an extended period of time, and that may, although not necessarily, result in substantially constant blood levels of a drug over an extended time period. The period of time may be as long as a month or more and should be a release which is longer than the same amount of agent administered in bolus form. For sustained release, the compounds may be formulated with a suitable polymer or hydrophobic material which provides sustained release properties to the compounds. As such, the compounds for use the method of the invention may be administered in the form of microparticles, for example, by injection or in the form of wafers or discs by implantation. In certain embodiments of the invention, the compounds of the invention are administered to a patient, alone or in combination with another pharmaceutical agent, using a sustained release formulation.

The term delayed release is used herein in its conventional sense to refer to a drug formulation that provides for an initial release of the drug after some delay following drug administration and that may, although not necessarily, includes a delay of from about 10 minutes up to about 12 hours. The term pulsatile release is used herein in its conventional sense to refer to a drug formulation that provides release of the drug in such a way as to produce pulsed plasma profiles of the drug after drug administration. The term immediate release is used in its conventional sense to refer to a drug formulation that provides for release of the drug immediately after drug administration.

As used herein, short-term refers to any period of time up to and including about 8 hours, about 7 hours, about 6 hours, about 5 hours, about 4 hours, about 3 hours, about 2 hours, about 1 hour, about 40 minutes, about 20 minutes, or about 10 minutes and any or all whole or partial increments thereof after drug administration.

As used herein, rapid-offset refers to any period of time up to and including about 8 hours, about 7 hours, about 6 hours, about 5 hours, about 4 hours, about 3 hours, about 2 hours, about 1 hour, about 40 minutes, about 20 minutes, or about 10 minutes, and any and all whole or partial increments thereof after drug administration.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures, embodiments, claims, and examples described herein. Such equivalents were considered to be within the scope of this invention and covered by the claims appended hereto. For example, it should be understood, that modifications in reaction conditions, including but not limited to reaction times, reaction size/volume, and experimental reagents, such as solvents, catalysts, pressures, atmospheric conditions, *e.g.*, nitrogen atmosphere, and reducing/oxidizing agents, with art-recognized alternatives and using no more than routine experimentation, are within the scope of the present application.

It is to be understood that wherever values and ranges are provided herein, all values and ranges encompassed by these values and ranges, are meant to be encompassed within the scope of the present invention. Moreover, all values that fall within these ranges, as well as the upper or lower limits of a range of values, are also contemplated by the present application.

The following examples further illustrate aspects of the present invention. However, they are in no way a limitation of the teachings or disclosure of the present invention as set forth herein.

## EXAMPLES

The invention is now described with reference to the following Examples. These Examples are provided for the purpose of illustration only, and the invention is not limited to these Examples, but rather encompasses all variations that are evident as a result of the teachings provided herein.

### Methods and Materials:

#### *ENPP1-asj GACI mouse model:*

Heterozygous ENPP1-asj/+ breeding pairs were maintained on the 'acceleration

diet' (TD00.442, Harlan Laboratories, Madison WI) throughout the entire experiment to generate ENPP1-WT and ENPP1-asj/asj sibling pairs that had been exposed to the acceleration diet *in utero*. Litters were genotyped on day 8 and weaned at day 21. Following weaning, sibling pairs were divided into experimental cohorts and all experimental animals were maintained on the acceleration diet through completion of the study.

#### ***ENPP1-Fc design:***

Modified, human and mouse NPP1 (Human: NCBI accession NP\_006199; Mouse: NCBI accession NP\_03839) modified to express soluble, recombinant protein was fused to IgG1 by subcloning into pFUSE-hIgG1-Fc1 or pFUSE-mIgG1-Fc1 plasmids (InvivoGen, San Diego CA), respectively.

#### ***Protein Production:***

***Shaking flasks:*** Stable transfections of the ENPP1-Fc were established in HEK293 cells under zeocin selection, and adherent HEK293 cells were adapted for suspension growth. Adapted cells were used to seed liquid culture growths in FreeStyle medium (Gibco #12338-018) in shaker flasks at 37 °C and 5% CO<sub>2</sub>, agitated at 120 rpm with high humidity. The culture was gradually expanded to the desired target volume and then maintained for another 12 days to accumulate extracellular protein. During the maintenance phase, cultures were supplemented with CD EfficientFeed C AGT (Gibco #A13275-05) to enhance protein production.

***Bioreactor:*** Cells were propagated in a 10 liter bioreactor equipped with dissolved oxygen and pH control. Dissolved oxygen was kept at 40% air saturation by supplying the culture with mixture of air and oxygen not exceeding 3 liter per minute at an agitation rate of 80 RPM. pH was controlled at 7.4 by sparging CO<sub>2</sub> when the pH was higher than 7.4. Culture growth was followed by measuring cell number, cell viability, glucose and lactate concentrations. Final yields for both methods of production were approximately 5 mg of purified ENPP1-Fc per liter of culture.

#### ***Protein Purification:***

The liquid cultures were centrifuged at 4300 x g for 15 min and the supernatants

were filtered through a 0.2  $\mu\text{m}$  membrane and concentrated via tangential flow using a Pellicon®3 0.11  $\text{m}^2$  Ultracell® 30 kD cassette (Millipore, Billerica MA). The concentrated supernatant was then purified by a combination of chromatographic techniques in a multi-step process. These techniques are performed sequentially and may include any of the following:

5 affinity chromatography with protein A or protein G, cation-exchange chromatography, anion-exchange chromatography, size exclusion chromatography, hydrophobic exchange chromatography, high-pressure liquid chromatography (HPLC), precipitation steps, extractions steps, lyophilizations steps, and/or crystallization steps. Using any one of these steps in series, one schooled in the art of protein chemistry can purify the compositions of matter described to

10 homogeneity such that there are no contaminating protein bands on a silver stained gel (in a non-limiting exemplification, FIG. 10). The resulting protein samples then tested with Pierce LAL Chromogenic Endotoxin Quantitation Kit (cat. 88282) to verify that all were free of endotoxin.

#### *Enzymology:*

15 The steady state hydrolysis of ATP by human NPP1 was determined by HPLC. Briefly, enzyme reactions were started by addition of 10 nM NPP1 to varying concentrations of ATP in the reaction buffer containing 20mM Tris, pH 7.4, 150 mM NaCl, 4.5 mM KCl, 14 $\mu\text{M}$  ZnCl<sub>2</sub>, 1mM MgCl<sub>2</sub> and 1mM CaCl<sub>2</sub>. At various time points, 50  $\mu\text{L}$  reaction solution were removed and quenched with an equal volume of 3M formic acid. The quenched reaction

20 solution was loaded on a C-18 (5  $\mu\text{m}$  250 X 4.6 mm) column (Higgins Analytical) equilibrated in 15 mM ammonium acetate (pH 6.0) solution and eluted with a 0% to 20% methanol gradient. Substrate and products were monitored by UV absorbance at 259 nm and quantified according to the integration of their correspondent peaks and standard curves.

#### *Vehicle:*

25 mENPP1-Fc was formulated in vehicle such that the volume of vehicle delivered was 16  $\mu\text{L}$  vehicle/gram of body weight. Vehicle consisted of americanBio 10X PBS (Stock# AB11072) diluted to 1x with endotoxin free water and supplemented with 14 $\mu\text{M}$  CaCl<sub>2</sub> and 14  $\mu\text{M}$  ZnCl<sub>2</sub>.

#### *Dosing:*

Animals were dosed either with vehicle or with mouse ENPP1-Fc (mENPP1-Fc) formulated in vehicle. Mice were dosed with daily subcutaneous injections starting on day 14 at dose levels of 500 au/Kg mENPP1-Fc.

#### 5 **Enzyme Activity:**

In certain embodiments, enzymes useful within the invention have enzymatic activity with the Michaelis Menton constants as described in Albright, *et al.*, 2015, Nature Comm. 6:10006 ( $K_M \sim 2 \mu\text{M}$  for ATP hydrolysis;  $k_{cat}$  of  $3.46 (\pm 0.44) \text{ s}^{-1}$ ).

#### 10 **Quantification of plasma PPI:**

ENPP1-WT and dosed ENPP1-asj/asj animals were terminally bled retro-orbitally using heparinized micropipettes, and the blood was immediately dispensed into heparin-treated eppendorf tubes and placed on wet ice. The samples were spun in a  $4^\circ\text{C}$  pre-cooled microcentrifuge at 4000 rpm for 5 minutes, and plasma was collected and diluted in one volume of 50 mM Tris-Acetate pH=8.0 and frozen at  $-80^\circ\text{C}$ . Quantitation of serum PPI was performed using as described previously (Cheung & Suhadolnik, 1977, Anal Biochem 83:61-63).

#### **Micro-CT scans:**

***In vivo*  $^{99\text{m}}$ PYP imaging:** The bone imaging agent  $^{99\text{m}}$ Tc-pyrophosphate (Pharmalucence, Inc) was evaluated in cohorts of animals using a preclinical microSPECT/CT hybrid imaging system with dual 1 mm pinhole collimators (X-SPECT, Gamma Medica-Ideas). Each animal was injected ip with 2-5 mCi of the radiolabeled tracer and imaged 1-1.5 hr after injection. A CT scan (512 projections at 50 kVp, 800 uA and a magnification factor of 1.25) was acquired for anatomical co-localization with the SPECT image. The SPECT imaging was acquired with  $180^\circ$  per collimator head in a counter-clockwise rotation, 32 projections, 60 seconds per projection with an ROR of 7.0 cm, FOV of 8.95 cm and an energy window of  $140 \text{ keV} \pm 20$ . CT images were reconstructed with the FLEX X-O CT software (Gamma Medica-Ideas) using a filtered back-projection algorithm. SPECT images were reconstructed using FLEX SPECT software (5 iterations, 4 subsets) and subsequently fused with the CT images and analyzed using AMIRA software and offline in-house script. Data was corrected for decay and injected dose to achieve % injected dose (%ID).

**Quantification of <sup>99m</sup>PYP uptake:** For the <sup>99m</sup>PYP murine scans, the animals were imaged two hours postinjection. The resulting SPECT scans were imported into NIH's ImageJ image processing software and ROI's were drawn around each animal's head (target organ) and whole body. Percent injected activity (PIA), often referred to as "percent injected dose" (%ID) was calculated by comparing the ratio of counts in the head to the counts in the whole body, and expressed as %ID to give a measure as of the affinity with which the radiotracer is taken up by the ROI (head). The total counts in each scan were taken as the whole body measure of injected dose.

### Sequences:

#### NPP1 Amino Acid Sequence (NCBI accession NP\_006199) (SEQ ID NO:1)

MERDGCAGGSRGGEGGRAPREGPAGNGRDRGRSHAAEAPGDPQAAASLLAPMDVGEEPLEKAARAR  
TAKDPNTYKVLSSLVLSVCVLTITLGCIFGLKPSCAKEVKCKGRGCFERTFGNCRCDAAACVELGNCCCL  
DYQETCIEPEHIWTCNKFRGCEKRLTRSLCACSDDCCKDKGDCCINYSVCQGEKSWVEEPCESINEP  
QCPAGFETPPTLLFSLDGFRAEYLHTWGGLLPVISKLLKCGTYTKNMRPVYPTKTFPNHYSIVTGLY  
PESHGIIIDNKMYDPKMNASFSLKSKEKFNPEWYKGEPIWVTAKYQGLKSGTFFWPGSDVEINGIFPD  
IYKMYNGSVPFEEERILAVLQWLQLPKDERPHFYTLYLEEPDSSGHSYGVPVSSEVIKALQRVDGMVGM  
LMDGLKELNLHRCNLILISDHGMEQGSCKYIYLNKYLGDVKNIKVIYGPAARLRPSDVPDKYYSF  
NYEGIARNLSCREPNQHFKPYLKHFLPKRLHFAKSDRIEPLTFYLDQPWQLALNPSEKRYCGSGFHG  
SDNVFSNMQALFVGYPGFKHGIEADTFENIEVYNLMCDLLNLTPAPNNGTHGSLNHLLKNPVYTPK  
HPKEVHPLVQCPFTTRNPRDNLGCSCNP SILPIEDFQTQFNLTVAEEKI IKHETLPYGRPRVLQKENT  
ICLLSQHQFMSGYSQDILMPLWTSYTVDRNDSFSTEDFSNCLYQDFRIPLSPVHKCSFYKNNTKVSY  
GFLSPPQLNKNSSGIYSEALLTTNIVPMYQSFQVIWRYFHDTLRLKYAEERNGVNVVSGPVDFDFDYD  
GRCDLENLRQKRRVIRNQEILIPTHFFIVLTSCKDTSTQPLHCENLDTLAFILPHRTDNSESCVHG  
KHDSSWVEELMLHRARITDVEHITGLSFYQQRKEPVSDILKLKTHLPTFSQED

#### NPP2 Amino Acid Sequence (NCBI accession NP\_001124335) (SEQ ID NO:2)

MARRSSFQSCQIISLFTFAVGVNICLGFTAHRIKRAEGWEEGPPTVLSDSPWTNISGCKGRGCFELQ  
EAGPPDCRCDNLCKSYTSCCHDFDELCLKTARGWECTKDRCGEVRNEENACHCEDCLARGDCCTNY  
QVVCKGESHWVDDDC EEI KAAEC PAGFVRPPLII FSV DGF RASYM KKGSKVMPNIEKLRSCGTHSPY  
MRPVYPTKTFPNLYTLATGLYPESHGIVGNSMYDPVDFDATFHLRGREKFNHRWWGGQPLWITATKQG



VKAGTFFWSVVI PHERRILTILQWLTLPDHERPSVYAFYSEQPDFSGHKYGPFGPEMTNPLREIDKI  
 VGQLMDGLKQLKLHRCVNVI FVGDHGMEDVTCDRTEFLSNYL TNVDDITLVP GTLGRIRSKFSNNAK  
 YDPKAI IANLTCKKPDQHFKPYLKQHL PKRLHYANNRRIEDIHL LVERRWHVARKPLDVYKKPSGKC  
 FFQGDHGF DNKVN SMQTVFVG YGSTFKYKTKVPPFENIELYNVMCDLLGLKPAPNNGTHGSLNHLLR  
 5 TNTFRPTMPEEVTRPNYPGIMYLSDFDLGCTCDDKVEPKNKLDELNKRLHTKGSTEAETRKFGRGS  
 NENKENINGNFEPKREHLLYGRPAVLYRTRYDILYHTDFESGYSEIFLMPLWTSYTVSKQAEVSSV  
 PDHLTSCVRPDVRVSPSFSQNCLAYKNDKQMSYGFLFPPYLSSSPEAKYDAFLVTNMVPMYPAFKRV  
 WNYFQRLVVKKYASERNGVNVISGP IFDYDYDGLHDTEDKIKQYVEGSSIPVPTHYYSIITSCLDFT  
 QPADKCDGPLSVSSFILPHRPDNEESCNSSEDESKWVEELMKMHTARVRDIEHLTSLDFFRKTSRSY  
 10 PEILTLKTYLHTYESEI

**NPP4 Amino Acid Sequence (NCBI accession AAH18054.1) (SEQ ID NO:3)**

MKLLVILLFSG LITGFRSDSSSSSLPPKLLLV SFDGFRADYLKNYEFPHLQNF IKEGVLVEHVKNVFI  
 TKTFPNHYSIVTGLYEESHGIVANS MYDAVTKKHFSDSNDKDPFWWNEAVPIWVTNQLQENRSSAAA  
 15 MWPGTDVPIHDTISSYFMNYNSSVSFEERLNNITMWLNNSNP FVTFATLYWEEP DASGHKYGPEDKE  
 NMSRVLKKIDDLIGDLVQRLKMLGLWENLNVIITSDHGMTQCSQDR LINLDSCIDHSYYTLIDLSPV  
 AAILPKINRTEVYNKLKNCSPHMNVYLKEDI PNRFYYQHNDRIQPIILVADEGWTIVLNESSQKLGD  
 HGYDNSLPSMHPFLAAHGPAFHKG YKHSTINIVDIYPMCHILGLKPHPNNGTFGHTKCLLVDQWCI  
 NLPEAIAIVIGSLLVLTMLTCLIIIMQNRLSVPRPFSRLQLQEDDDDPLIG

20

**(DSS)<sub>n</sub> (SEQ ID NO:4)**, wherein n is an integer ranging from 1 to 10

**(ESS)<sub>n</sub> (SEQ ID NO:5)**, wherein n is an integer ranging from 1 to 10

**(RQQ)<sub>n</sub> (SEQ ID NO:6)**, wherein n is an integer ranging from 1 to 10

**(KR)<sub>n</sub> (SEQ ID NO:7)**, wherein n is an integer ranging from 1 to 10

25 **R<sub>m</sub> (SEQ ID NO:8)**, wherein m is an integer ranging from 1 to 15

**DSSSEKFLRRIGRFG (SEQ ID NO:9)**

**EEEEEEEPRGDT (SEQ ID NO:10)**

**APWHLSSQYSRT (SEQ ID NO:11)**

**STLPIPHFSRE (SEQ ID NO:12)**

30 **VTKHLNQISQSY (SEQ ID NO:13)**

**E<sub>m</sub> (SEQ ID NO:14)**, wherein m is an integer ranging from 1 to 15

**NPP121 Amino Acid Sequence (SEQ ID NO:15)**

1 M E R D G C A G G G S R G G E G G R A P  
 21 R E G P A G N G R D R G R S H A A E A P  
 41 G D P Q A A A S L L A P M D V G E E P L  
 61 E K A A R A R T A K D P N T Y K I I S L  
 5 81 F T F A V G V N I C L G\*\*F T A G L K P S  
 101 C A K E V K S C K G R C F E R T F G N C  
 121 R C D A A C V E L G N C C L D Y Q E T C  
 141 I E P E H I W T C N K F R C G E K R L T  
 161 R S L C A C S D D C K D K G D C C I N Y  
 10 181 S S V C Q G E K S W V E E P C E S I N E  
 201 P Q C P A G F E T P P T L L F S L D G F  
 221 R A E Y L H T W G G L L P V I S K L K K  
 241 C G T Y T K N M R P V Y P T K T F P N H  
 261 Y S I V T G L Y P E S H G I I D N K M Y  
 15 281 D P K M N A S F S L K S K E K F N P E W  
 301 Y K G E P I W V T A K Y Q G L K S G T F  
 321 F W P G S D V E I N G I F P D I Y K M Y  
 341 N G S V P F E E R I L A V L Q W L Q L P  
 361 K D E R P H F Y T L Y L E E P D S S G H  
 20 381 S Y G P V S S E V I K A L Q R V D G M V  
 401 G M L M D G L K E L N L H R C L N L I L  
 421 I S D H G M E Q G S C K K Y I Y L N K Y  
 441 L G D V K N I K V I Y G P A A R L R P S  
 461 D V P D K Y Y S F N Y E G I A R N L S C  
 25 481 R E P N Q H F K P Y L K H F L P K R L H  
 501 F A K S D R I E P L T F Y L D P Q W Q L  
 521 A L N P S E R K Y C G S G F H G S D N V  
 541 F S N M Q A L F V G Y G P G F K H G I E  
 561 A D T F E N I E V Y N L M C D L L N L T  
 30 581 P A P N N G T H G S L N H L L K N P V Y  
 601 T P K H P K E V H P L V Q C P F T R N P  
 621 R D N L G C S C N P S I L P I E D F Q T  
 641 Q F N L T V A E E K I I K H E T L P Y G

661 R P R V L Q K E N T I C L L S Q H Q F M  
 681 S G Y S Q D I L M P L W T S Y T V D R N  
 701 D S F S T E D F S N C L Y Q D F R I P L  
 721 S P V H K C S F Y K N N T K V S Y G F L  
 5 741 S P P Q L N K N S S G I Y S E A L L T T  
 761 N I V P M Y Q S F Q V I W R Y F H D T L  
 781 L R K Y A E E R N G V N V V S G P V F D  
 801 F D Y D G R C D S L E N L R Q K R R V I  
 821 R N Q E I L I P T H F F I V L T S C K D  
 10 841 T S Q T P L H C E N L D T L A F I L P H  
 861 R T D N S E S C V H G K H D S S W V E E  
 881 L L M L H R A R I T D V E H I T G L S F  
 901 Y Q Q R K E P V S D I L K L K T H L P T  
 921 F S Q E D

15 Singly Underlined: residues swapped with NPP2 residues 1-27 to afford cleavage at transition position (\*\*); Doubly Underlined: NPP1 protein (beginning and end).

#### NPP121-Fc Amino Acid Sequence (SEQ ID NO:16)

1 M E R D G C A G G G S R G G E G G R A P  
 20 21 R E G P A G N G R D R G R S H A A E A P  
 41 G D P Q A A A S L L A P M D V G E E P L  
 61 E K A A R A R T A K D P N T Y K I I S L  
 81 F T F A V G V N I C L G\*\*F T A G L K P S  
 101 C A K E V K S C K G R C F E R T F G N C  
 25 121 R C D A A C V E L G N C C L D Y Q E T C  
 141 I E P E H I W T C N K F R C G E K R L T  
 161 R S L C A C S D D C K D K G D C C I N Y  
 181 S S V C Q G E K S W V E E P C E S I N E  
 201 P Q C P A G F E T P P T L L F S L D G F  
 30 221 R A E Y L H T W G G L L P V I S K L K K  
 241 C G T Y T K N M R P V Y P T K T F P N H  
 261 Y S I V T G L Y P E S H G I I D N K M Y  
 281 D P K M N A S F S L K S K E K F N P E W

301 Y K G E P I W V T A K Y Q G L K S G T F  
 321 F W P G S D V E I N G I F P D I Y K M Y  
 341 N G S V P F E E R I L A V L Q W L Q L P  
 361 K D E R P H F Y T L Y L E E P D S S G H  
 5 381 S Y G P V S S E V I K A L Q R V D G M V  
 401 G M L M D G L K E L N L H R C L N L I L  
 421 I S D H G M E Q G S C K K Y I Y L N K Y  
 441 L G D V K N I K V I Y G P A A R L R P S  
 461 D V P D K Y Y S F N Y E G I A R N L S C  
 10 481 R E P N Q H F K P Y L K H F L P K R L H  
 501 F A K S D R I E P L T F Y L D P Q W Q L  
 521 A L N P S E R K Y C G S G F H G S D N V  
 541 F S N M Q A L F V G Y G P G F K H G I E  
 561 A D T F E N I E V Y N L M C D L L N L T  
 15 581 P A P N N G T H G S L N H L L K N P V Y  
 601 T P K H P K E V H P L V Q C P F T R N P  
 621 R D N L G C S C N P S I L P I E D F Q T  
 641 Q F N L T V A E E K I I K H E T L P Y G  
 661 R P R V L Q K E N T I C L L S Q H Q F M  
 20 681 S G Y S Q D I L M P L W T S Y T V D R N  
 701 D S F S T E D F S N C L Y Q D F R I P L  
 721 S P V H K C S F Y K N N T K V S Y G F L  
 741 S P P Q L N K N S S G I Y S E A L L T T  
 761 N I V P M Y Q S F Q V I W R Y F H D T L  
 25 781 L R K Y A E E R N G V N V V S G P V F D  
 801 F D Y D G R C D S L E N L R Q K R R V I  
 821 R N Q E I L I P T H F F I V L T S C K D  
 841 T S Q T P L H C E N L D T L A F I L P H  
 861 R T D N S E S C V H G K H D S S W V E E  
 30 881 L L M L H R A R I T D V E H I T G L S F  
 901 Y Q Q R K E P V S D I L K L K T H L P T  
 921 F S Q E D L I N D K T H T C P P C P A P  
 941 E L L G G P S V F L F P P K P K D T L M

961 I S R T P E V T C V V V D V S H E D P E  
 981 V K F N W Y V D G V E V H N A K T K P R  
 1001 E E Q Y N S T Y R V V S V L T V L H Q D  
 1021 W L N G K E Y K C K V S N K A L P A P I  
 5 1041 E K T I S K A K G Q P R E P Q V Y T L P  
 1061 P S R E E M T K N Q V S L T C L V K G F  
 1081 Y P S D I A V E W E S N G Q P E N N Y K  
 1101 T T P P V L D S D G S F F L Y S K L T V  
 1121 D K S R W Q Q G N V F S C S V M H E A L  
 10 1141 H N H Y T Q K S L S L S P G K

Singly Underlined: residues swapped with NPP2 residues 1-27 to afford cleavage at transition position (\*\*); Doubly Underlined: NPP1 protein (beginning and end); **Bold**: hIgG1 (Fc)

#### NPP71 Amino Acid Sequence (SEQ ID NO:17)

15 1 MRGPAVLLTV ALATLLAPGA GAGLKPSCAK EVKSCKGRCF ERTFGNCRCD  
 51 AACVELGNCC LDYQETCIEP EHIWTCNKFR CGEKRLTRSL CACSDDCKDK  
 101 GDCCINYSSV CQGEKSWVEE PCESINEPQC PAGFETPPTL LFSLDGFRAE  
 151 YLHTWGGLLP VISKLKCCGT YTKNMRPVYP TKTFPNHYSI VTGLYPESHG  
 201 IIDNKMYDPK MNASFSLKSK EKFNPEWYKG EPIWVTAKYQ GLKSGTFFWP  
 20 251 GSDVEINGIF PDIYKMYNGS VPFEERILAV LQWLQLPKDE RPHFYTLYLE  
 301 EPDSSGHSYG PVSSEVIKAL QRVDGMVGML MDGLKELNLH RCLNLILISD  
 351 HGMEQGSCCK YIYLNKYLGD VKNIKVIYGP AARLRPSDVP DKYYSFNYEG  
 401 IARNLSCREP NQHFKPYLKH FLPKRLHFAK SDRIEPLTFY LDPQWQLALN  
 451 PSERKYCGSG FHGSDNVFSN MQALFVGYP GFKHGIEADT FENIEVYNLM  
 25 501 CDLLNLTPAP NNGTHGSLNH LLKNPVYTPK HPKEVHPLVQ CPFTRNPRDN  
 551 LGCSCNPSIL PIEDFQTQFN LTVAEEKIIK HETLPYGRPR VLQKENTICL  
 601 LSQHQFMSGY SQDILMPLWT SYTVDRNDSF STEDFSNCly QDFRIPLSPV  
 651 HKCSFYKNNT KVSYGFLSPP QLNKNSSGIY SEALLTTNIV PMYQSFQVIW  
 701 RYFHD TLLRK YAEERNGVNV VSGPVDFDFD DGRCDLENL RQKRRVIRNQ  
 30 751 EILIPTHFFI VLTSCKDTSQ TPLHCENLDT LAFILPHRTD NSESCVHGKH  
 801 DSSWVEELLM LHRARITDVE HITGLSFYQQ RKEPVSDILK LKTHLPTFSQ  
 851 ED

Singly Underlined: NPP7; Doubly Underlined: NPP1 protein (beginning and end).

**NPP71-Fc Amino Acid Sequence (SEQ ID NO:18)**

1      MRGPAVLLTV ALATLLAPGA GAGLKPSCAK    EVKSCKGRCF    ERTFGNCRCD  
 51      AACVELGNCC    LDYQETCIEP    EHIWTCNKFR    CGEKRLTRSL    CACSDDCKDK  
 5    101    GDCCINYSSV    CQGEKSWVEE    PCESINEPQC    PAGFETPPTL    LFSLDGFRAE  
      151    YLHTWGGLLP    VISKLKCCGT    YTKNMRPVYP    TKTFPNHYSI    VTGLYPESHG  
      201    IIDNKMYDPK    MNASFSLKSK    EKFNPEWYKG    EPIWVTAKYQ    GLKSGTFFWP  
      251    GSDVEINGIF    PDIYKMYNGS    VPFEERILAV    LQWLQLPKDE    RPHFYTTYLE  
      301    EPDSSGHSYG    PVSSEVIKAL    QRVDGMVGML    MDGLKELNLH    RCLNLILISD  
 10    351    HGMEQGSCCK    YIYLNKYLGD    VKNIKVIYGP    AARLRPSDVP    DKYYSFNIEG  
      401    IARNLSCREP    NQHFKPYLKH    FLPKRLHFAK    SDRIEPLTFY    LDPQWQLALN  
      451    PSERKYCGSG    FHGSDNVFSN    MQALFVGYP    GFKHGIEADT    FENIEVYNLM  
      501    CDLLNLTPAP    NNGTHGSLNH    LLKNPVYTPK    HPKEVHPLVQ    CPFTRNPRDN  
      551    LGCSCNPSIL    PIEDFQTQFN    LTVAEEKIHK    HETLPYGRPR    VLQKENTICL  
 15    601    LSQHQFMSGY    SQDILMPLWT    SYTVDRNDSF    STEDFSNCY    QDFRIPLSPV  
      651    HKCSFYKNNT    KVSYGFLSPP    QLNKNSSGIY    SEALLTNIV    PMYQSFQVIW  
      701    RYFHDITLLRK    YAEERNGVNV    VSGPVDFDY    DGRCDLENL    RQKRRVIRNQ  
      751    EILIPTHFFI    VLTSCKDTSQ    TPLHCENLDT    LAFILPHRTD    NSESCVHGKH  
      801    DSSWVEELLM    LHRARITDVE    HITGLSFYQQ    RKEPVSDILK    LKTHLPTFSQ  
 20    851    EDLINDKTH    CPPCPAPELL    GGPSVFLFPP    **KPKDTLMISR**    **TPEVTCVVVD**  
      901    **VSHEDPEVKF**    **NWYVDGVEVH**    **NAKTKPREEQ**    **YNSTYRVVSV**    **LTVLHQDWLN**  
      951    **GKEYKCKVSN**    **KALPAPIEKT**    **ISKAKQPRE**    **PQVYTLPPSR**    **EEMTKNQVSL**  
      1001    **TCLVKGFYPS**    **DIAVEWESNG**    **QPENNYKTP**    **PVLDSGDSFF**    **LYSKLTVDKS**  
      1051    **RWQQGNVFSC**    **SVMHEALHNH**    **YTQKSLSLSP**    **GK**  
 25    Singly Underlined: NPP7; Doubly Underlined: NPP1 protein (beginning and end); **Bold:** hIgG1 (Fc).

**(NPP71 lacking NPP1 N-Terminus GLK) Amino Acid Sequence (SEQ ID NO:19)**

1      MRGPAVLLTV ALATLLAPGA GA    PSCAK    EVKSCKGRCF    ERTFGNCRCD  
 30    51      AACVELGNCC    LDYQETCIEP    EHIWTCNKFR    CGEKRLTRSL    CACSDDCKDK  
      101    GDCCINYSSV    CQGEKSWVEE    PCESINEPQC    PAGFETPPTL    LFSLDGFRAE  
      151    YLHTWGGLLP    VISKLKCCGT    YTKNMRPVYP    TKTFPNHYSI    VTGLYPESHG  
      201    IIDNKMYDPK    MNASFSLKSK    EKFNPEWYKG    EPIWVTAKYQ    GLKSGTFFWP

251 GSDVEINGIF PDIYKMYNGS VPFEERILAV LQWLQLPKDE RPHFYTLYLE  
 301 EPDSSGHSYG PVSSEVIKAL QRVDGMVGML MDGLKELNLH RCLNLILISD  
 351 HGMEQGSCKK YIYLNKYLGD VKNIKVIYGP AARLRPSDVP DKYYSFNYEG  
 401 IARNLSCREP NQHFKPYLKH FLPKRLHFAK SDRIEPLTFY LDPQWQLALN  
 5 451 PSERKYCGSG FHGSDNVFSN MQALFVGYP GFKHGIEADT FENIEVYNLM  
 501 CDLLNLTPAP NNGTHGSLNH LLKNPVYTPK HPKEVHPLVQ CPFTRNPRDN  
 551 LGCSNPSIL PIEDFQTQFN LTVAEEKIHK HETLPYGRPR VLQKENTICL  
 601 LSQHQFMSGY SQDILMPLWT SYTVDRNDSF STEDFSNCLY QDFRIPLSPV  
 651 HKCSFYKNNT KVSYGFLSPP QLNKNSSGIY SEALLTTNIV PMYQSFQVIW  
 10 701 RYFHDTLRLK YAEERNGVNV VSGPVFDFDY DGRCDLENL RQKRRVIRNQ  
 751 EILIPTHFFI VLTCKDTSQ TPLHCENLDT LAFILPHRTD NSESCVHGKH  
 801 DSSWVEELLM LHRARITDVE HITGLSFYQQ RKEPVSDILK LKTHLPTFSQ  
 851 ED  
Singly Underlined: NPP7; Doubly Underlined: NPP1 protein (beginning and end) (first 3-amino  
 15 acids at the N-terminus of NPP1, GLK, are omitted).

**(NPP71 lacking NPP1 N-Terminus GLK)-Fc Amino Acid Sequence (SEQ ID NO:20)**

1 MRGPAVLLTV ALATLLAPGA GA PSCAK EVKSCKGRCF ERTFGNCRCD  
 51 AACVELGNCC LDYQETCIEP EHIWTCNKFR CGEKRLTRSL CACSDDCKDK  
 20 101 GDCCINYSSV CQGEKSWVEE PCESINEPQC PAGFETPPTL LFSLDGFRAE  
 151 YLHTWGGLLP VISKLKCGT YTKNMRPVYP TKTFPNHYSI VTGLYPESHG  
 201 IIDNKMYDPK MNASFSLKSK EKFNPEWYKG EPIWVTAKYQ GLKSGTFFWP  
 251 GSDVEINGIF PDIYKMYNGS VPFEERILAV LQWLQLPKDE RPHFYTLYLE  
 301 EPDSSGHSYG PVSSEVIKAL QRVDGMVGML MDGLKELNLH RCLNLILISD  
 25 351 HGMEQGSCKK YIYLNKYLGD VKNIKVIYGP AARLRPSDVP DKYYSFNYEG  
 401 IARNLSCREP NQHFKPYLKH FLPKRLHFAK SDRIEPLTFY LDPQWQLALN  
 451 PSERKYCGSG FHGSDNVFSN MQALFVGYP GFKHGIEADT FENIEVYNLM  
 501 CDLLNLTPAP NNGTHGSLNH LLKNPVYTPK HPKEVHPLVQ CPFTRNPRDN  
 551 LGCSNPSIL PIEDFQTQFN LTVAEEKIHK HETLPYGRPR VLQKENTICL  
 30 601 LSQHQFMSGY SQDILMPLWT SYTVDRNDSF STEDFSNCLY QDFRIPLSPV  
 651 HKCSFYKNNT KVSYGFLSPP QLNKNSSGIY SEALLTTNIV PMYQSFQVIW  
 701 RYFHDTLRLK YAEERNGVNV VSGPVFDFDY DGRCDLENL RQKRRVIRNQ  
 751 EILIPTHFFI VLTCKDTSQ TPLHCENLDT LAFILPHRTD NSESCVHGKH

801 DSSWVEELLM LHRARITDVE HITGLSFYQQ RKEPVSDILK LKTHLPTFSQ  
 851 EDLINDKTHT CPPCPAPELL GGPSVFLEFP KPKDTLMISR TPEVTCVVVD  
 901 VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN  
 951 GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR EEMTKNQVSL  
 5 1001 TCLVKGFYPS DIAVEWESNG QPENNYKTFP PVLDSDGSFF LYSKLTVDKS  
 1051 RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK

Singly Underlined: NPP7; Doubly Underlined: NPP1 protein (beginning and end) (first 3-amino acids at the N-terminus of NPP1 are omitted); **Bold**: hlgG1 (Fc).

# 10 NPP121-ALB Amino Acid Sequence (SEQ ID NO:21)

**MERDGCAGGSGRGEGGRAPREGPAGNGRDRGRSHAAEAPGDFQAAASLLAPMDVGEEPLEKAARAR**  
**TAKDENTYKIIIS**LFTFAVGVNICLG\*\*FTAGLKPSCAKEVKSCKGRCFERTFGNCRCDAAACVELGNC  
 CLDYQETCIEPEHIWTCNKFRGCEKRLTRSLCACSDCKDKGDCCINYSVCQGEKSWVEEPCESIN  
 EPQCPAGFETPPTLLFSLDGFRAEYLHTWGGLLPVISKLKCGTYTKNMRPVYPTKTFPNHYSIVTG  
 15 LYPESHGIIDNKMYDPKMNASFSLKSKEKFNPEWYKGEPIWVTAKYQGLKSGTFFWPGSDVEINGIF  
 PDIYKMYNGSVPFEEIRILAVLQWLQLPKDERPHFYTLYLEEPDSSGHSYGPVSSEVIKALQVRDGMV  
 GMLMDGLKELNLHRCNLNLILISDHGMEQGSCCKYIYLNKYLGDVKNIKVIYGPAARLRPSDVPDKYY  
 SFNYEGIARNLSCREPNQHFKPYLKHFLPKRLHFAKSDRIEPLTFYLDLPQWQLALNPSEKRYCGSGF  
 HGSDNVFSNMQALFVGYPGFGKHGIEADTFENIEVYNLMCDLLNLTPAPNNGTHGSLNHLLKNPVYT  
 20 PKHPKEVHPLVQCPFTRNPRDNLGCSCNPSILPIEDFQTQFNLTVAEEKI IKHETLPYGRPRVLQKE  
 NTICLLSQHQFMSGYSQDILMPLWTSYTVDRNDSFSTEDFSNCLYQDFRIPLSPVHKCSFYKNNTKV  
 SYGFLSPQNLKNSSGIYSEALLTTNIVPMYQSFQVIWRYFHDTLRKYAEERNGVNVVSGPVDFED  
 YDGRCDLENLRQKRRVIRNQEILIPHTFFIVLTSCKDTSQTPLHCENLDTLAFILPHRTDNSESC  
 HGKHDSSWVEELMLHRARITDVEHITGLSFYQQRKEPVSDILKLKTHLPTFSQEDRSGSGGSMKWV  
 25 **TFLLLL**LVSGSAFSGVFRREAHKSEIAHRYNDLGEQHFKGLVLIAFSQYLQKCSYDEHAKLVQEV  
 DFAKTCVADESAANCDKSLHTLFGDKLCAIPNLRENYGELADCCTKQEPERNECFLQHKDDNPSLPP  
 FERPEAEAMCTSFKENPTTFMGHYLHEVARRHPYFYAPELLYYAEQYNEILTQCCAEADKESCLTPK  
 LDGVKEKALVSSVRQRMKCSSMQKFGERAFAKAWAVARLSQTFPNADFAEITKLATDLTKVNKECCHG  
 DLLECADDRAELAKYMCENQATISSKLQTCCKPLLKKAHCLSEVEHDTMPADLPAIAADFVEDQEV  
 30 CKNYAEAKDVFLGTFLEYEYSRRHPDYSVSLLLRLAKKYEATLEKCCAANPPACYGTVLAEFQPLVE  
 EPKNLVKTNCDLYEKLGEYGFQNAILVRYTQKAPQVSTPTLVEAARNLGRVGTKCCTLPEDQRLPCV  
 EDYLSAILNRVCLLHEKTPVSEHVTKCCSGSLVERRPCFSALTVDETYVPKEFKAETFTFHSDICTL



PEKEKQIKKQTALAEVLKHKPKATAEQLKTVMD DFAQLDTCCKAADKDTCFSTEGPNLVTRCKDAL  
ARSWSHPOFEK

***Bold Italics***: NPP1 cytoplasmic and transmembrane; Singly Underlined: Swapped residues with  
NPP2 residues 1-27 to give cleavage at transition position (\*\*); Doubly Underlined: NPP1  
transmembrane; Plain: NPP1 Extracellular Domain; **Bold Underlined**: Linker; **Bold**: Albumin

**(NPP71 lacking NPP1 N-Terminus GLK)-ALB Amino Acid Sequence (SEQ ID NO:22)**

MRGPAVLLTVALATLLAPGAGAPSCAKEVKSCKGRCFERTFGNCRCDAAACVELGNCCLDYQETCIEP  
EHIWTCNKFRGCEKRLTRSLCACSDCKDKGDCCINYSVCQGEKSWVEEPCESINEPQCPAGFETP  
PTLLFSLDGFRAEYLHTWGGLLPVISKLKCGTYTKNMRPVYPTKTFPNHYSIVTGLYPESHGIIDN  
KMYDPKMNASFSLKSKEKFNPEWYKGEPIWVTAKYQGLKSGTFFWPGSDVEINGIFPDIYKMYNGSV  
PFEERILAVLQWLQLPKDERPHFYTLYLEEDSSGHSYGPVSSEVIKALQRVDGMVGMMLMDGLKELN  
LHRCLNLILISDHGMEQGSCKYIYLNKYLGDVKNIKVIYGPAARLRPSDVPDKYYSFNYEGIARNL  
SCREPNQHFKPYLKHFLPKRLHFAKSDRIEPLTFYLDPOWQLALNPSEKRYCGSGFHGSDNVFSNMQ  
ALFVGYGPGFKHGIEADTFENIEVYNLMCDLLNLTPAPNNGTHGSLNHLLKNPVYTPKHPKEVHPLV  
QCPFTRNPRDNLGCSCNPSILPIEDFQTQFNLTVAEEKI IKHETLPYGRPRVLQKENTICLLSQHQF  
MSGYSQDILMPLWTSYTVDRNDSFSTEDFSNCLYQDFRIPLSPVHKCSFYKNNTKVSYGFLSPPQLN  
KNSSGIYSEALLTTNIVPMYQS FQVIWRYFHD TLLRKYAEERNGVNVVSGPVFDFDYDGRCDLENL  
RQKRRVIRNQEILIPTHFFIVLTSCKDTSQTPLHCENLDTLAFILPHRTDNSESCVHGKHDSSWVEE  
LLMLHRARITDVEHITGLSFYQQRKEPVSDILKLKTHLPTFSQED **RSGSGGSMKWVTFLLLLFVSGS**  
AFSRGVFRREAHKSEIAHRYNDLGEQHFKGLVLIAFSQYLQKCSYDEHAKLVQEVTDFAKTCVADES  
AANCDKSLHTLFGDKLCAIPNLRENYGELADCCTKQEPERNECF LQHKDDNPSLP PFERPEAEAMCT  
SFKENPTTFMGHYLHEVARRHPYFYAPELLYAEQYNEILTQCCAEADKESCLTPKLDGVKEKALVS  
SVRQRMKCSSMQKFGERAFKAWAVARLSQTFPNADFAEITKLATDLTKVNKECCHGDLLECADDRAE  
LAKYMCENQATISSKLQTCCKPLLKKAHCLSEVEHDTMPADLPAIAADFVEDQEVCKNYAEAKDVF  
LGTFLYEYSRRHPDYSVSLLLRLAKKYEATLEKCCAEANPPACYGTVLAEFQPLVEEPKNLVKTNCD  
LYEKLGEYGFQNAILVRYTQKAPQVSTPTLVEAARNLGRVGTKCCTLPEDQRLPCVEDYLSAILNRV  
CLLHEKTPVSEHVTKCCSGSLVERRPCFSALTVDETYVPKEFKAETFTFHSDICTLPEKEKQIKKQT  
ALAEVLKHKPKATAEQLKTVMD DFAQLDTCCKAADKDTCFSTEGPNLVTRCKDALARSWSHPOFEK

Doubly Underlined: NPP7; Plain Text: NPP1; **Bold**: spacer sequence; Singly Underlined:  
albumin

**HSLFTFAVGVNICLGFTA (SEQ ID NO:23)**

**NPP51 Amino Acid Sequence (SEQ ID NO:24)**

MTSKFLLVSFILAALSLSLSTTFSLQPSCAKEVKSCKGRCFERTFSNCRCDAAACVSLGNCCCLDFQETCV  
 EPTHIWTCNKFRFCGEKRLSRFVCSCADDCKTHNDCCINYSSVCQDKKSWVEETCESIDTPECPAEFE  
 5 SPPTLLFSLDGFRAEYLHTWGGLLPVISKLNCGTYTKNMRPMYPTKTFPNHYSIVTGLYPESHGII  
 DNKMYDPKMNASFSLKSKEKFNPLWYKGQPIWVTANHQEVSQTYFWPGSDVEIDGILPDIYKVYNG  
 SVPFEERILAVLEWLQLPSHERPHFYTLYLEEDSSGSHSGPVSSEVIKALQKVDRVLVGMMLMDGLKD  
 LGLDKCLNLILISDHGMEQGSCKKYVYLNKYLGDVNNVKVYGPAAARLRPTDVPETYYSFNYEALAK  
 NLSCREPNQHFRPYLKPFLPKRLHFAKSDRIEPLTFYLDLPQWQLALNPSEKRYCGSGFHGSDNLFSN  
 10 MQALFIGYGPAFKHGAEVDSFENIEVYNLMCDLLGLIPAPNNGSHGSLNHLLKKPIYNPSHPKEEGF  
 LSQCPIKSTSNLDGCTCDPWIVPIKDFEKQLNLTTEDVDDIYHMTVPYGRPRILLKQHRVCLLQQQQ  
 FLTGYSLDLLMPLWASYTFLSNDQFSRDDFSNCLYQDLRIPLSPVHKCSYYKSNSKLSYGFLTTPRL  
 NRVSNHIYSEALLTSNIVPMYQSFQVIWHYLHDTLLQRYAHERNGINVVSGPVDFDYGGRYDSLEI  
 LKQNSRVIRSQEILIPTHFFIVLTSCKQLSETPLECSALESSAYILPHRPDNIESCTHGKRESSWVE  
 15 ELLTLHRARVTDVELITGLSFYQDRQESVSELLRLKTHLPIFSQED

Underlined: NPP5; Plain: NPP1

**NPP51-ALB Amino Acid Sequence (SEQ ID NO:25)**

MTSKFLLVSFILAALSLSLSTTFSLQPSCAKEVKSCKGRCFERTFSNCRCDAAACVSLGNCCCLDFQETCV  
 20 EPTHIWTCNKFRFCGEKRLSRFVCSCADDCKTHNDCCINYSSVCQDKKSWVEETCESIDTPECPAEFE  
 SPPTLLFSLDGFRAEYLHTWGGLLPVISKLNCGTYTKNMRPMYPTKTFPNHYSIVTGLYPESHGII  
 DNKMYDPKMNASFSLKSKEKFNPLWYKGQPIWVTANHQEVSQTYFWPGSDVEIDGILPDIYKVYNG  
 SVPFEERILAVLEWLQLPSHERPHFYTLYLEEDSSGSHSGPVSSEVIKALQKVDRVLVGMMLMDGLKD  
 LGLDKCLNLILISDHGMEQGSCKKYVYLNKYLGDVNNVKVYGPAAARLRPTDVPETYYSFNYEALAK  
 25 NLSCREPNQHFRPYLKPFLPKRLHFAKSDRIEPLTFYLDLPQWQLALNPSEKRYCGSGFHGSDNLFSN  
 MQALFIGYGPAFKHGAEVDSFENIEVYNLMCDLLGLIPAPNNGSHGSLNHLLKKPIYNPSHPKEEGF  
 LSQCPIKSTSNLDGCTCDPWIVPIKDFEKQLNLTTEDVDDIYHMTVPYGRPRILLKQHRVCLLQQQQ  
 FLTGYSLDLLMPLWASYTFLSNDQFSRDDFSNCLYQDLRIPLSPVHKCSYYKSNSKLSYGFLTTPRL  
 NRVSNHIYSEALLTSNIVPMYQSFQVIWHYLHDTLLQRYAHERNGINVVSGPVDFDYGGRYDSLEI  
 30 LKQNSRVIRSQEILIPTHFFIVLTSCKQLSETPLECSALESSAYILPHRPDNIESCTHGKRESSWVE  
 ELLTLHRARVTDVELITGLSFYQDRQESVSELLRLKTHLPIFSQEDGGSGGSMKWTELLLLLFVSGS  
AFSRGVFRREAHKSEIAHRYNDLGEQHFKGLVLIAFSQYLQKCSYDEHAKLVQEVTDFAKTCVADES  
AANCDKSLHTLFGDKLCAIPNLRENYGELADCCTKQEPERNECFLOHKDDNPSLPFERPEAEAMCT

SFKENPTTFMGHYLHEVARRHPYFYAPELLYYAEQYNEILTQCCAEADKESCLTPKLDGVKEKALVS  
SVRQRMKCSSMQKFGERAFAKAWAVARLSQTFPNADFAEITKLATDLTKVNKECCHGDLLECADDRAE  
LAKYMCENQATISSKLQTCCKPLLKKAHCLSEVEHDTMPADLPAIAADFVEDQEVCKNYAEAKDVF  
LGTFLYEYSRRHPDYSVSLLLRLAKKYEATLEKCCAEANPPACYGTVLAEFQPLVEEPKKNLVKTNCD  
5 LYEKLGEYGFQNAILVRYTQKAPQVSTPTLVEAARNLGRVGTKCCTLPEDQRLPCVEDYLSAILNRV  
CLLHEKTPVSEHVTKCCSGSLVERRPCFSALTVDETYVPKEFKAETFTFHSDICTLPEKEKQIKKQT  
ALAELVKHKPKATAEQLKTVMDFAQFLDTCKAADKDTCFSTEGPNLVTRCKDALARSWSHPOFEK  
Doubly Underlined: NPP5; Plain: NPP1; **Bold:** Spacer; Singly Underlined: Albumin

10 **Human IgG Fc domain, Fc (SEQ ID NO:26)**

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNA  
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS  
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGN  
VFSCSVMEALHNHYTQKSLSLSPGK

15

**ALB (SEQ ID NO:27)**

MKWVTFLLLLFVSGSAFSGVFRREAHKSEIAHRYNDLGEQHFKGLVLIAFSQYLQKCSYDEHAKLV  
QEVTDFAKTCVADESAANCDKSLHTLFGDKLCAIPNLRENYGELADCCTKQEPERNECFLOHKDDNP  
SLPPFERPEAEAMCTSFKENPTTFMGHYLHEVARRHPYFYAPELLYYAEQYNEILTQCCAEADKESC  
20 LTPKLDGVKEKALVSSVRQRMKCSSMQKFGERAFAKAWAVARLSQTFPNADFAEITKLATDLTKVNKE  
CCHGDLLECADDRAELAKYMCENQATISSKLQTCCKPLLKKAHCLSEVEHDTMPADLPAIAADFVE  
DQEVCKNYAEAKDVF LGTFLYEYSRRHPDYSVSLLLRLAKKYEATLEKCCAEANPPACYGTVLAEFQ  
PLVEEPKKNLVKTNCDLYEKLGEYGFQNAILVRYTQKAPQVSTPTLVEAARNLGRVGTKCCTLPEDQR  
LPCVEDYLSAILNRVCLLHEKTPVSEHVTKCCSGSLVERRPCFSALTVDETYVPKEFKAETFTFHSD  
25 ICTLPEKEKQIKKQTALAELVKHKPKATAEQLKTVMDFAQFLDTCKAADKDTCFSTEGPNLVTRC  
KDALARSWSHPOFEK

**LIN (SEQ ID NO:28)**

**GGSGGS (SEQ ID NO:29)**

30 **RSGSGGS (SEQ ID NO:30)**

**Example 1: ENPP1-asj PXE Mouse Model**

Certain polypeptides of the invention (such as ENPP1-Fc) were tested in mouse models of PXE and osteoarthritis (OA). The PXE mice present the loss of function mutation in the multi-pass membrane transporter ABCC6, in a similar fashion to humans with PXE. ANK mice were used as a mammalian model for OA.

Heterozygous ENPP1-asj/+ breeding pairs were maintained on the “acceleration diet” (TD00.442, Harlan Laboratories, Madison WI) throughout the entire experiment to generate ENPP1-WT and ENPP1-asj/asj sibling pairs that had been exposed to the acceleration diet *in utero*. Litters were genotyped on day 8 and weaned at day 21. Following weaning, sibling pairs were divided into experimental cohorts and all experimental animals were maintained on the acceleration diet through completion of the study. Selected polypeptides of the invention were administered to study animals, as described herein, and bones are analyzed.

As illustrated in FIG. 7, both PXE and ANK mice initially have low PPi, a biomarker that is reported in the literature to account for the pathogenesis of PXE (Jansen, *et al.*, 2014, *Arterioscler. Thromb. Vasc. Biol.* 34:1985-1989).

Two PXE mice were dosed for one week with ENPP1-Fc, and the mean plasma PPi in these animals increased to about 4  $\mu$ M. This indicates that administration of the polypeptides of the invention to mammals raises their extracellular levels of PPi and treats PXE.

The polypeptide's ability to elevate PPi was not expected, because the biological mechanism for low PPi was thought to be associated with low ATP concentrations (Jansen, *et al.*, 2013, *PNAS U S A* 110(50):20206–20211). In fact, it was proposed in the prior art that correction of plasma PPi in PXE is sufficient to treat the disease (Jansen, *et al.*, 2013, *PNAS U S A* 110(50):20206–20211). Based on the prior art at the time of the invention, one skilled in the art would contemplate that ENPP1 enzyme is not able to generate PPi in the setting of PXE due to lack of sufficient substrate in the extracellular space. As demonstrated herein, this is clearly not the case.

**Example 2:**

When fed an acceleration diet, the daily weights of ENPP1-asj/asj mice diverged from WT siblings pairs at day 26, when the ENPP1-asj/asj mice experienced a “failure to thrive” event and began to lose weight (FIG. 1A). After day 26 the ENPP1-asj/asj animals displayed

progressive stiffness and reductions in physical activity. All of the ENPP1-asj/asj animals died between days 35-71, with a median lifespan of 58 days (FIG. 1G). The presence of calcifications in ENPP1-asj/asj and ENPP1-WT mice was evaluated post mortem by micro-CT scans and histologic sections taken from the heart, aorta, and kidneys. Approximately one-third of the ENPP1-asj/asj mice had visible calcifications in their hearts, and two-thirds had visible calcifications in their aortas, by micro-CT imaging (Table 2). These percentages increased to 100% upon histologic examination, which also showed that many of the animals had dramatic nearly circumferential calcifications in their aortic walls (FIGs. 1D-1E). Histologic examination also revealed that 100% of the coronary arteries possessed arterial wall calcifications, and that 70% of the animals had focal or confluent areas of myocardial necrosis consistent with myocardial infarction (FIGs. 1F-1G). Conversely, the ENPP1-WT mice displayed none of these abnormalities. These findings demonstrate that the animal model recapitulates GACI in humans, which is characterized by prominent calcifications of the large and medium sized arteries and a cardiac demise.

To produce soluble, recombinant ENPP1 for *in vivo* use, ENPP1 was fused to the Fc domain of IgG1 (hereafter referred to as ENPP1-Fc, FIG. 2B) and the fusion protein was expressed in stable mammalian (HEK293) cell lines. The combined effect of switching protein expression from insect cells to mammalian cells and fusion of ENPP1 to the Fc domain of IgG1 altered the Michaelis Menton kinetics by increasing the affinity of ENPP1 for ATP substrate by over two orders of magnitude, while also reducing the  $K_{cat}$  by a factor of 3-4 (FIGs. 2C-2E). The activity of ENPP1-Fc was noted to diminish over a 30 day period when stored at 4° C but the enzyme could be frozen at -80° C and retain nearly complete activity upon thawing (FIG. 2C). The enzyme was therefore stored as a frozen stock solution after purification until needed.

Following purification, ENPP1-Fc was dialyzed into PBS supplemented with Zn and Mg (PBS<sub>plus</sub>) concentrated to between 5 and 7 mg/ml, and frozen at -80° C in aliquots of 200-500  $\mu$ l. Aliquots were thawed immediately prior to use and the specific activity of the solution was adjusted to 31.25 au/ml (or about 0.7 mg/ml depending on the preparation) by dilution in PBS<sub>plus</sub>.

Dosing was performed according to activity units (au) per Kg animal weight to account for variations in specific activity in different protein preparations. The specific activity of the enzyme varied with each protein preparation, and because the clinical response was noted

to be highly dependent on enzyme specific activity, protein preparations with specific activities of less than 40 au/mg were rejected. To establish initial dosing levels for the proof of concept study, dose escalation trials were performed in limited numbers of animals (1-2 per dose level). While both the human and mouse version ENPP1 was used in the dose escalation trials, the proof of concept study was performed with the mouse isoform of ENPP1-Fc (mENPP1-Fc). ENPP1-asj/asj mice were dosed daily on the 14<sup>th</sup> day of life with subcutaneous injections of mENPP1-Fc and weekly with intra-peritoneal (I.P.) injections of GK 1.5, the latter added to minimize immune rejection of recombinant protein. Subcutaneous doses of mEnpp1-Fc at 500 au/Kg qD demonstrated a strong early response in weight with an absence of the observed “failure to thrive” crisis observed in undosed ENPP1-asj/asj animals.

Based on the results of the dose escalation trials, a cohort of 8 NPP1-asj/asj animals was dosed with mNPP1-Fc at 500 au/Kg qD and weekly IP injections with GK1.5 (FIG. 3). A control group (NPP1-WT + vehicle and NPP1-asj/asj + vehicle) was dosed daily with vehicle and weekly with GK1.5 in an identical manner as the dosed cohort, and the study duration was shortened to 55 days. All 8 treated ENPP1-asj/asj animals survived the full 55 days of the trial, with a dramatic clinical response observed in treated, while the median life span of the untreated NPP1 asj/asj animals decreased from 58 days to 37 days in the therapeutic trial, perhaps resulting from the weekly IP injections of the GK1.5 immunosuppressive. The untreated ENPP1-asj/asj animals also all experienced a failure to thrive crisis day 26, characterized by weight loss and mobility restriction progressing variably to paralysis and death over the next 30 days. All but one untreated ENPP1-asj/asj animal expired over the 55 day trial, while in contrast all treated ENPP1-asj/asj mice gained weight comparable to the ENPP1-WT mice and displayed no signs of reduced mobility or stiffness.

At the conclusion of the study, 100% of the ENPP1-asj/asj mice treated with vehicle displayed calcifications in their hearts, aortas, and coronary arteries, and 77% of the animals displayed histologic evidence of myocardial infraction (Table 1). In most cases this took the form of small areas of myocardial cell necrosis and drop out in the vicinity of the cardiac calcifications (FIGs. 3C-3D, FIGs. 4C-4E), but in two animals (22%) there were large, full thickness myocardial infarctions in the free wall of the right ventricle (FIGs. 4C-4D). Myocardial fibrosis in myocardial tissue adjacent to coronary artery calcifications was a common finding (FIG. 4E), illustrating that ischemia from coronary artery calcification likely accounts for

the myocardial disease. In contrast, none of the ENPP1-asj/asj animals treated with ENPP1-Fc displayed cardiac, arterial, or aortic calcification on histology or post-mortem micro-CT (Table 1, and FIGs. 3D and 4D).

In addition to survival, daily animal weights, and terminal histology, treatment response was also assessed via post-mortem high resolution micro-CT scans to image vascular calcifications, plasma [PPi] concentrations, and Tc99 PPi ( $^{99m}\text{PYP}$ ) uptake (FIG. 5 and Table 1). The biochemical and physiologic response was complete as measured by all of these parameters. None of the WT or treated ENPP1-asj/asj animals were noted to possess any vascular calcifications via micro-CT, in contrast to the dramatic calcifications noted in the aortas, coronary arteries, and hearts of the untreated ENPP1-asj/asj cohort (FIG. 5A). In addition, serum PPi concentrations of treated ENPP1-asj/asj animals were elevated above WT animals (about 30  $\mu\text{M}$  in the treated ENPP1-asj/asj vs. about 10  $\mu\text{M}$  in WT), and well above untreated ENPP1-asj/asj levels ( $< 0.5 \mu\text{M}$ ) (FIG. 5B). In addition, serum PPi concentrations of treated ENPP1-asj/asj animals (about 30  $\mu\text{M}$ ) were elevated well above untreated ENPP1-asj/asj levels ( $< 0.5 \mu\text{M}$ ), and above that of WT animals (about 10  $\mu\text{M}$ ) (FIG. 5B).

$^{99m}\text{PYP}$ , an imaging agent typically employed in cardiac imaging and bone remodeling, was used as a marker for treatment response because one would expect that  $^{99m}\text{PYP}$  uptake in animals lacking functional ENPP1 should be increased as they would be expected to have reduce plasma [PPi] and more 'open' PPi binding sites at sites of ectopic mineralization. To test this hypothesis, *in vivo*  $^{99m}\text{PYP}$  imaging was performed weekly in ENPP1-WT and undosed ENPP1-asj/asj animals to detect differences in PYP uptake between the sibling pairs (FIGs. 5C-5D). Analysis of  $^{99m}\text{PYP}$  uptake was limited to the head, which is comprised of both enchondral bone (skull) and soft tissue (vibrissae), which are known sites of ectopic calcification in mouse models of this GACI. In addition, the analysis was limited to the head to simplify data collection, as the head does not overlap with internal organs showing transient  $^{99m}\text{PYP}$  uptake (such as the bladder, heart, and diaphragm) during the 180° camera rotation that occurs during data collection.

Weekly serial imaging of ENPP1-WT and untreated ENPP1-asj/asj animals demonstrated that the percent uptake of the injected dose of  $^{99m}\text{PYP}$  in skulls was greater in ENPP1-asj/asj animals than in ENPP1-WT animals and changes in  $^{99m}\text{PYP}$  uptake within experimental groups did not vary significantly over the course of the study (FIGs. 5C-5D).

<sup>99m</sup>PYP Uptake in treated and untreated ENPP1-asj/asj animals was compared at two time points—days 30-35 and at the completion of the study (days 50-65). Comparison of these experimental groups demonstrates that ENPP1-Fc treatment returned <sup>99m</sup>PYP uptake in GACI mice to WT levels (FIGs. 5E-5F), suggesting that ENPP1-Fc treatment is able to abrogate unregulated tissue and skull mineralization in ENPP1-asj/asj mice by saturating open PPi binding sites with ‘cold’ PPi, which presumably originates from increased plasma PPi concentrations induced by the therapeutic.

**Table 1: Cardiovascular Pathology, Proof of Concept Study**

	WT+Vehicle	asj/asj+vehicle	asj/asj+mENPP1-Fc
Calcifications Heart (CT/Histology)	0 / 0	55%/100%	0/0
Calcifications Aorta (CT/Histology)	0 / 0	66%/100%	0/0
Calcifications in Coronary Arteries (CT/histology)	0 / 0	43%/100%	0/0
% myocardial infarction (Histology)	0 / 0	77%	0

**Table 2: Cardiovascular Pathology, Natural History Study**

	WT	asj/asj
% Calcifications in heart (CT / histology)	0 / 0	37% / 100%
Calcifications in Aorta (CT/histology)	0 / 0	62% / 100%
Calcifications in Coronary Arteries (Histology)	0 / 0	100%
% myocardial infarction (Histology)	0	70%

### Example 3: Expression of Albumin Fusion Protein

Human serum albumin (HSA), a protein of 585 amino acids, is responsible for a significant proportion of the osmotic pressure of serum and also functions as a carrier of endogenous and exogenous ligands. At present, HSA for clinical use is produced by extraction from human blood. Production of recombinant HSA (rHSA) in microorganisms has been disclosed in EP 0 330 451 and EP 0 361 991.



The role of albumin as a carrier molecule and its inert nature are desirable properties for use as a stabilizer and transporter of polypeptides. Use of albumin as a component of a fusion protein for stabilizing other proteins has been disclosed in WO 93/15199, WO 93/15200, and EP 0 413 622. The use of N-terminal fragments of HSA for fusions to polypeptides has also been disclosed (EP 0 399 666). Fusion to the polypeptide is achieved by genetic manipulation, such that the DNA coding for HSA, or a fragment thereof, is joined to the DNA coding for the polypeptide. A suitable host is then transformed or transfected with the fused nucleotide sequences, so arranged on a suitable plasmid as to express a fusion polypeptide. Nomura, *et al.*, 1995, Biosci. Biotechnol. Biochem. 59(3):532-4 attempted to express human apolipoprotein E in *S. cerevisiae* as a fusion protein with HSA or fragments of HSA, using the HSA pre-sequence to direct secretion. Whilst fusion to full length HSA resulted in the secretion of low levels of the protein into the medium (maximum yield of 6.3 mg per liter), fusion to HSA (1-198) or HSA (1-390) did not result in secretion into the medium.

The human serum albumin may be a variant of normal HSA (termed hereinafter "HSA"). As used herein, "variants" include insertions, deletions and substitutions, either conservative or non-conservative, where such changes do not, substantially alter one or more of the oncotic, useful ligand-binding and non-immunogenic properties of albumin. In particular, "variants" include naturally-occurring polymorphic variants of human albumin and fragments of human albumin, for example those fragments disclosed in EP 0 322 094 (namely HA (1-n), where n is 369 to 419). The albumin or growth hormone (GH) may be from any vertebrate, especially any mammal, for example human, cow, sheep, pig, hen or salmon. The albumin and GH parts of the fusion may be from differing animals.

By "conservative substitutions" is intended swaps within groups such as Gly / Ala; Val / Ile / Leu; Asp / Glu; Asn / Gln; Ser / Thr; Lys / Arg; and Phe / Tyr. The variant usually has at least 75% (such as at least 80%, 90%, 95% or 99%) sequence identity with a length of normal HSA that is the same length as the variant and that is more identical thereto than any other length of normal HSA, once the allowance is made for deletions and insertions as is customary in this art. Generally speaking, an HSA variant is at least 100 amino acids long, in some embodiments at least 150 amino acids long. The HSA variant may consist of or comprise at least one whole domain of HSA, for example domains 1 (1-194), 2 (195-387), 3 (388-585), 1+2 (1-387), 2+3 (195-585) or 1+3 (1-194,+388-585). Each domain is itself made up of two

homologous subdomains namely 1-105, 120-194, 195-291, 316-387, 388-491 and 512-585, with flexible inter-subdomain linker regions comprising residues Lys106 to Glu199, Glu292 to Val315 and Glu492 to Ala511. In some embodiments, the HSA part of the NPP1 fusion comprises at least one subdomain or domain of HA or conservative modifications thereof.

5 Many expression systems are known, including bacteria (for example *E. coli* and *Bacillus subtilis*), yeasts (for example *Saccharomyces cerevisiae*, *Kluyveromyces lactis* and *Pichia pastoris*), filamentous fungi (for example *Aspergillus*), plant cells, animal cells and insect cells.

The desired protein can be produced in conventional ways, for example from a coding sequence inserted in the host chromosome or on a free plasmid.

10 The yeasts can be transformed with a coding sequence for the desired protein in any of the usual ways, for example electroporation. Methods for transformation of yeast by electroporation are disclosed in Becker & Guarente, 1990, Methods Enzymol. 194:182. Successfully transformed cells, *i.e.*, cells that contain a DNA construct of the present invention, can be identified by well-known techniques. For example, cells resulting from the introduction of an expression construct can be grown to produce the desired polypeptide. Cells can be harvested and lysed and their DNA content examined for the presence of the DNA using a method, such as that described by Southern, 1975, J. Mol. Biol. 98:503 and/or Berent, *et al.*, 1985, Biotech 3:208. Alternatively, the presence of the protein in the supernatant can be detected using antibodies.

20 Useful yeast plasmid vectors include pRS403–406 and pRS413–416 and are generally available from Stratagene Cloning Systems, La Jolla, CA, USA. Plasmids pRS403, pRS404, pRS405 and pRS406 are Yeast Integrating plasmids (YIps) and incorporate the yeast selectable markers HIS3, TRP1, LEU2 and URA3. Plasmids pRS413–416 are Yeast Centromere plasmids (YCps).

25 A variety of methods have been developed to operably link DNA to vectors via complementary cohesive termini. For instance, complementary homopolymer tracts can be added to the DNA segment to be inserted to the vector DNA. The vector and DNA segment are then joined by hydrogen bonding between the complementary homopolymeric tails to form recombinant DNA molecules.

Synthetic linkers containing one or more restriction sites provide an alternative method of joining the DNA segment to vectors. The DNA segment, generated by endonuclease restriction digestion, is treated with bacteriophage T4 DNA polymerase or *E. coli* DNA polymerase I, which are enzymes that remove protruding, 3'-single-stranded termini with their 3'-5'-exonucleolytic activities, and fill in recessed 3'-ends with their polymerizing activities.

The combination of these activities thus generates blunt-ended DNA segments. The blunt-ended segments are then incubated with a large molar excess of linker molecules in the presence of an enzyme that is able to catalyze the ligation of blunt-ended DNA molecules, such as bacteriophage T4 DNA ligase. Thus, the products of the reaction are DNA segments carrying polymeric linker sequences at their ends. These DNA segments are then cleaved with the appropriate restriction enzyme and ligated to an expression vector that has been cleaved with an enzyme that produces termini compatible with those of the DNA segment.

Synthetic linkers containing a variety of restriction endonuclease sites are commercially available from a number of sources including International Biotechnologies Inc, New Haven, CT, USA.

A desirable way to modify the DNA in accordance with the invention, if, for example HA variants are to be prepared, is to use the polymerase chain reaction as disclosed by Saiki, *et al.*, 1988, Science 239:487-491. In this method the DNA to be enzymatically amplified is flanked by two specific oligonucleotide primers that themselves become incorporated into the amplified DNA. The specific primers may contain restriction endonuclease recognition sites which can be used for cloning into expression vectors using methods known in the art.

***ENPPI-ALB design:***

Modified, human and mouse NPP1 (Human: NCBI accession NP\_006199; Mouse: NCBI accession NP\_03839) modified to express soluble, recombinant protein is fused to human serum albumin (HSA) by sub cloning into pFUSE plasmids (InvivoGen, San Diego CA), respectively.

***Protein Production:***

*Shaking flasks:* Stable transfections of the ENPPI-ALB are established in HEK293 cells under zeocin selection, and adherent HEK293 cells can be adapted for suspension growth. Adapted cells are used to seed liquid culture growths in FreeStyle medium (Gibco #12338-018) in shaker flasks at 37 °C and 5% CO<sub>2</sub>, agitated at 120 rpm with high humidity. The

culture is gradually expanded to the desired target volume and then maintained for another 12 days to accumulate extracellular protein. During the maintenance phase, cultures are supplemented with CD EfficientFeed C AGT (Gibco #A13275-05) to enhance protein production.

5                    *Bioreactor:* Cells are propagated in a 10 liter bioreactor equipped with dissolved oxygen and pH control. Dissolved oxygen is kept at 40% air saturation by supplying the culture with mixture of air and oxygen not exceeding 3 liter per minute at an agitation rate of 80 RPM. pH is controlled at 7.4 by sparging CO<sub>2</sub> when the pH is higher than 7.4. Culture growth is followed by measuring cell number, cell viability, glucose and lactate concentrations.

10    ***Protein Purification:***

                    The liquid cultures are centrifuged at 4300 x g for 15 min and the supernatants are filtered through a 0.2 µm membrane and concentrated via tangential flow using a Pellicon®3 0.11 m2 Ultracell® 30 kD cassette (Millipore, Billerica MA). The concentrated supernatant is loaded onto a protein-AG column and can be eluted with a buffer comprising 50 mM Sodium  
15    Citrate, 150 mM NaCl, 3 mM ZnCl<sub>2</sub>, 3 mM CaCl<sub>2</sub>, pH=3.5. Fractions containing enzymatic activity are pooled and dialyzed against IX PBS buffer pH 7.4, 11 µM ZnCl<sub>2</sub>, 20 µM CaCl<sub>2</sub>, then concentrated to 6 mg/ml, distributed into small aliquots and stored at -80 °C.

                    The resulting protein samples are tested with Pierce LAL Chromogenic Endotoxin Quantitation Kit (cat. 88282) to verify that all are free of endotoxin.

20    ***Enzymology***

                    The NPPI-albumin fusion protein after purification are characterized following the experimental protocols discussed in Examples 1 and 2, described elsewhere herein.

                    The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety. While this invention has  
25    been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.

## CLAIMS

What is claimed is:

1. A compound of formula (I), or a salt thereof:  

PROTEIN-Z-DOMAIN-X-Y (I), wherein in (I):

PROTEIN comprises residues 23-849 of SEQ ID NO:20, residues 23-852 of SEQ ID NO:18, or residues 93-925 of SEQ ID NO:16;

DOMAIN is selected from the group consisting of a human IgG Fc domain (Fc) and human serum albumin protein (ALB);

X and Z are independently absent or a polypeptide comprising 1-20 amino acids;

Y is absent; and

the compound lacks a negatively-charged bone-targeting sequence.
2. The compound of claim 1, wherein DOMAIN is a Fc.
3. The compound of claim 1, wherein DOMAIN is an ALB.
4. The compound of any one of claims 1 to 3, wherein the nuclease domain of the PROTEIN is absent.
5. The compound of any one of claims 1 to 3, wherein the nuclease domain from about residue 524 to about residue 885 relative to SEQ ID NO:1 is absent in the PROTEIN.
6. The compound of any one of claims 1 to 5, wherein a segment of the extracellular region of ENPP2 containing a furin or signal peptide cleavage site is, or is not, substituted into the PROTEIN.
7. The compound of claim 1, wherein DOMAIN is a Fc, and wherein PROTEIN-Z-DOMAIN comprises (SEQ ID NO:15)-Z-Fc, (SEQ ID NO:17)-Z-Fc, (SEQ ID NO:19)-Z-Fc, OR (SEQ ID NO:24)-Z-Fc.
8. The compound of claim 7, wherein the DOMAIN comprises the amino acid

sequence as set forth is SEQ ID NO:26.

9. The compound of claim 1, wherein DOMAIN is an ALB, and wherein PROTEIN-Z-DOMAIN comprises (SEQ ID NO:15)-Z-ALB, (SEQ ID NO:17)-Z-ALB, (SEQ ID NO:19)-Z-ALB, or (SEQ ID NO:24)-Z-ALB.

10. The compound of claim 9, wherein the DOMAIN comprises the amino acid sequence as set forth in SEQ ID NO:27.

11. The compound of any one of claims 1 to 10, which has a  $k_{cat}$  value greater than or equal to  $3.4 (\pm 0.4) \text{ s}^{-1} \text{ enzyme}^{-1}$ , wherein the  $k_{cat}$  is determined by measuring the compound's ATP hydrolysis rate.

12. The compound of any one of claims 1 to 11, which has a  $K_M$  value less than or equal to  $2 \mu\text{M}$ , wherein the  $K_M$  is determined by measuring the compound's ATP hydrolysis rate.

13. A method of treating and/or preventing a disease or disorder associated with pathological calcification and/or pathological ossification in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of at least one compound of any of claims 1-12, wherein the disease or disorder comprises at least one selected from the group consisting of Generalized Arterial Calcification of Infancy (GACI), Idiopathic Infantile Arterial Calcification (IIAC), Ossification of the Posterior Longitudinal Ligament (OPLL), Chronic Kidney Disease (CKD), hypophosphatemic rickets, osteoarthritis, and calcification of atherosclerotic plaques.

14. Use of at least one compound of any of claims 1-12 in the preparation of a medicament for treating and/or preventing a disease or disorder in a subject, wherein the disease or disorder comprises at least one selected from the group consisting of Generalized Arterial Calcification of Infancy (GACI), Idiopathic Infantile Arterial Calcification (IIAC), Ossification of the Posterior Longitudinal Ligament (OPLL), Chronic Kidney Disease (CKD), hypophosphatemic rickets, osteoarthritis, and calcification of atherosclerotic plaques.

15. A method of treating and/or preventing a disease or disorder associated with pathological calcification and/or pathological ossification in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of at least one compound of any of claims 1-12, wherein the disease or disorder comprises at least one selected from the group consisting of PXE, hereditary and non-hereditary forms of osteoarthritis, ankylosing spondylitis, hardening of the arteries occurring with aging, calciphylaxis resulting from end stage renal disease (ESRD) and progeria.

16. Use of at least one compound of any of claims 1-12 in the preparation of a medicament for treating and/or preventing a disease or disorder in a subject, wherein the disease or disorder comprises at least one selected from the group consisting of PXE, hereditary and non-hereditary forms of osteoarthritis, ankylosing spondylitis, hardening of the arteries occurring with aging, calciphylaxis resulting from end stage renal disease (ESRD) and progeria.

17. A method of reducing and/or preventing cardiac calcifications, arterial calcifications and/or elastic fiber mineralizations in a subject afflicted with at least one disease or disorder selected from the group consisting of GACI and PXE, the method comprising administering to the subject a therapeutically effective amount of at least one compound of any one of claims 1-14, whereby the administering of the at least one compound increases extracellular pyrophosphate (PPi) concentrations in the subject, thus reducing or preventing cardiac calcifications, arterial calcifications and/or elastic fiber mineralization in the subject.

18. Use of at least one compound of any one of claims 1-12 in the preparation of a medicament for reducing and/or preventing cardiac calcifications, arterial calcifications and/or elastic fiber mineralizations in a subject, whereby administering of the medicament to the subject increases extracellular pyrophosphate (PPi) concentrations in the subject, thus reducing or preventing cardiac calcifications, arterial calcifications and/or elastic fiber mineralization in the subject.

19. The method of any one of claims 13, 15 or 17, wherein the administering is subcutaneous.

20. The method of claim 19, wherein the administering restores the subject's extracellular pyrophosphate concentrations to a level within the range found in a subject not afflicted with GACI and/or PXE.

21. The method of any one of claims 13, 15, 17, 19 or 20, or the use of any one of claims 14, 16 or 18, wherein the compound is a cleavage product of a precursor ENPP1 polypeptide comprising an ecto-nucleotide pyrophosphate/phosphodiesterase-2 (ENPP2) transmembrane domain.

22. The method or use of claim 21, wherein the ENPP2 transmembrane domain is residues 12-30 of NCBI accession no. NP\_001124335 (SEQ ID NO:2), which corresponds to SEQ ID NO:23.

23. A method of reducing and/or preventing cardiac calcifications, arterial calcifications and/or elastic fiber mineralizations in a subject afflicted with at least one disease or disorder selected from the group consisting of GACI and PXE, the method comprising administering to the infant a therapeutically effective amount of a given polypeptide comprising a ENPP1 polypeptide and an ALB, wherein the given polypeptide lacks a polyaspartic acid domain, wherein the administering increases extracellular PPI concentrations in the subject, thus reducing or preventing cardiac calcifications, arterial calcifications and/or elastic fiber mineralization in the subject.

24. The method of claim 23, wherein the administering comprises subcutaneous administration.

25. The method of claim 23 or 24, wherein the administering restores the subject's extracellular pyrophosphate concentrations to a level within the range found in a subject not afflicted with GACI and/or PXE.



26. Use of a polypeptide comprising a ENPP1 polypeptide and an ALB, wherein the given polypeptide lacks a polyaspartic acid domain in the preparation of a medicament for reducing and/or preventing cardiac calcifications, arterial calcifications and/or elastic fiber mineralizations in a subject afflicted with at least one disease or disorder selected from the group consisting of GACI and PXE, wherein the polypeptide lacks a polyaspartic acid domain, and wherein administering the medicament to the subject increases extracellular PPi concentrations in the subject, thus reducing or preventing cardiac calcifications, arterial calcifications and/or elastic fiber mineralization in the subject

27. A method of reducing and/or preventing cardiac calcifications, arterial calcifications and/or elastic fiber mineralizations in a subject afflicted with a disease characterized by having a loss of function mutation in ABCC6, the method comprising administering to the subject a therapeutically effective amount of at least one compound of any of claims 1-12, thereby reducing and/or preventing cardiac calcifications, arterial calcifications and/or elastic fiber mineralizations in the subject.

28. Use of at least one compound of any of claims 1-12 in the preparation of a medicament for reducing and/or preventing cardiac calcifications, arterial calcifications and/or elastic fiber mineralizations in a subject afflicted with a disease characterized by having a loss of function mutation in ABCC6.

29. A method of reducing and/ or preventing cardiac calcifications, arterial calcifications and/or elastic fiber mineralizations in a subject afflicted with a disease characterized by having a loss of function mutation in ENPP1, the method comprising administering to the subject a therapeutically effective amount of at least one compound of any of claims 1-12, thereby reducing and/ or preventing cardiac calcifications, arterial calcifications and/or elastic fiber mineralizations in the subject.

30. Use of at least one compound of any of claims 1-12 in the preparation of a medicament for reducing and/ or preventing cardiac calcifications, arterial calcifications and/or

elastic fiber mineralizations in a subject afflicted with a disease characterized by having a loss of function mutation in ENPP1.

**Yale University**  
**Patent Attorneys for the Applicant/Nominated Person**  
**SPRUSON & FERGUSON**

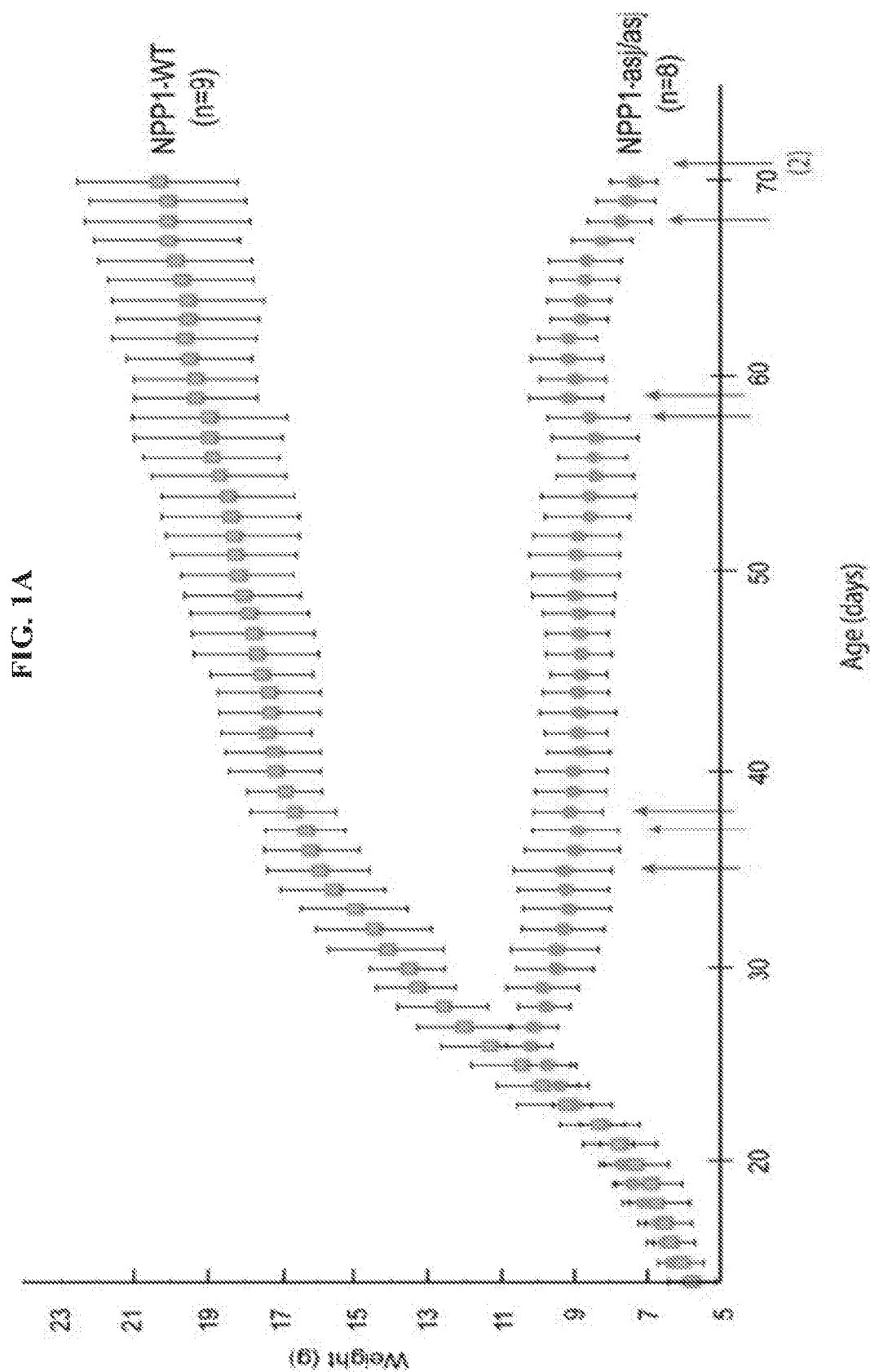


FIG. 1B

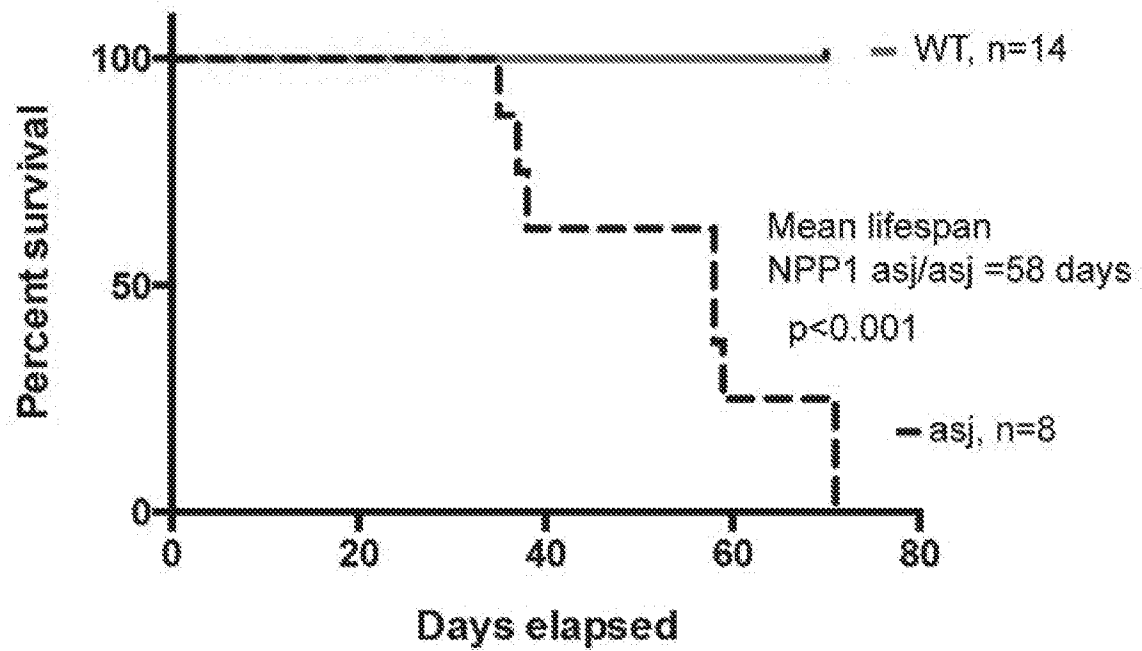


FIG. 1C

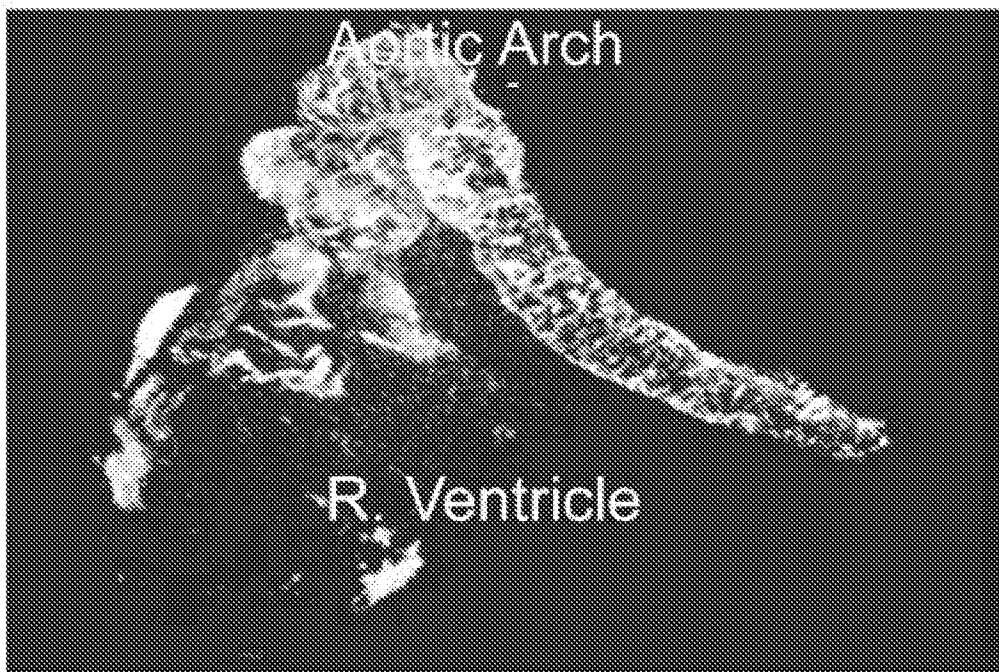


FIG. 1D

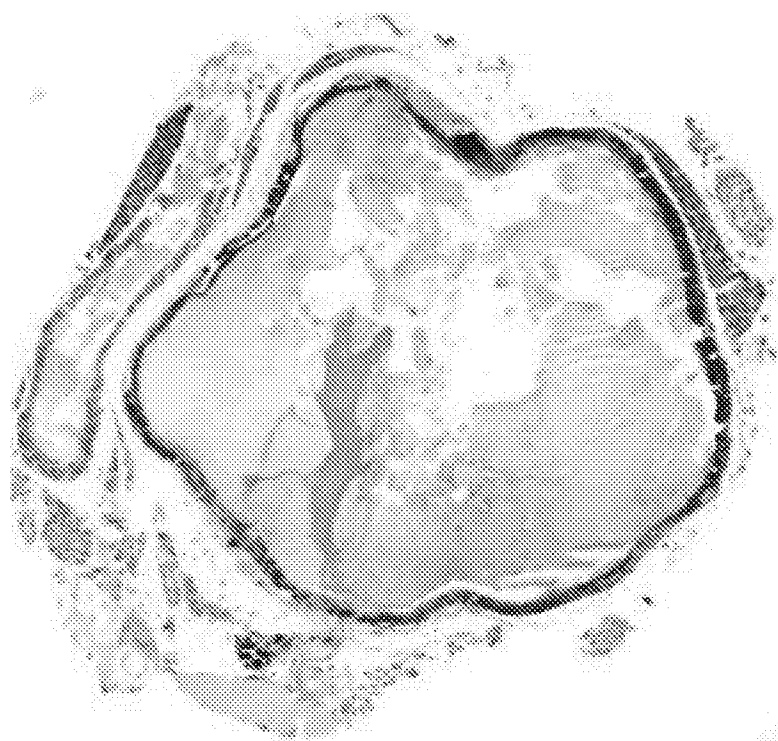


FIG. 1E

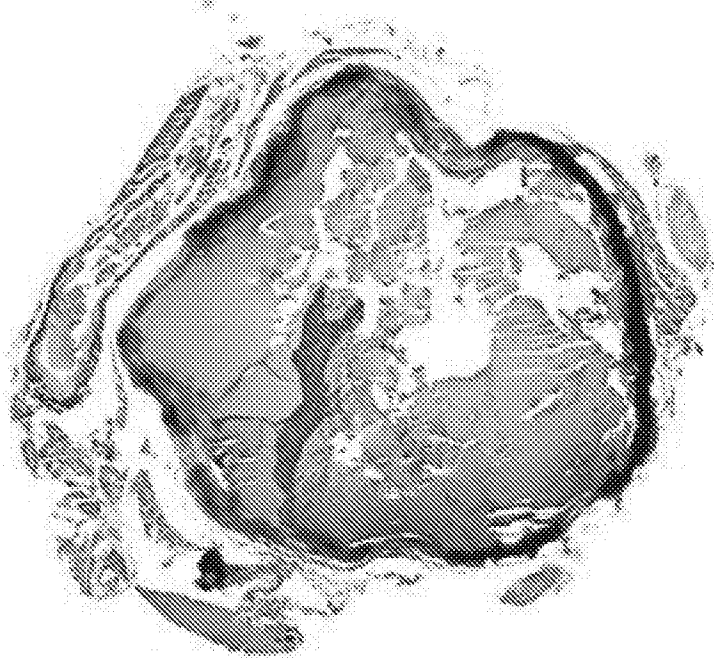


FIG. 1F

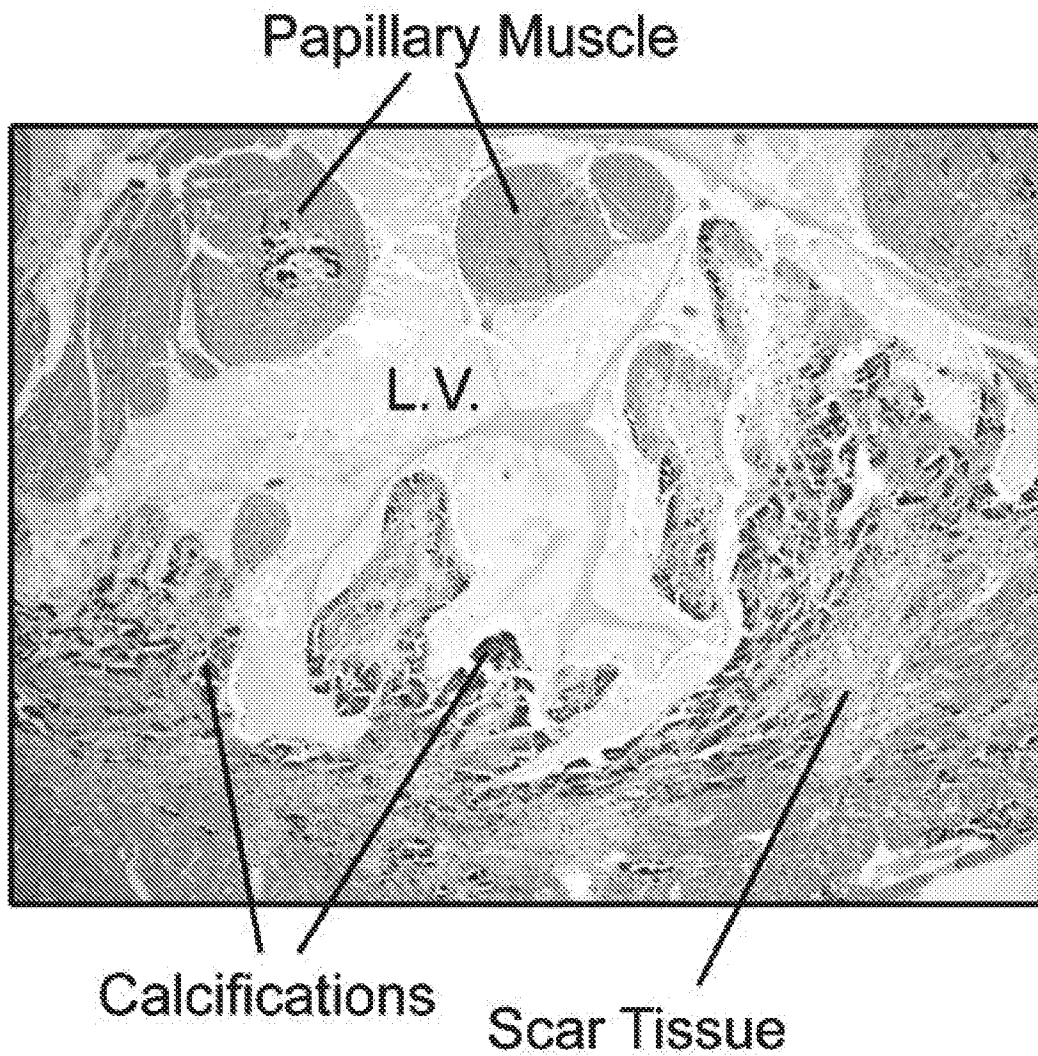


FIG. 1G

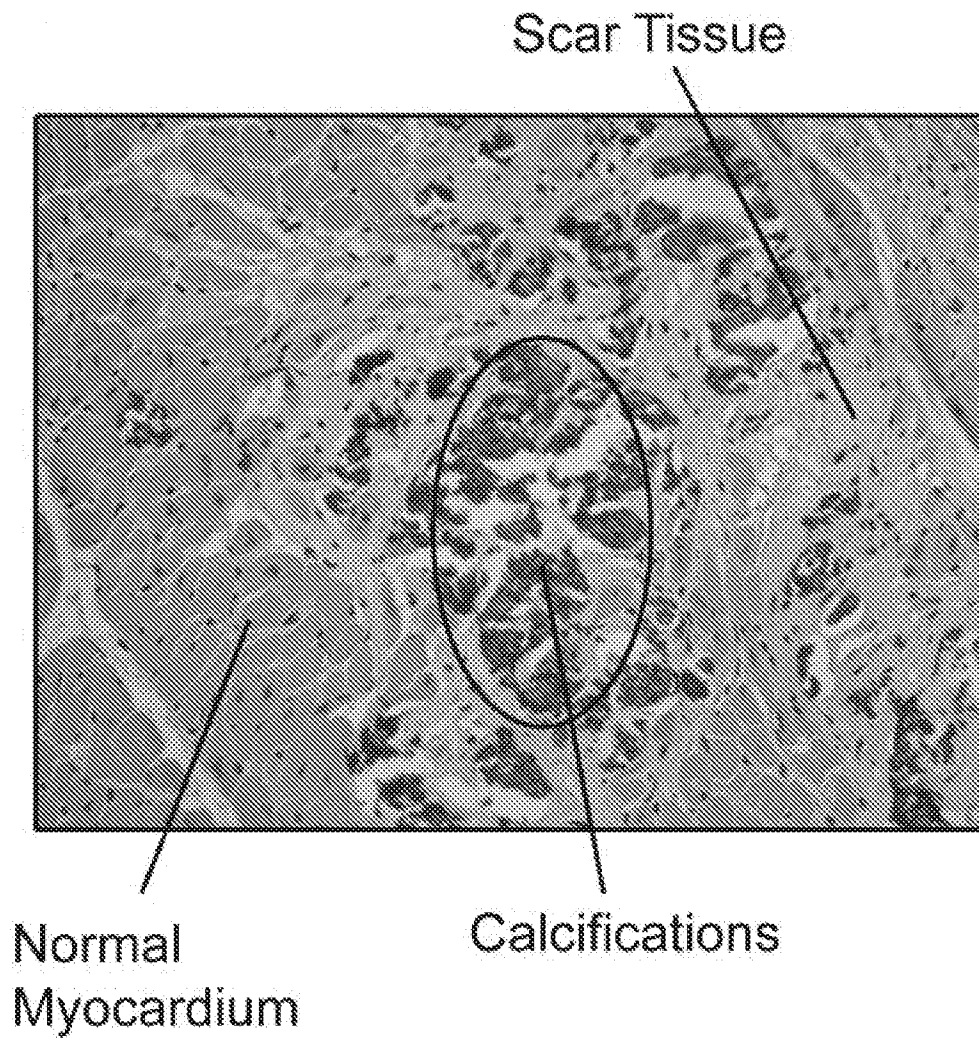


FIG. 2A

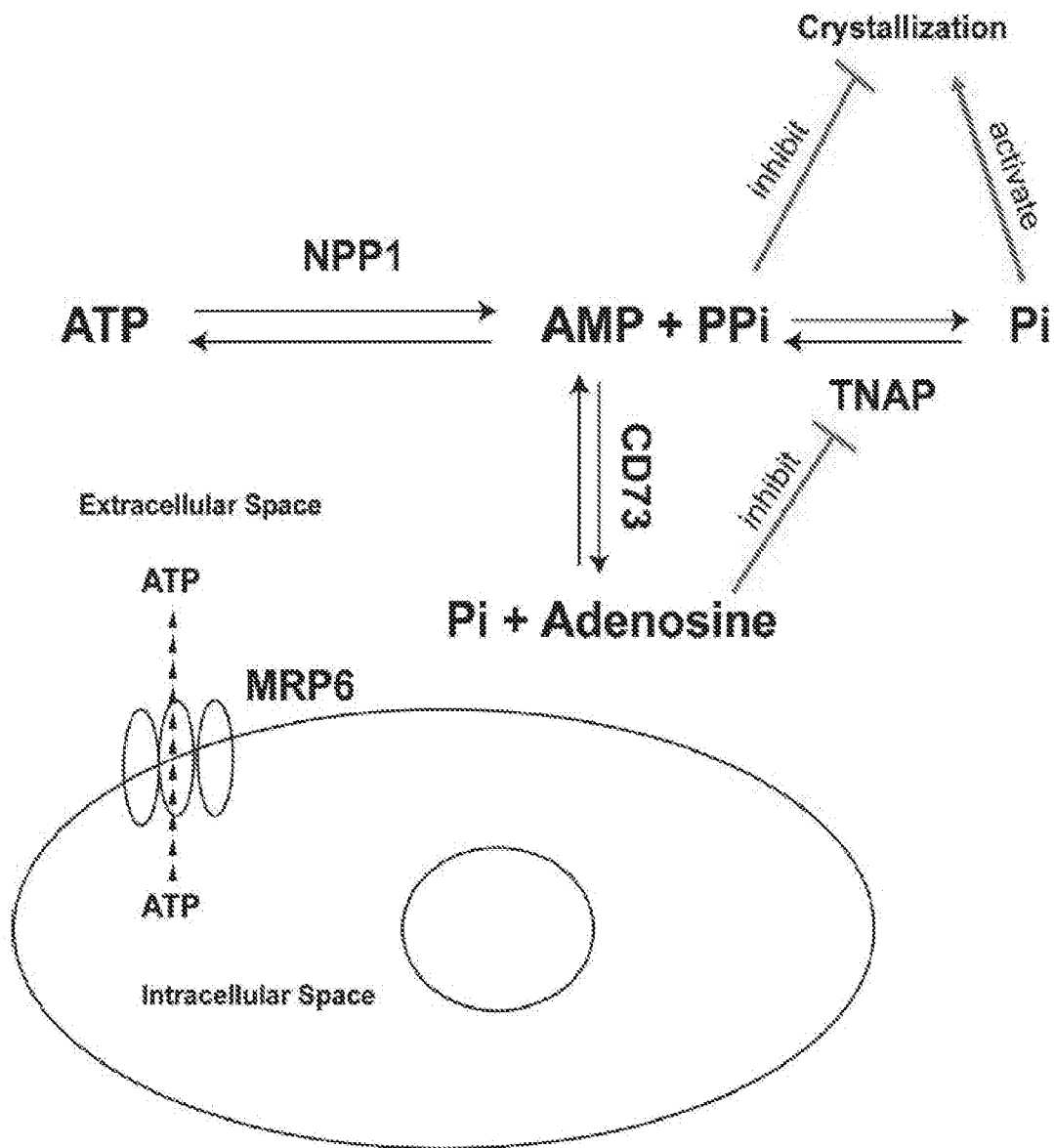




FIG. 2B

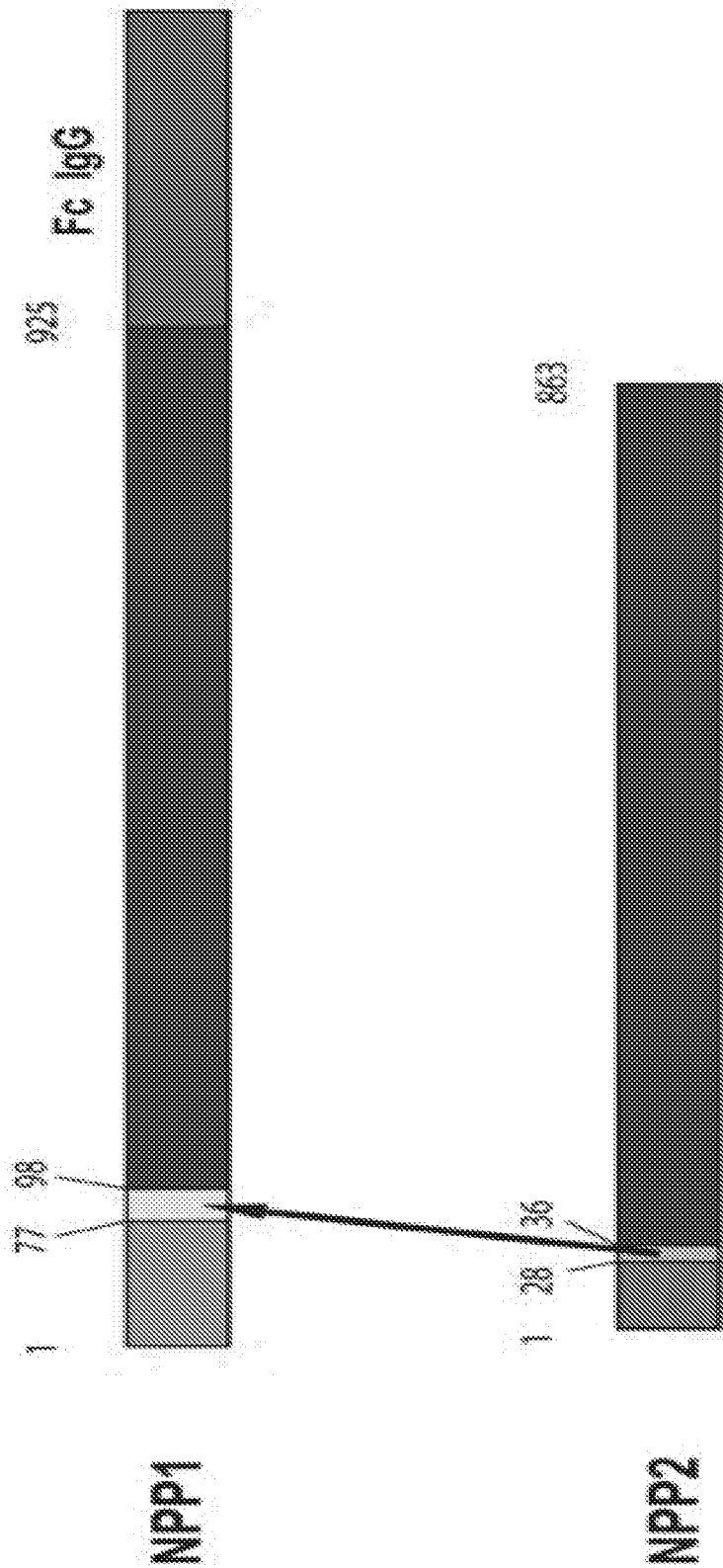


FIG. 2C

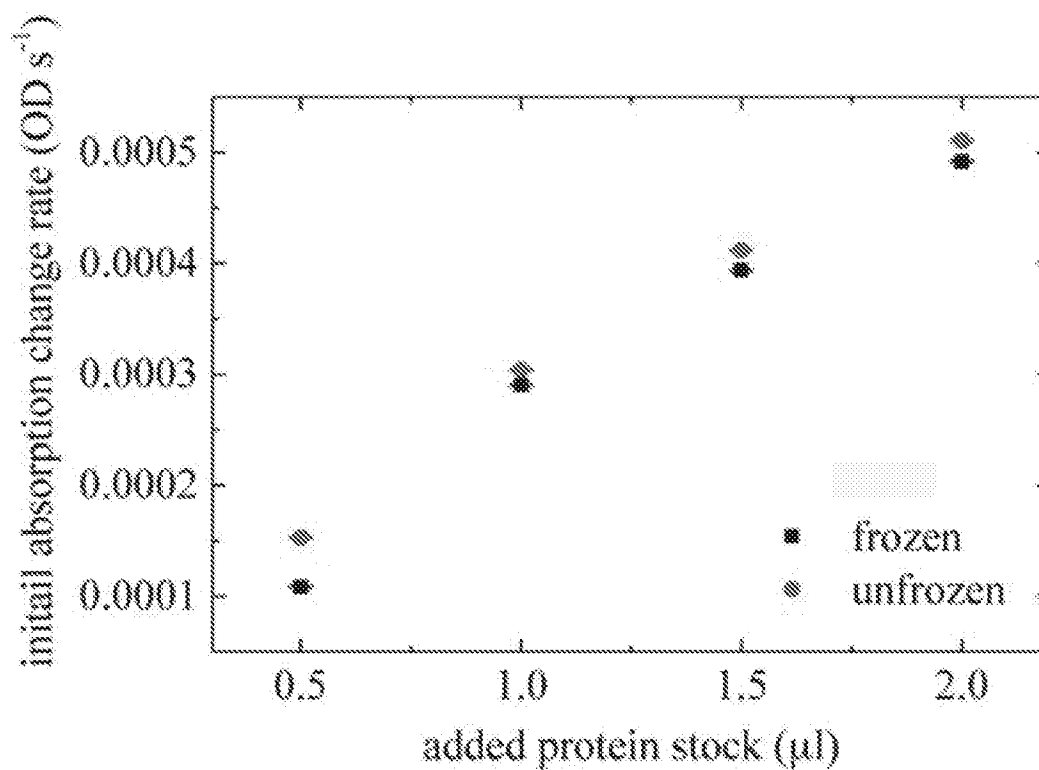


FIG. 2D

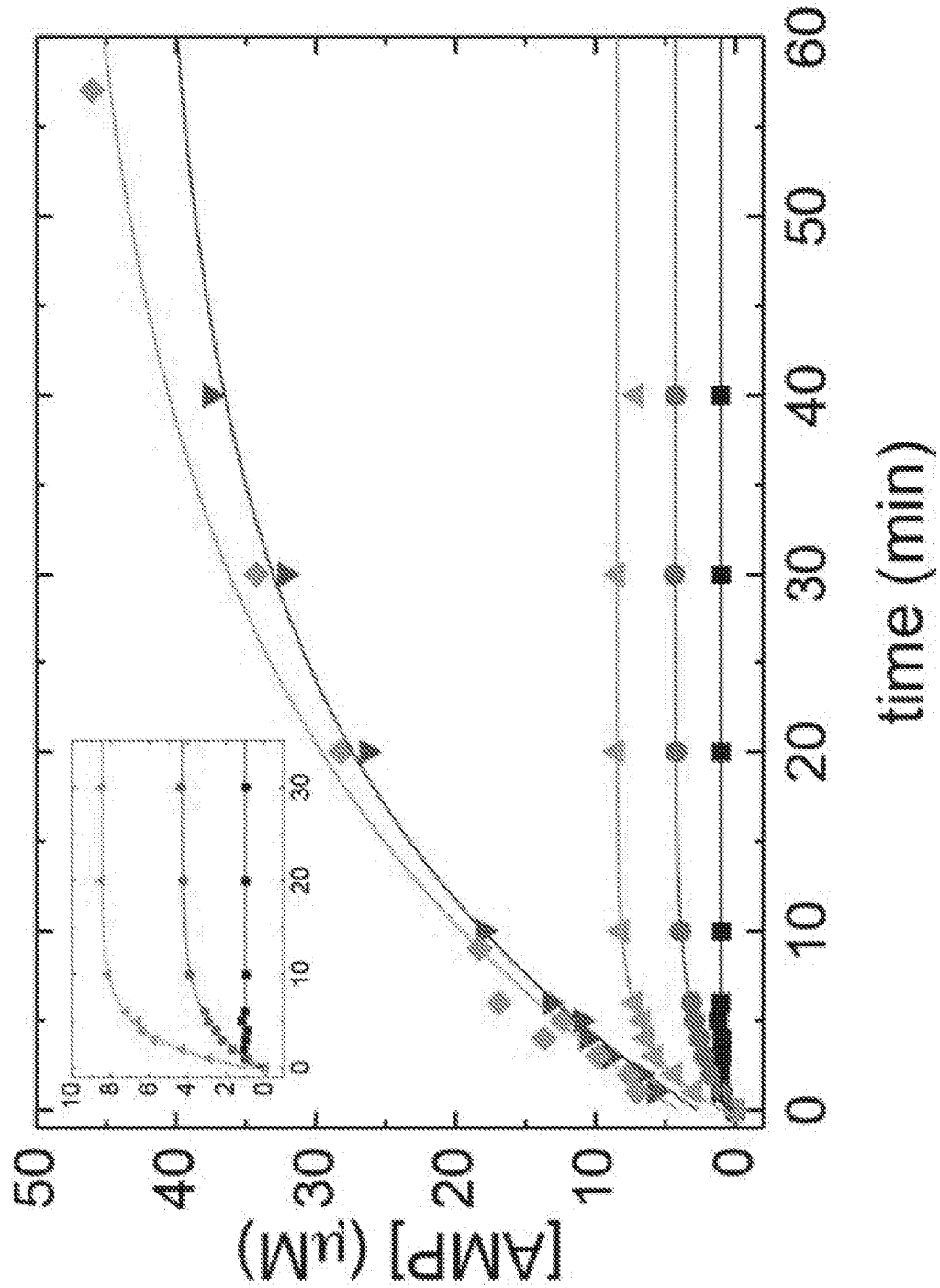


FIG. 2E

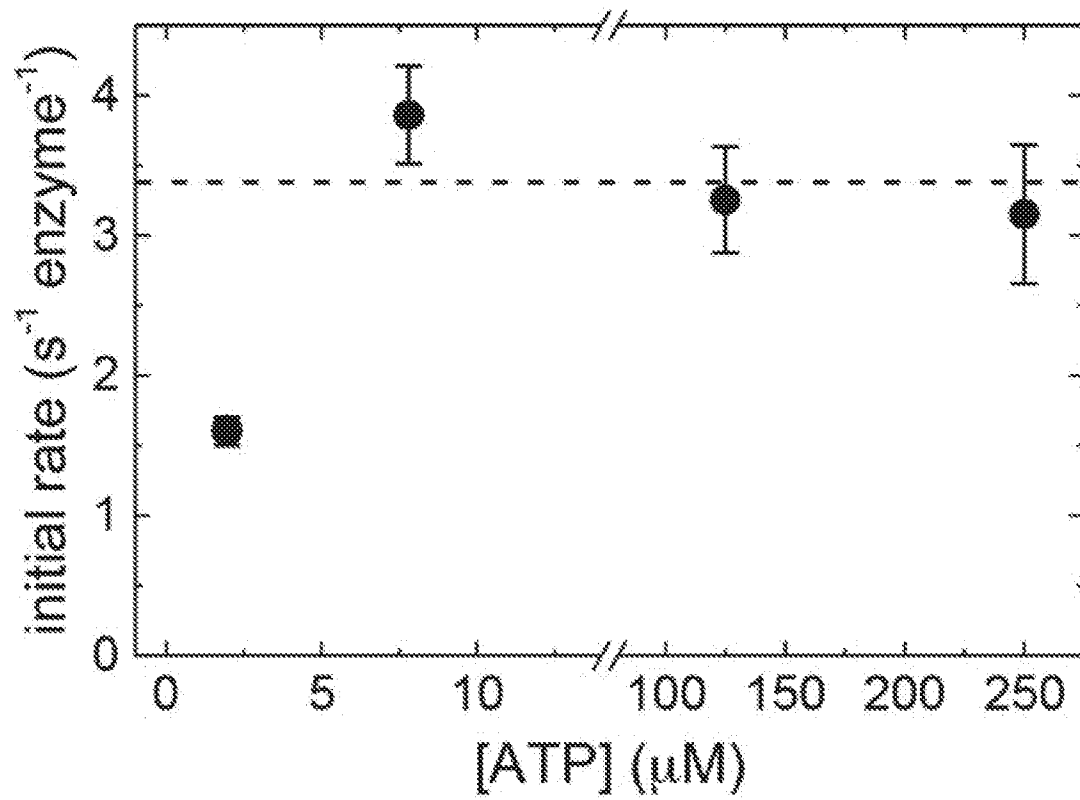


FIG. 3A

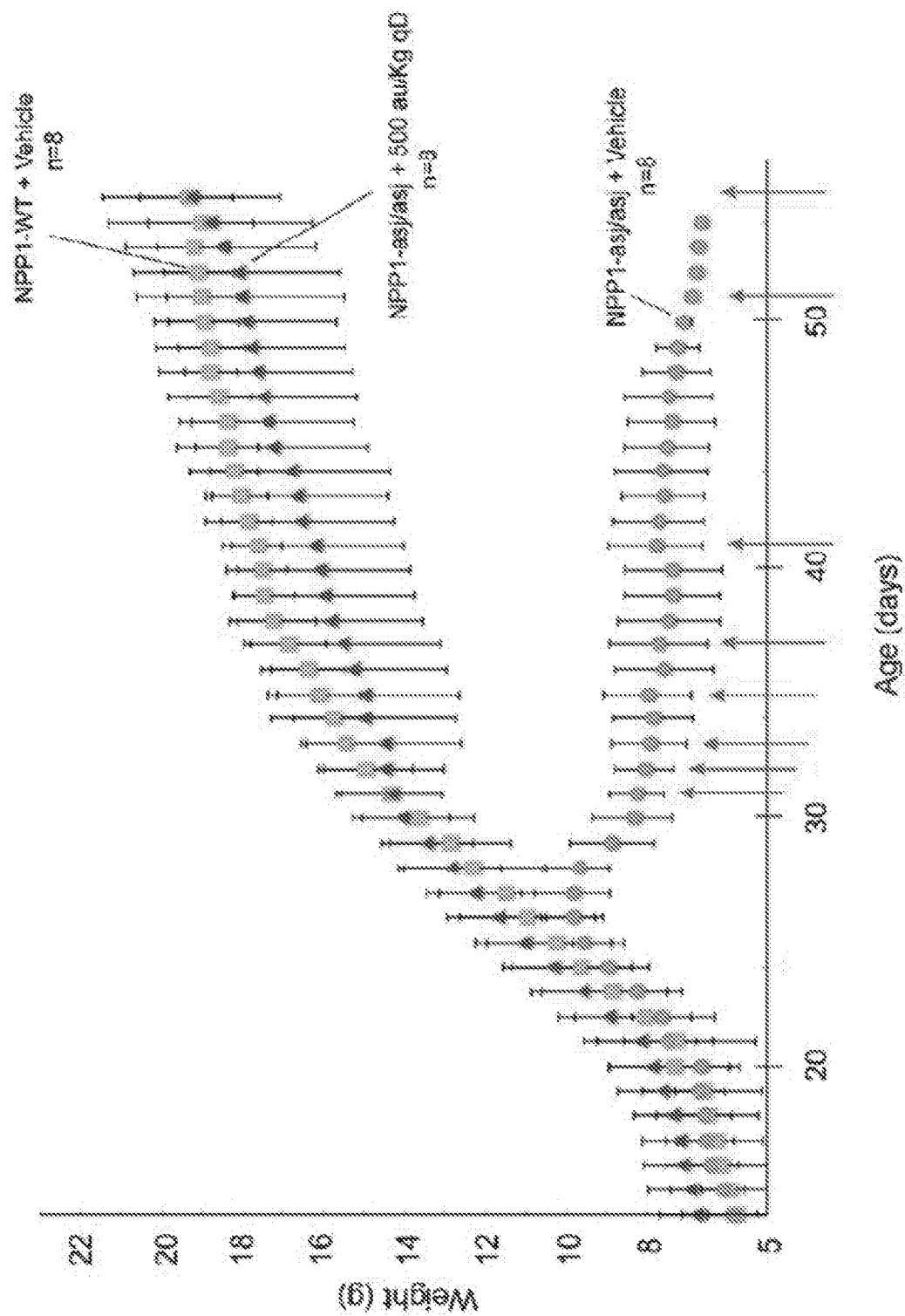


FIG. 3B

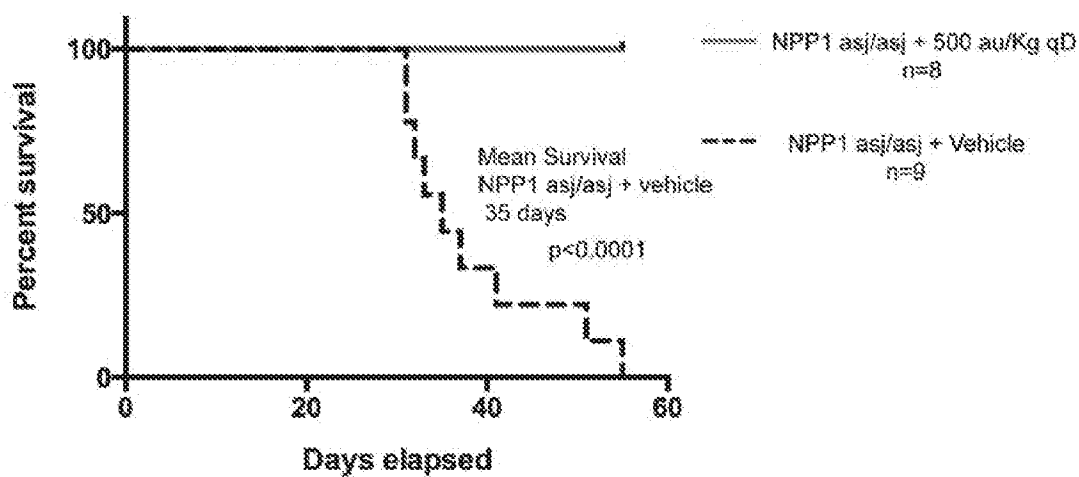
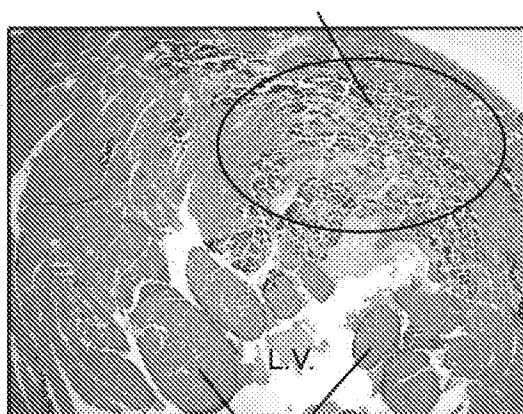
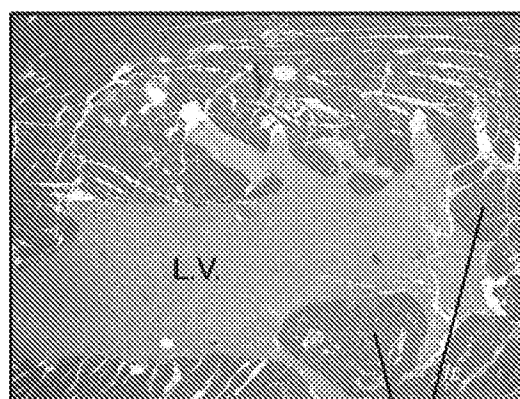


FIG. 3C



Papillary Muscles

FIG. 3D



Papillary Muscles

FIG. 4A

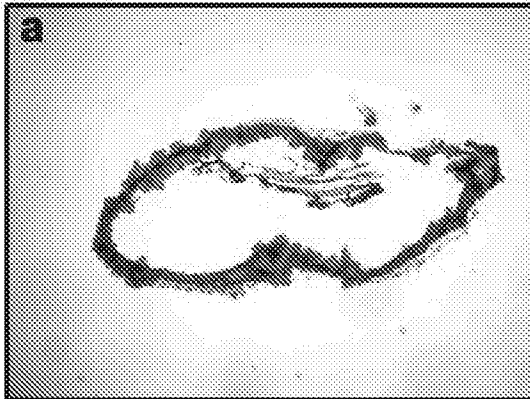


FIG. 4B

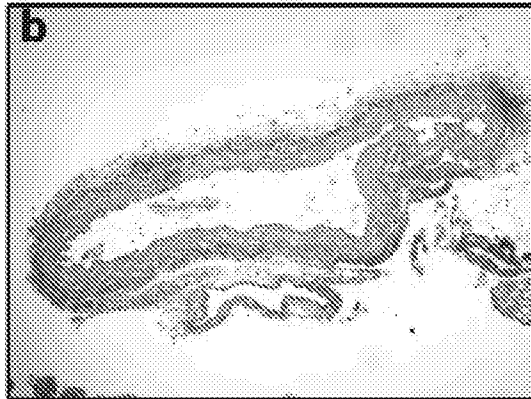


FIG. 4C

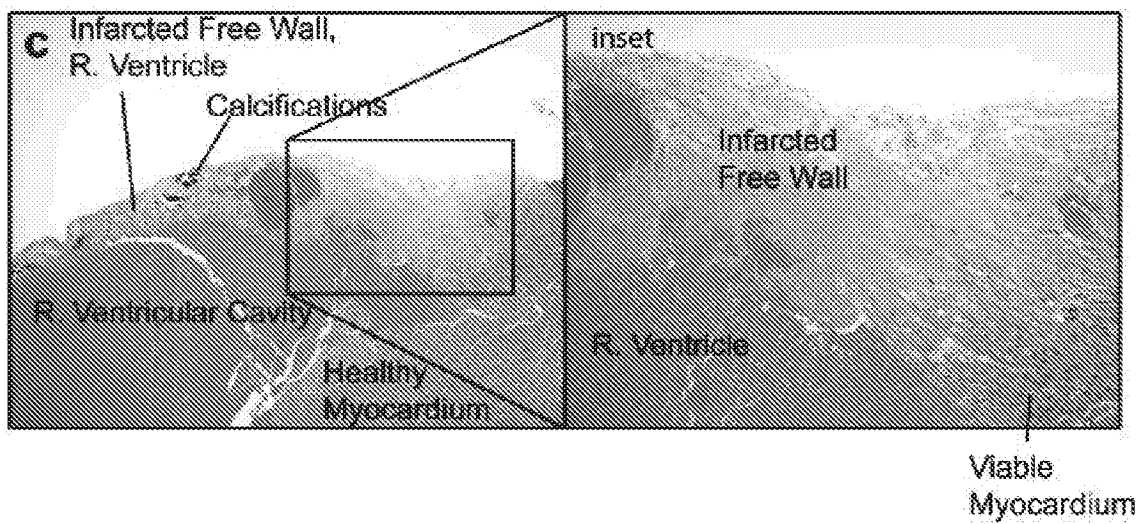


FIG. 4D

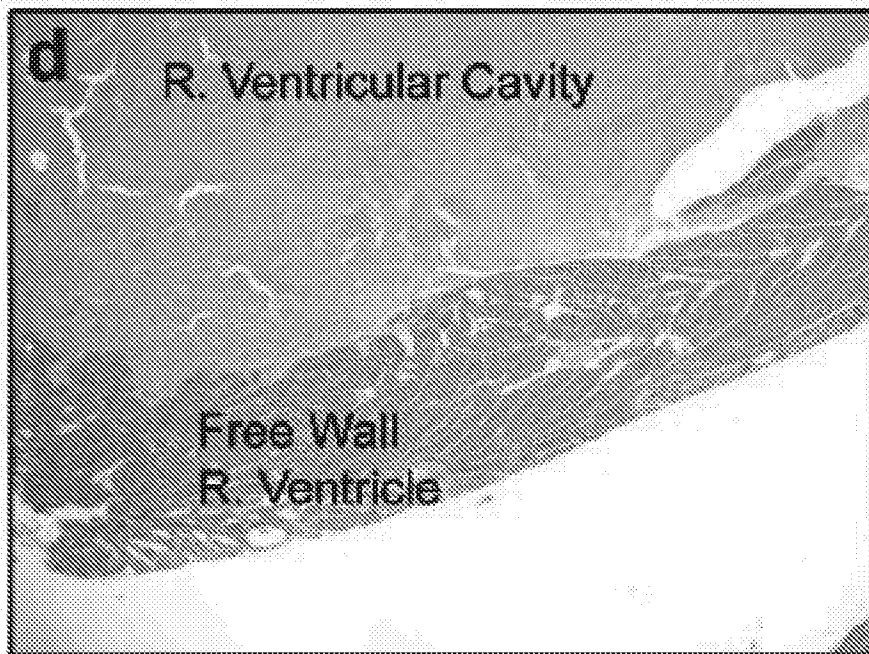


FIG. 4E

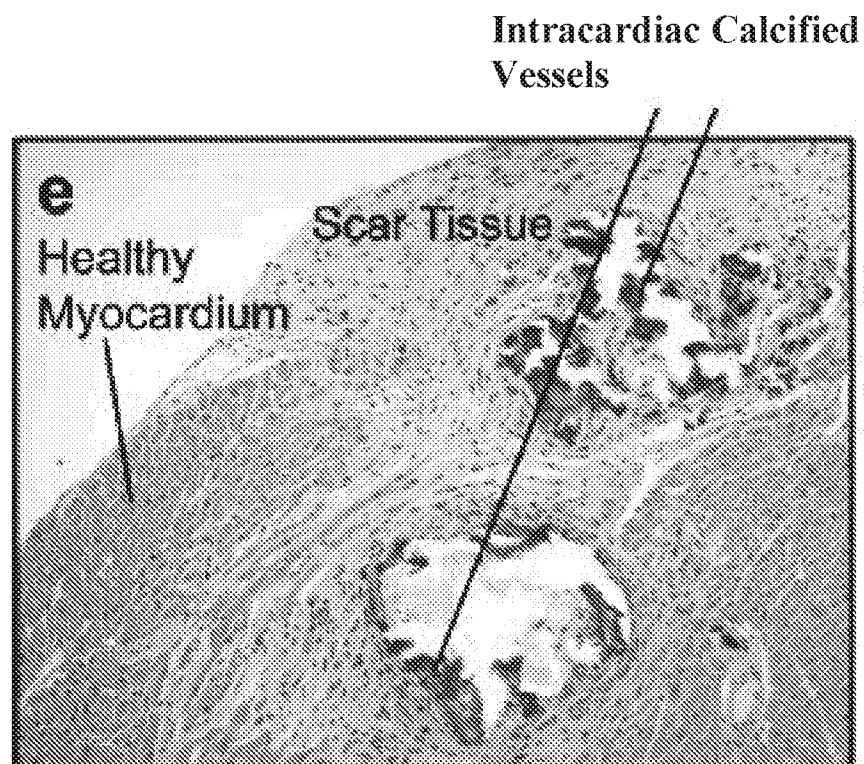




FIG. 4F

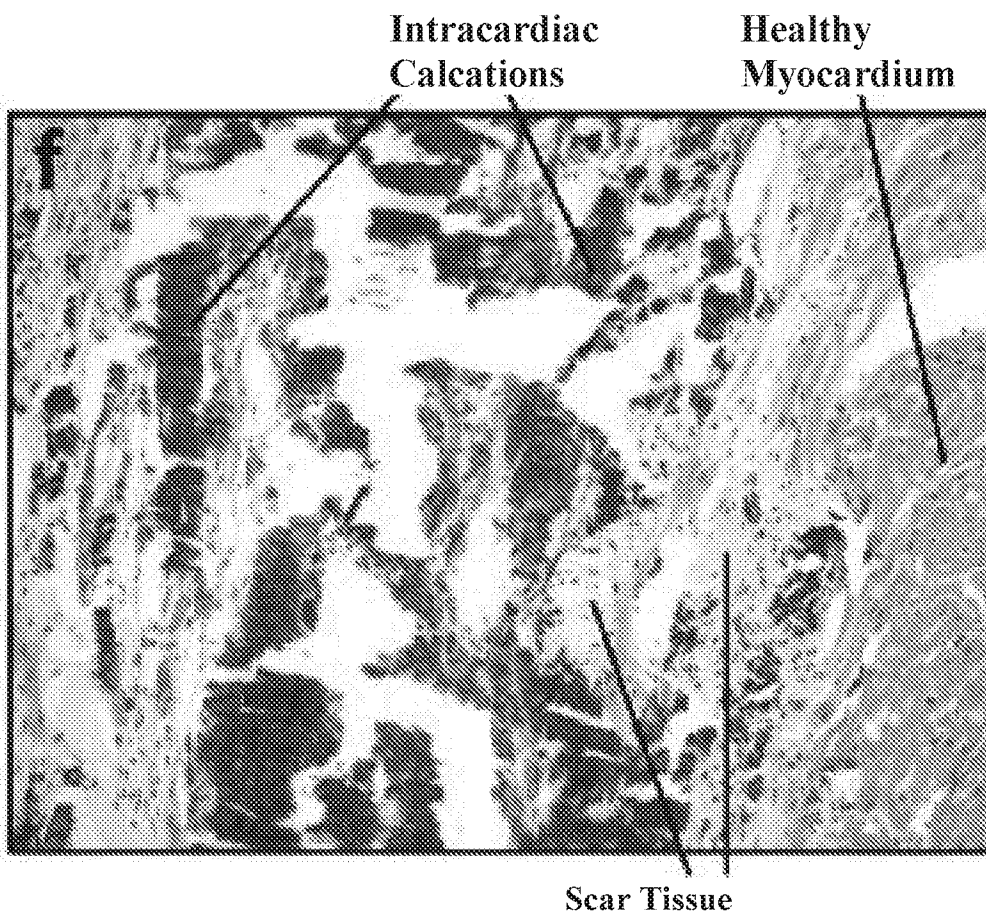


FIG. 4G

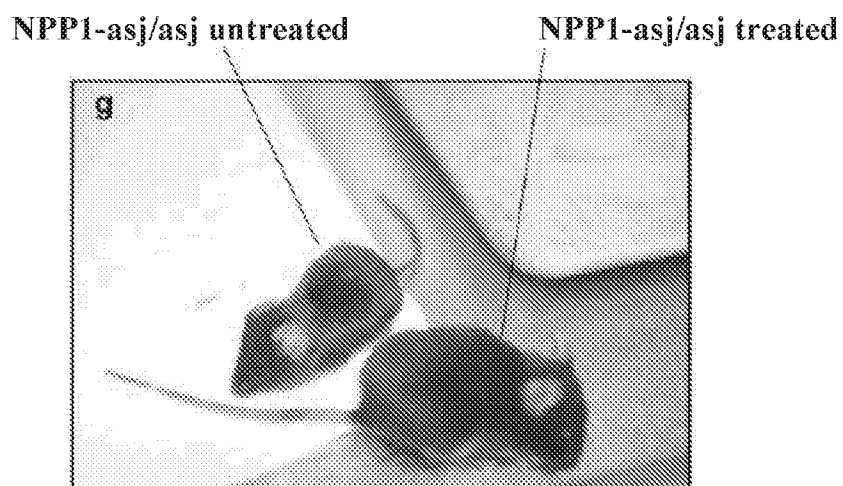


FIG. 5A

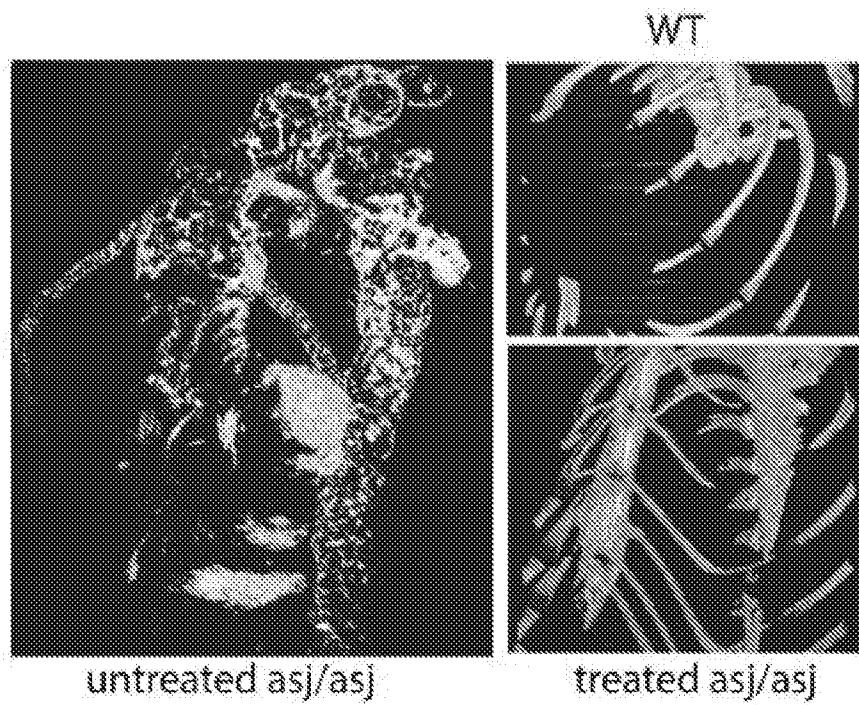


FIG. 5B

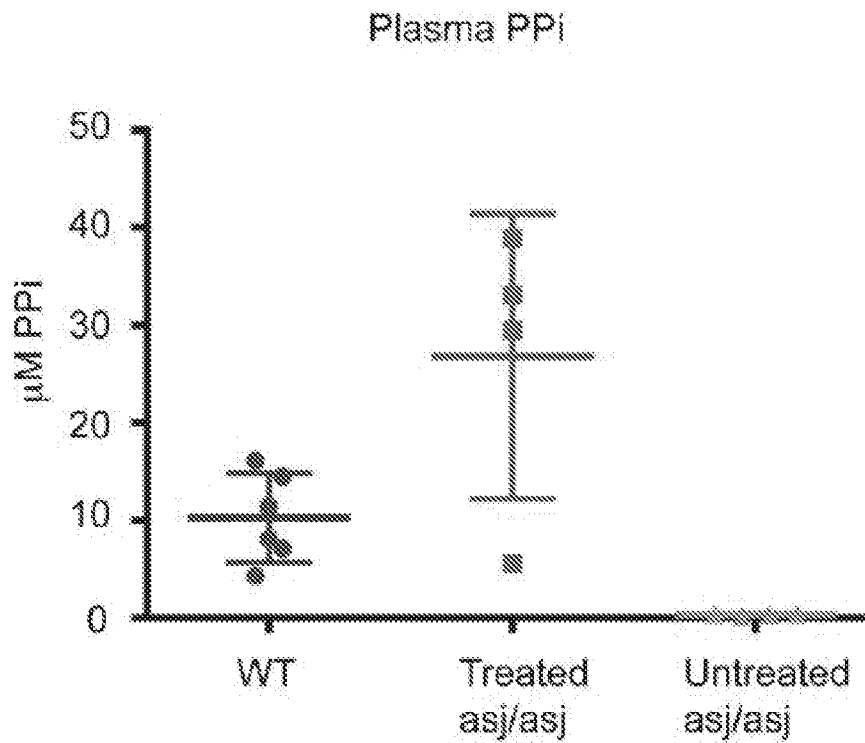


FIG. 5C

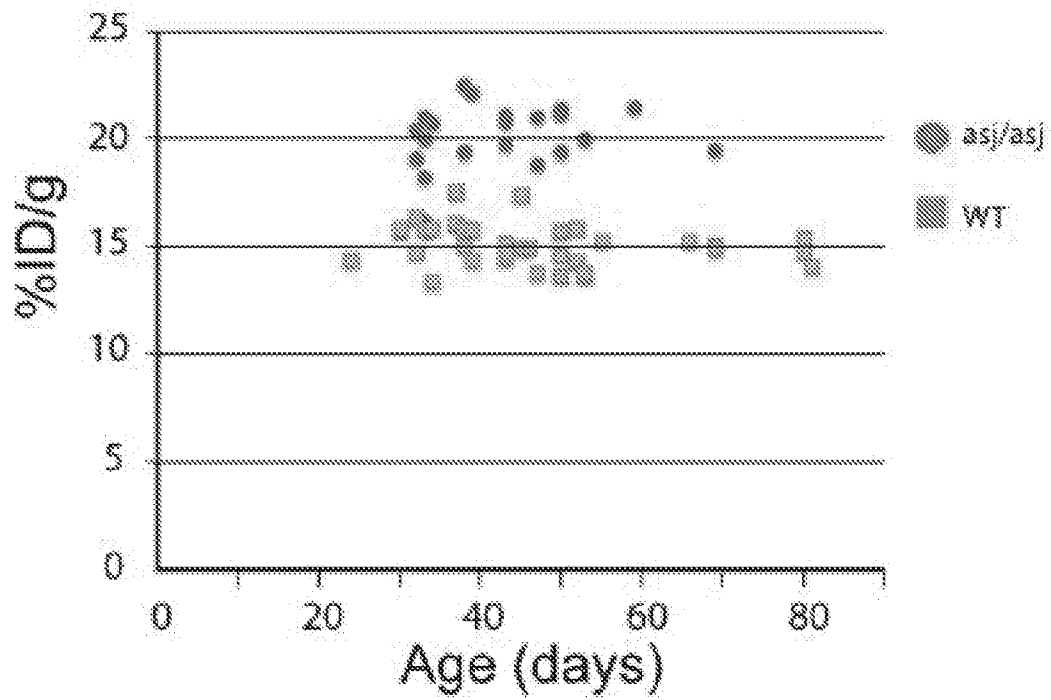


FIG. 5D

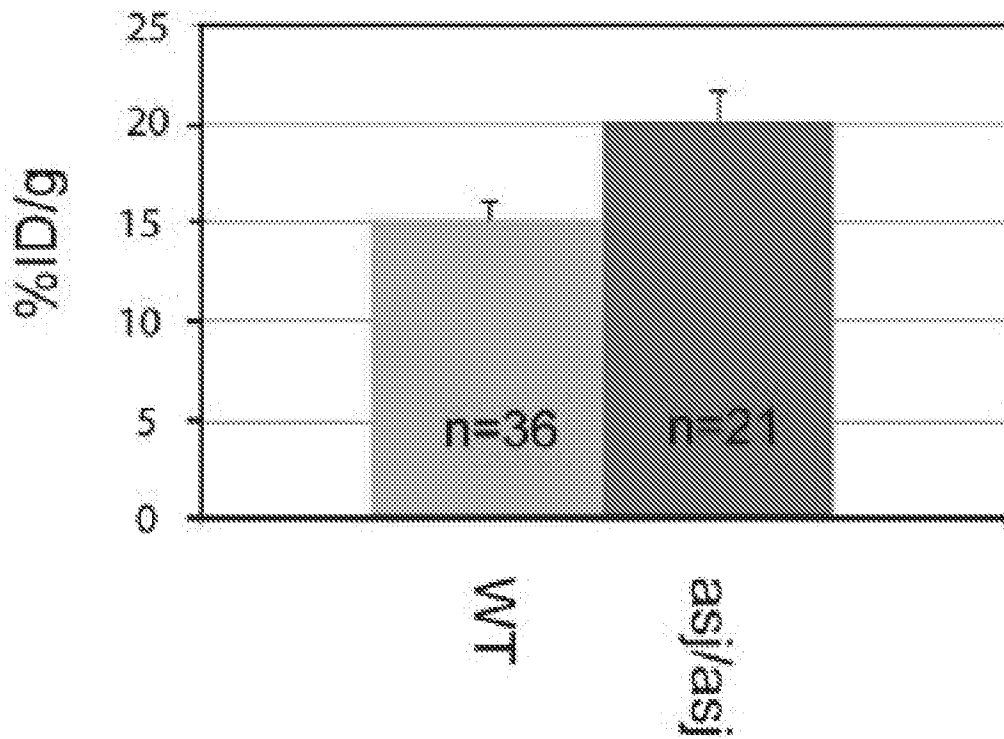


FIG. 5E

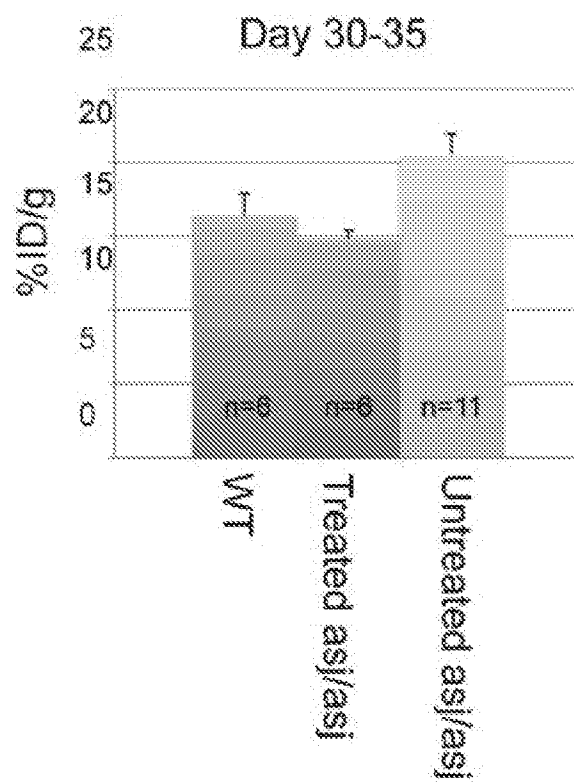


FIG. 5F

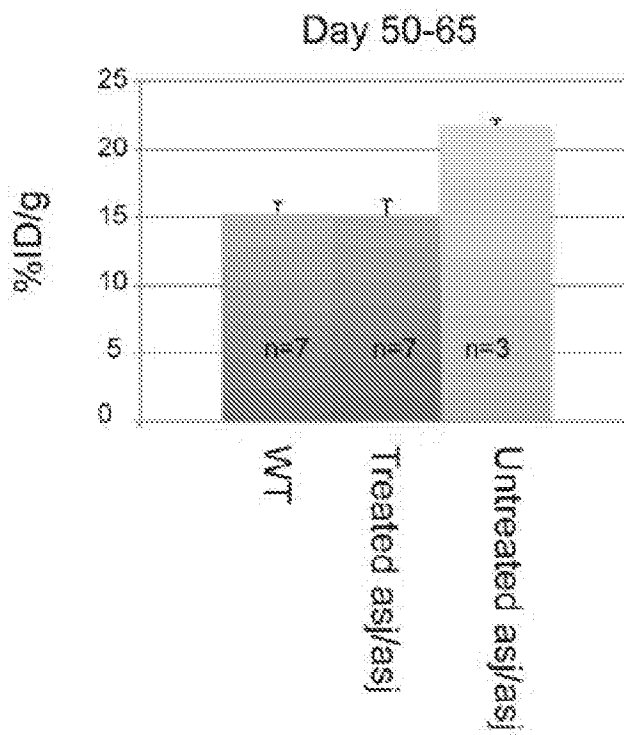


FIG. 6

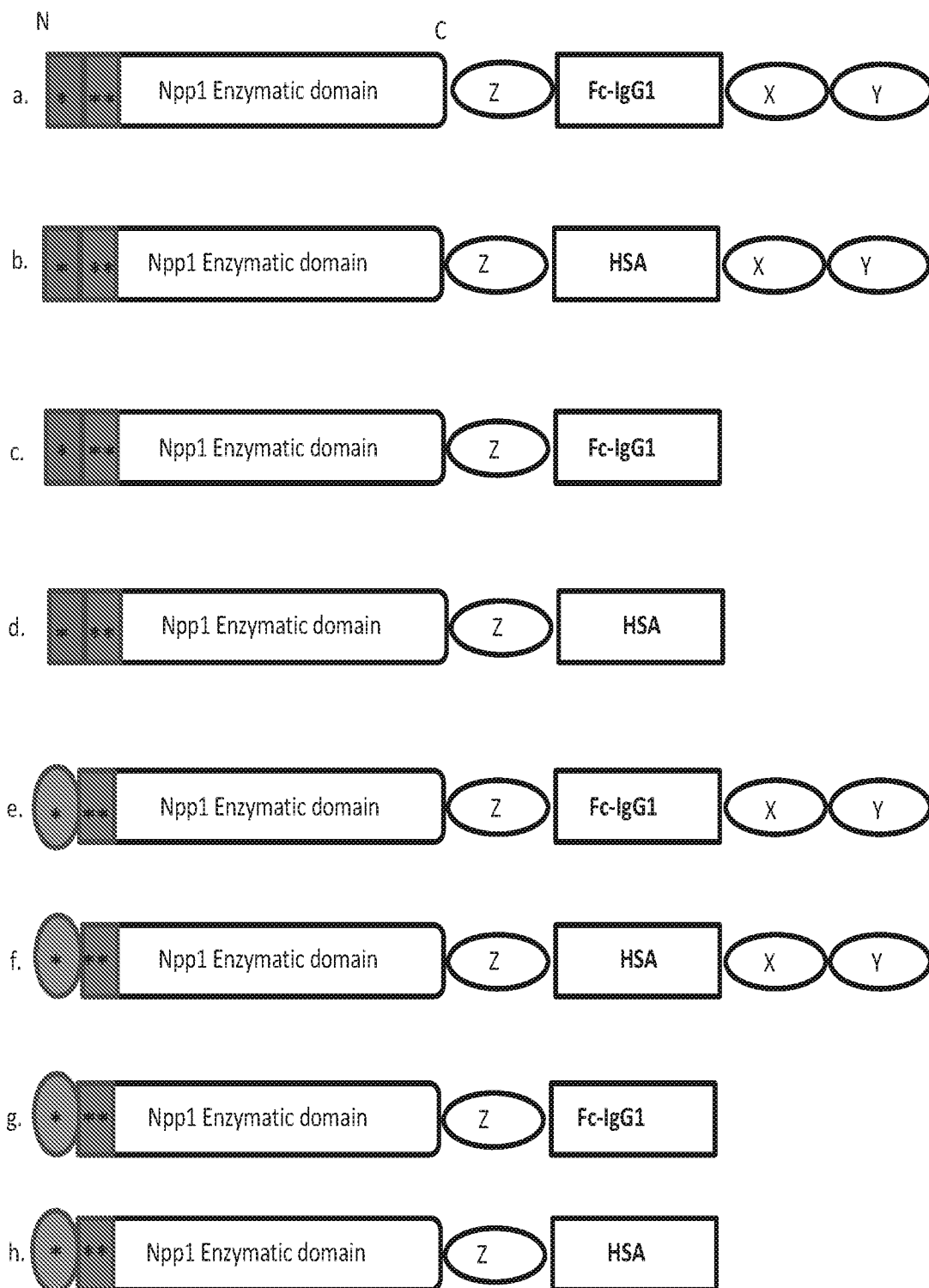


FIG. 7

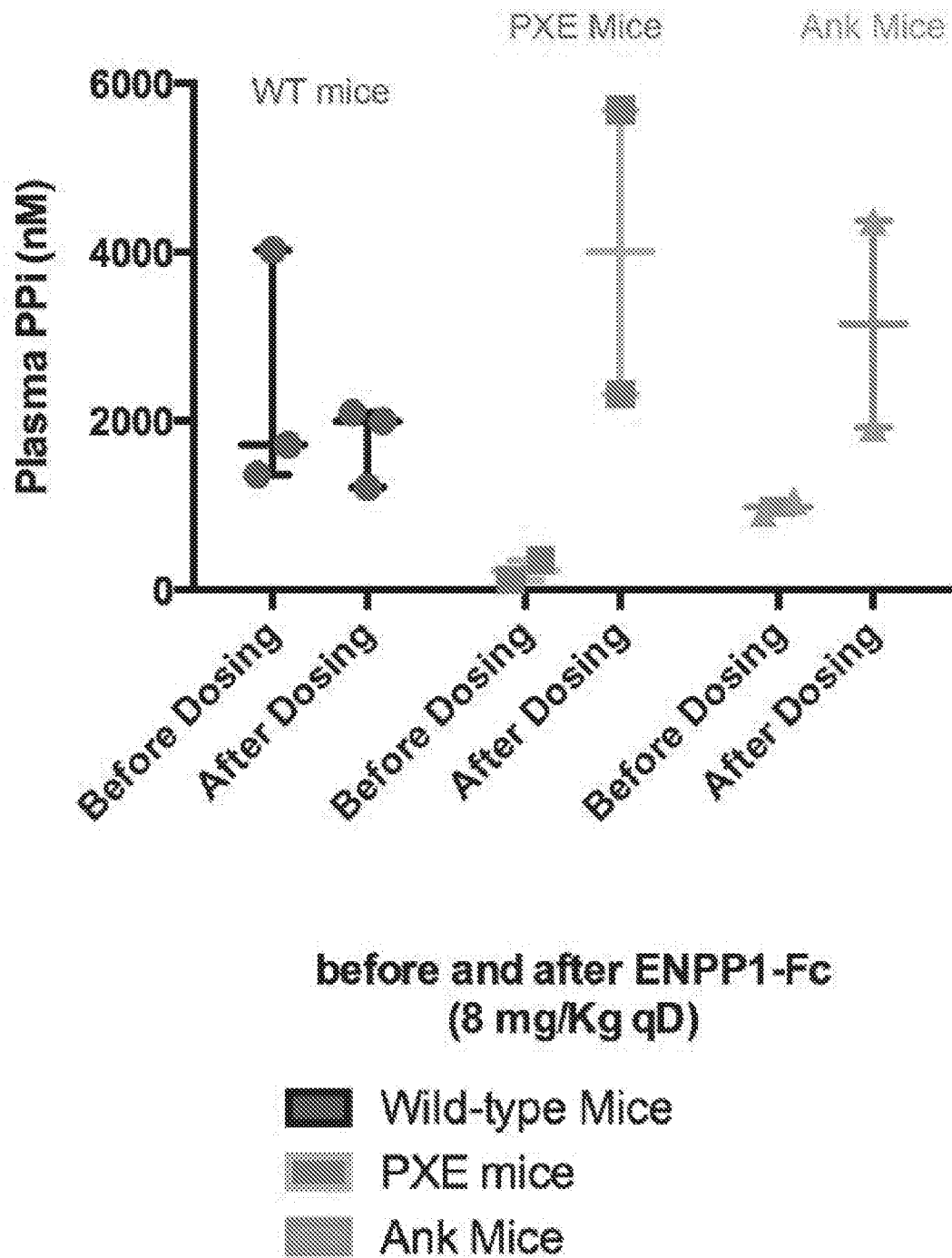


FIG. 8

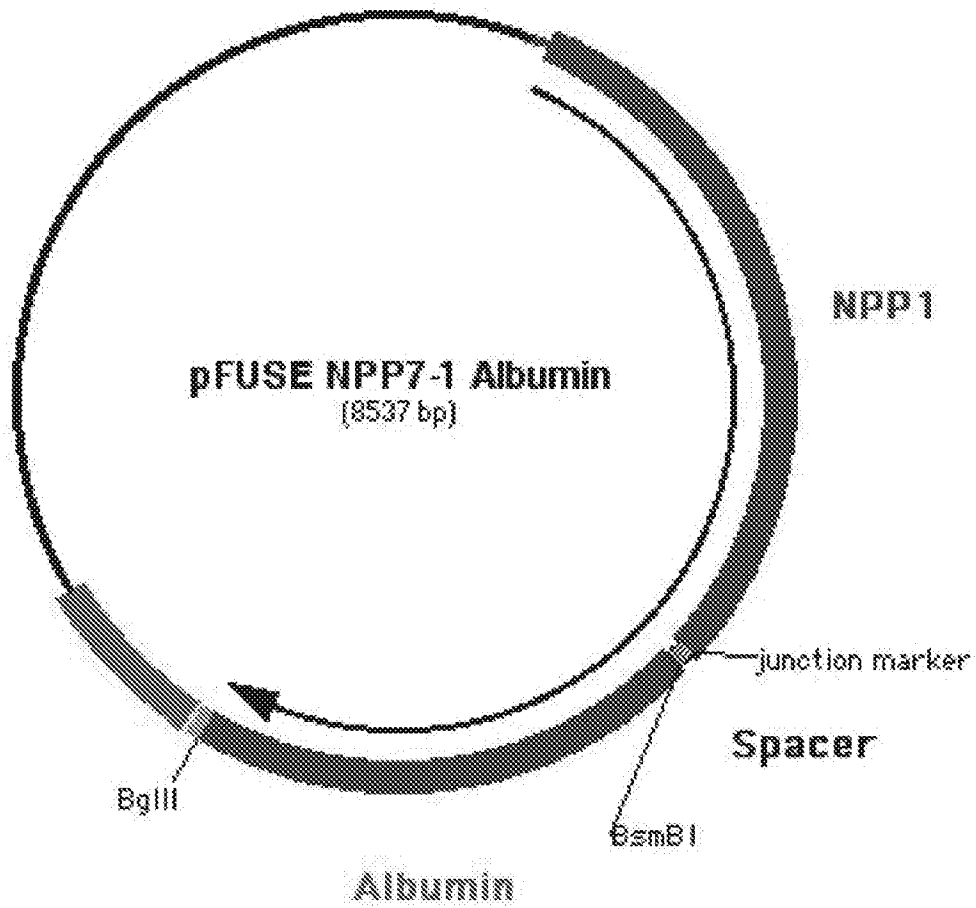


FIG. 9

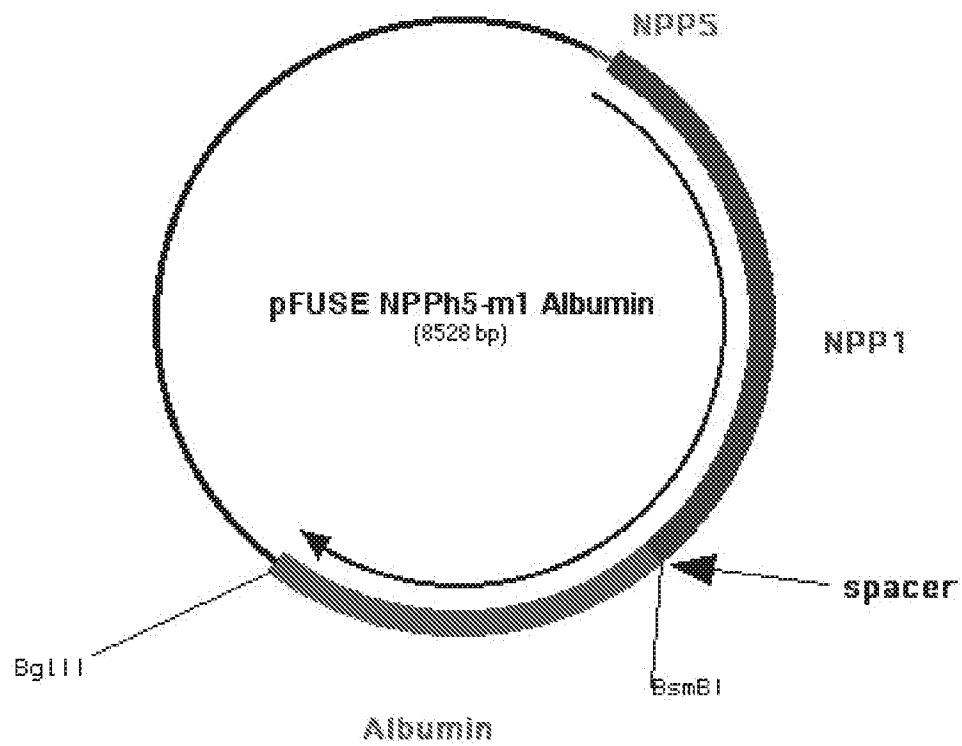
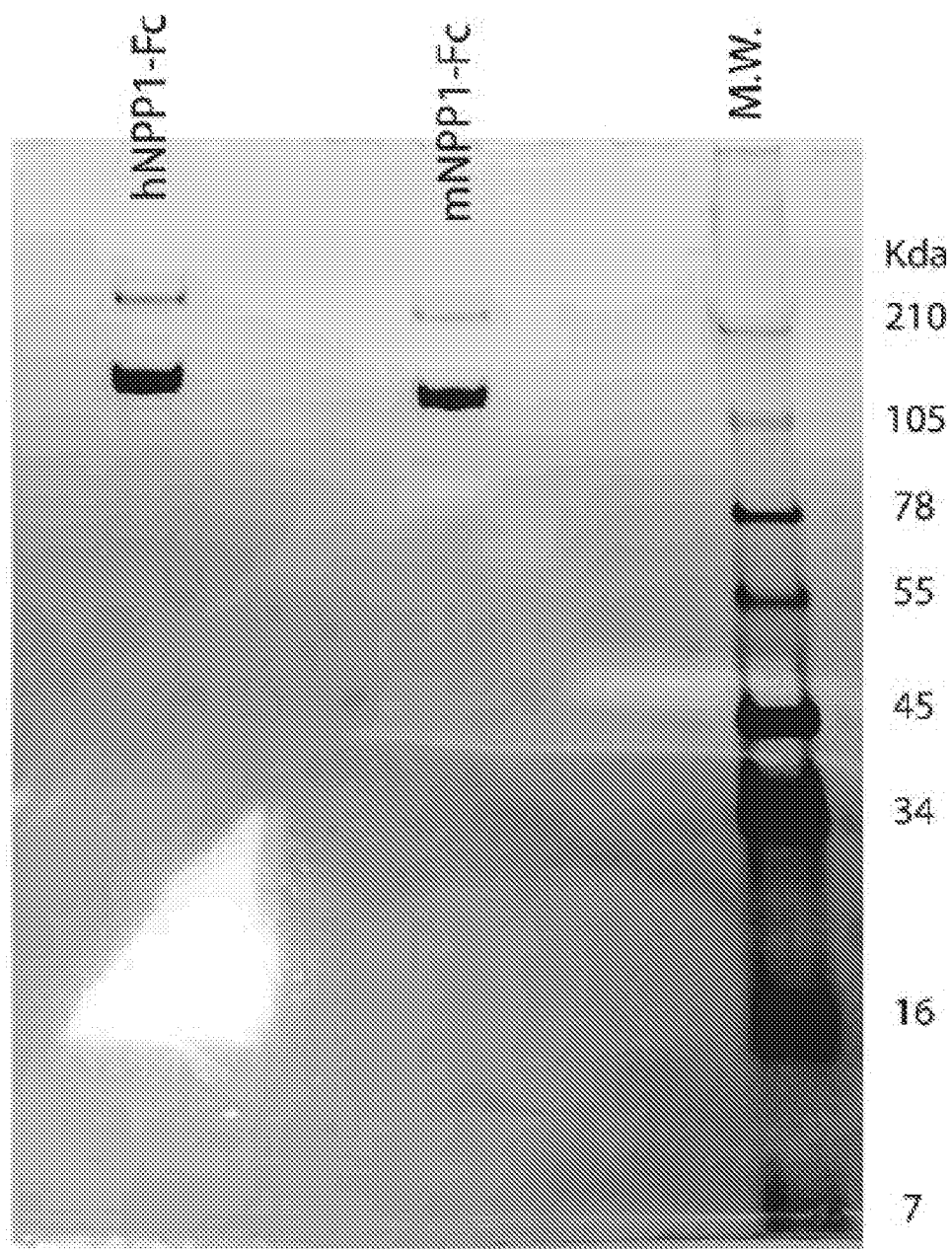




FIG. 10



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SEQUENCE LISTING

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Braddock, Demetrios  
Albright, Ronald

<120> Compositions for Treating Pathological Calcification Conditions,  
and Methods Using Same

<130> 047162-7065W01(00401)

<150> US 62/163,500

<151> 2015-05-19

<160> 30

<170> PatentIn version 3.5

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35 40 45

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047162-7065W01\_Seq\_Listing.txt

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 210 215 220  
 Leu His Thr Trp Gly Gly Leu Leu Pro Val Ile Ser Lys Leu Lys Lys  
 225 230 235 240  
 Cys Gly Thr Tyr Thr Lys Asn Met Arg Pro Val Tyr Pro Thr Lys Thr  
 245 250 255  
 Phe Pro Asn His Tyr Ser Ile Val Thr Gly Leu Tyr Pro Glu Ser His  
 260 265 270  
 Gly Ile Ile Asp Asn Lys Met Tyr Asp Pro Lys Met Asn Ala Ser Phe  
 275 280 285  
 Ser Leu Lys Ser Lys Glu Lys Phe Asn Pro Glu Trp Tyr Lys Gly Glu  
 290 295 300  
 Pro Ile Trp Val Thr Ala Lys Tyr Gln Gly Leu Lys Ser Gly Thr Phe  
 305 310 315 320  
 Phe Trp Pro Gly Ser Asp Val Glu Ile Asn Gly Ile Phe Pro Asp Ile  
 325 330 335  
 Tyr Lys Met Tyr Asn Gly Ser Val Pro Phe Glu Glu Arg Ile Leu Ala  
 340 345 350  
 Val Leu Gln Trp Leu Gln Leu Pro Lys Asp Glu Arg Pro His Phe Tyr  
 355 360 365  
 Thr Leu Tyr Leu Glu Glu Pro Asp Ser Ser Gly His Ser Tyr Gly Pro  
 370 375 380  
 Val Ser Ser Glu Val Ile Lys Ala Leu Gln Arg Val Asp Gly Met Val  
 385 390 395 400  
 Gly Met Leu Met Asp Gly Leu Lys Glu Leu Asn Leu His Arg Cys Leu  
 405 410 415  
 Asn Leu Ile Leu Ile Ser Asp His Gly Met Glu Gln Gly Ser Cys Lys  
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Arg	Glu	Pro	Asn	Gln	His	Phe	Lys	Pro	Tyr	Leu	Lys	His	Phe	Leu	Pro
				485					490					495	
Lys	Arg	Leu	His	Phe	Ala	Lys	Ser	Asp	Arg	Ile	Glu	Pro	Leu	Thr	Phe
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Tyr	Leu	Asp	Pro	Gln	Trp	Gln	Leu	Ala	Leu	Asn	Pro	Ser	Glu	Arg	Lys
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Tyr	Cys	Gly	Ser	Gly	Phe	His	Gly	Ser	Asp	Asn	Val	Phe	Ser	Asn	Met
	530					535					540				
Gln	Ala	Leu	Phe	Val	Gly	Tyr	Gly	Pro	Gly	Phe	Lys	His	Gly	Ile	Glu
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Ala	Asp	Thr	Phe	Glu	Asn	Ile	Glu	Val	Tyr	Asn	Leu	Met	Cys	Asp	Leu
				565					570					575	
Leu	Asn	Leu	Thr	Pro	Ala	Pro	Asn	Asn	Gly	Thr	His	Gly	Ser	Leu	Asn
			580					585					590		
His	Leu	Leu	Lys	Asn	Pro	Val	Tyr	Thr	Pro	Lys	His	Pro	Lys	Glu	Val
		595					600					605			
His	Pro	Leu	Val	Gln	Cys	Pro	Phe	Thr	Arg	Asn	Pro	Arg	Asp	Asn	Leu
	610					615					620				
Gly	Cys	Ser	Cys	Asn	Pro	Ser	Ile	Leu	Pro	Ile	Glu	Asp	Phe	Gln	Thr
625					630					635					640
Gln	Phe	Asn	Leu	Thr	Val	Ala	Glu	Glu	Lys	Ile	Ile	Lys	His	Glu	Thr
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		675					680					685			
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	690					695					700				
Thr	Glu	Asp	Phe	Ser	Asn	Cys	Leu	Tyr	Gln	Asp	Phe	Arg	Ile	Pro	Leu
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Tyr Ser Glu Ala Leu Leu Thr Thr Asn Ile Val Pro Met Tyr Gln Ser  
755 760 765

Phe Gln Val Ile Trp Arg Tyr Phe His Asp Thr Leu Leu Arg Lys Tyr  
770 775 780

Ala Glu Glu Arg Asn Gly Val Asn Val Val Ser Gly Pro Val Phe Asp  
785 790 795 800

Phe Asp Tyr Asp Gly Arg Cys Asp Ser Leu Glu Asn Leu Arg Gln Lys  
805 810 815

Arg Arg Val Ile Arg Asn Gln Glu Ile Leu Ile Pro Thr His Phe Phe  
820 825 830

Ile Val Leu Thr Ser Cys Lys Asp Thr Ser Gln Thr Pro Leu His Cys  
835 840 845

Glu Asn Leu Asp Thr Leu Ala Phe Ile Leu Pro His Arg Thr Asp Asn  
850 855 860

Ser Glu Ser Cys Val His Gly Lys His Asp Ser Ser Trp Val Glu Glu  
865 870 875 880

Leu Leu Met Leu His Arg Ala Arg Ile Thr Asp Val Glu His Ile Thr  
885 890 895

Gly Leu Ser Phe Tyr Gln Gln Arg Lys Glu Pro Val Ser Asp Ile Leu  
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65 70 75 80

Lys Ser Tyr Thr Ser Cys Cys His Asp Phe Asp Glu Leu Cys Leu Lys  
85 90 95

Thr Ala Arg Gly Trp Glu Cys Thr Lys Asp Arg Cys Gly Glu Val Arg  
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Asn Glu Glu Asn Ala Cys His Cys Ser Glu Asp Cys Leu Ala Arg Gly  
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145 150 155 160

Phe Val Arg Pro Pro Leu Ile Ile Phe Ser Val Asp Gly Phe Arg Ala  
165 170 175

Ser Tyr Met Lys Lys Gly Ser Lys Val Met Pro Asn Ile Glu Lys Leu  
180 185 190

Arg Ser Cys Gly Thr His Ser Pro Tyr Met Arg Pro Val Tyr Pro Thr  
195 200 205

Lys Thr Phe Pro Asn Leu Tyr Thr Leu Ala Thr Gly Leu Tyr Pro Glu  
210 215 220

Ser His Gly Ile Val Gly Asn Ser Met Tyr Asp Pro Val Phe Asp Ala  
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245 250 255

Gly Gln Pro Leu Trp Ile Thr Ala Thr Lys Gln Gly Val Lys Ala Gly  
260 265 270

Thr Phe Phe Trp Ser Val Val Ile Pro His Glu Arg Arg Ile Leu Thr  
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Ile Leu Gln Trp Leu Thr Leu Pro Asp His Glu Arg Pro Ser Val Tyr  
290 295 300

Ala Phe Tyr Ser Glu Gln Pro Asp Phe Ser Gly His Lys Tyr Gly Pro  
 305 310 315 320  
 Phe Gly Pro Glu Met Thr Asn Pro Leu Arg Glu Ile Asp Lys Ile Val  
 325 330 335  
 Gly Gln Leu Met Asp Gly Leu Lys Gln Leu Lys Leu His Arg Cys Val  
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 370 375 380  
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 385 390 395 400  
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 Pro Asp Gln His Phe Lys Pro Tyr Leu Lys Gln His Leu Pro Lys Arg  
 420 425 430  
 Leu His Tyr Ala Asn Asn Arg Arg Ile Glu Asp Ile His Leu Leu Val  
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 465 470 475 480  
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 485 490 495  
 Tyr Lys Thr Lys Val Pro Pro Phe Glu Asn Ile Glu Leu Tyr Asn Val  
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 515 520 525  
 Gly Ser Leu Asn His Leu Leu Arg Thr Asn Thr Phe Arg Pro Thr Met  
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Gly 625 Arg Pro Ala Val Leu 630 Tyr Arg Thr Arg Tyr 635 Asp Ile Leu Tyr His 640

Thr Asp Phe Glu Ser 645 Gly Tyr Ser Glu Ile 650 Phe Leu Met Pro Leu 655 Trp

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His Leu Thr 675 Ser Cys Val Arg Pro 680 Asp Val Arg Val Ser 685 Pro Ser Phe

Ser Gln 690 Asn Cys Leu Ala Tyr 695 Lys Asn Asp Lys Gln 700 Met Ser Tyr Gly

Phe 705 Leu Phe Pro Pro Tyr 710 Leu Ser Ser Ser Pro 715 Glu Ala Lys Tyr Asp 720

Ala Phe Leu Val Thr 725 Asn Met Val Pro Met 730 Tyr Pro Ala Phe Lys 735 Arg

Val Trp Asn Tyr 740 Phe Gln Arg Val Leu 745 Val Lys Lys Tyr Ala 750 Ser Glu

Arg Asn Gly 755 Val Asn Val Ile Ser 760 Gly Pro Ile Phe Asp 765 Tyr Asp Tyr

Asp Gly 770 Leu His Asp Thr Glu 775 Asp Lys Ile Lys Gln 780 Tyr Val Glu Gly

Ser 785 Ser Ile Pro Val Pro 790 Thr His Tyr Tyr Ser 795 Ile Ile Thr Ser Cys 800

Leu Asp Phe Thr Gln 805 Pro Ala Asp Lys Cys 810 Asp Gly Pro Leu Ser Val 815

Ser Ser Phe Ile 820 Leu Pro His Arg Pro 825 Asp Asn Glu Glu Ser 830 Cys Asn

Ser Ser Glu 835 Asp Glu Ser Lys Trp 840 Val Glu Glu Leu Met 845 Lys Met His



Thr Ala Arg Val Arg Asp Ile Glu His Leu Thr Ser Leu Asp Phe Phe  
 850 855 860

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 865 870 875 880

Leu His Thr Tyr Glu Ser Glu Ile  
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Phe Asp Gly Phe Arg Ala Asp Tyr Leu Lys Asn Tyr Glu Phe Pro His  
 35 40 45

Leu Gln Asn Phe Ile Lys Glu Gly Val Leu Val Glu His Val Lys Asn  
 50 55 60

Val Phe Ile Thr Lys Thr Phe Pro Asn His Tyr Ser Ile Val Thr Gly  
 65 70 75 80

Leu Tyr Glu Glu Ser His Gly Ile Val Ala Asn Ser Met Tyr Asp Ala  
 85 90 95

Val Thr Lys Lys His Phe Ser Asp Ser Asn Asp Lys Asp Pro Phe Trp  
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Trp Asn Glu Ala Val Pro Ile Trp Val Thr Asn Gln Leu Gln Glu Asn  
 115 120 125

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 130 135 140

Asp Thr Ile Ser Ser Tyr Phe Met Asn Tyr Asn Ser Ser Val Ser Phe  
 145 150 155 160

Glu Glu Arg Leu Asn Asn Ile Thr Met Trp Leu Asn Asn Ser Asn Pro  
 165 170 175

Pro Val Thr Phe Ala Thr Leu Tyr Trp Glu Glu Pro Asp Ala Ser Gly  
 180 185 190

His Lys Tyr Gly Pro Glu Asp Lys Glu Asn Met Ser Arg Val Leu Lys  
 Page 8

195

200

205

Lys Ile Asp Asp Leu Ile Gly Asp Leu Val Gln Arg Leu Lys Met Leu  
 210 215 220

Gly Leu Trp Glu Asn Leu Asn Val Ile Ile Thr Ser Asp His Gly Met  
 225 230 235 240

Thr Gln Cys Ser Gln Asp Arg Leu Ile Asn Leu Asp Ser Cys Ile Asp  
 245 250 255

His Ser Tyr Tyr Thr Leu Ile Asp Leu Ser Pro Val Ala Ala Ile Leu  
 260 265 270

Pro Lys Ile Asn Arg Thr Glu Val Tyr Asn Lys Leu Lys Asn Cys Ser  
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Pro His Met Asn Val Tyr Leu Lys Glu Asp Ile Pro Asn Arg Phe Tyr  
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Tyr Gln His Asn Asp Arg Ile Gln Pro Ile Ile Leu Val Ala Asp Glu  
 305 310 315 320

Gly Trp Thr Ile Val Leu Asn Glu Ser Ser Gln Lys Leu Gly Asp His  
 325 330 335

Gly Tyr Asp Asn Ser Leu Pro Ser Met His Pro Phe Leu Ala Ala His  
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Gly Pro Ala Phe His Lys Gly Tyr Lys His Ser Thr Ile Asn Ile Val  
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Asp Ile Tyr Pro Met Met Cys His Ile Leu Gly Leu Lys Pro His Pro  
 370 375 380

Asn Asn Gly Thr Phe Gly His Thr Lys Cys Leu Leu Val Asp Gln Trp  
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Cys Ile Asn Leu Pro Glu Ala Ile Ala Ile Val Ile Gly Ser Leu Leu  
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Val Leu Thr Met Leu Thr Cys Leu Ile Ile Ile Met Gln Asn Arg Leu  
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Asp Pro Leu Ile Gly  
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 <223> the tripeptide is present n times, wherein n is integer ranging from 1 to 10

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<210> 11

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<223> chemically synthesized

<400> 11

Ala Pro Trp His Leu Ser Ser Gln Tyr Ser Arg Thr  
1 5 10

<210> 12

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> chemically synthesized

<400> 12

Ser Thr Leu Pro Ile Pro His Glu Phe Ser Arg Glu  
1 5 10

<210> 13

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> chemically synthesized

<400> 13

Val Thr Lys His Leu Asn Gln Ile Ser Gln Ser Tyr  
1 5 10

<210> 14

<211> 1

<212> PRT

<213> Artificial Sequence

<220>

<223> chemically synthesized

<220>

<221> REPEAT

<222> (1)..(1)

<223> the amino acid is present m times, wherein m is an integer ranging from 1 to 15

<400> 14

Glu  
1

<210> 15

<211> 925

<212> PRT

<213> Artificial Sequence

<220>

<223> chemically synthesized

<400> 15

047162-7065W01\_Seq\_Listing.txt

Met Glu Arg Asp Gly Cys Ala Gly Gly Gly Ser Arg Gly Gly Glu Gly  
1 5 10 15

Gly Arg Ala Pro Arg Glu Gly Pro Ala Gly Asn Gly Arg Asp Arg Gly  
20 25 30

Arg Ser His Ala Ala Glu Ala Pro Gly Asp Pro Gln Ala Ala Ala Ser  
35 40 45

Leu Leu Ala Pro Met Asp Val Gly Glu Glu Pro Leu Glu Lys Ala Ala  
50 55 60

Arg Ala Arg Thr Ala Lys Asp Pro Asn Thr Tyr Lys Ile Ile Ser Leu  
65 70 75 80

Phe Thr Phe Ala Val Gly Val Asn Ile Cys Leu Gly Phe Thr Ala Gly  
85 90 95

Leu Lys Pro Ser Cys Ala Lys Glu Val Lys Ser Cys Lys Gly Arg Cys  
100 105 110

Phe Glu Arg Thr Phe Gly Asn Cys Arg Cys Asp Ala Ala Cys Val Glu  
115 120 125

Leu Gly Asn Cys Cys Leu Asp Tyr Gln Glu Thr Cys Ile Glu Pro Glu  
130 135 140

His Ile Trp Thr Cys Asn Lys Phe Arg Cys Gly Glu Lys Arg Leu Thr  
145 150 155 160

Arg Ser Leu Cys Ala Cys Ser Asp Asp Cys Lys Asp Lys Gly Asp Cys  
165 170 175

Cys Ile Asn Tyr Ser Ser Val Cys Gln Gly Glu Lys Ser Trp Val Glu  
180 185 190

Glu Pro Cys Glu Ser Ile Asn Glu Pro Gln Cys Pro Ala Gly Phe Glu  
195 200 205

Thr Pro Pro Thr Leu Leu Phe Ser Leu Asp Gly Phe Arg Ala Glu Tyr  
210 215 220

Leu His Thr Trp Gly Gly Leu Leu Pro Val Ile Ser Lys Leu Lys Lys  
225 230 235 240

Cys Gly Thr Tyr Thr Lys Asn Met Arg Pro Val Tyr Pro Thr Lys Thr  
245 250 255

Phe Pro Asn His Tyr Ser Ile Val Thr Gly Leu Tyr Pro Glu Ser His  
260 265 270

047162-7065W01\_Seq\_Listing.txt

Gly Ile Ile Asp Asn Lys Met Tyr Asp Pro Lys Met Asn Ala Ser Phe  
275 280 285

Ser Leu Lys Ser Lys Glu Lys Phe Asn Pro Glu Trp Tyr Lys Gly Glu  
290 295 300

Pro Ile Trp Val Thr Ala Lys Tyr Gln Gly Leu Lys Ser Gly Thr Phe  
305 310 315 320

Phe Trp Pro Gly Ser Asp Val Glu Ile Asn Gly Ile Phe Pro Asp Ile  
325 330 335

Tyr Lys Met Tyr Asn Gly Ser Val Pro Phe Glu Glu Arg Ile Leu Ala  
340 345 350

Val Leu Gln Trp Leu Gln Leu Pro Lys Asp Glu Arg Pro His Phe Tyr  
355 360 365

Thr Leu Tyr Leu Glu Glu Pro Asp Ser Ser Gly His Ser Tyr Gly Pro  
370 375 380

Val Ser Ser Glu Val Ile Lys Ala Leu Gln Arg Val Asp Gly Met Val  
385 390 395 400

Gly Met Leu Met Asp Gly Leu Lys Glu Leu Asn Leu His Arg Cys Leu  
405 410 415

Asn Leu Ile Leu Ile Ser Asp His Gly Met Glu Gln Gly Ser Cys Lys  
420 425 430

Lys Tyr Ile Tyr Leu Asn Lys Tyr Leu Gly Asp Val Lys Asn Ile Lys  
435 440 445

Val Ile Tyr Gly Pro Ala Ala Arg Leu Arg Pro Ser Asp Val Pro Asp  
450 455 460

Lys Tyr Tyr Ser Phe Asn Tyr Glu Gly Ile Ala Arg Asn Leu Ser Cys  
465 470 475 480

Arg Glu Pro Asn Gln His Phe Lys Pro Tyr Leu Lys His Phe Leu Pro  
485 490 495

Lys Arg Leu His Phe Ala Lys Ser Asp Arg Ile Glu Pro Leu Thr Phe  
500 505 510

Tyr Leu Asp Pro Gln Trp Gln Leu Ala Leu Asn Pro Ser Glu Arg Lys  
515 520 525

Tyr Cys Gly Ser Gly Phe His Gly Ser Asp Asn Val Phe Ser Asn Met  
530 535 540

047162-7065W01\_Seq\_Listing.txt

Gln Ala Leu Phe Val Gly Tyr Gly Pro Gly Phe Lys His Gly Ile Glu  
545 550 555 560

Ala Asp Thr Phe Glu Asn Ile Glu Val Tyr Asn Leu Met Cys Asp Leu  
565 570 575

Leu Asn Leu Thr Pro Ala Pro Asn Asn Gly Thr His Gly Ser Leu Asn  
580 585 590

His Leu Leu Lys Asn Pro Val Tyr Thr Pro Lys His Pro Lys Glu Val  
595 600 605

His Pro Leu Val Gln Cys Pro Phe Thr Arg Asn Pro Arg Asp Asn Leu  
610 615 620

Gly Cys Ser Cys Asn Pro Ser Ile Leu Pro Ile Glu Asp Phe Gln Thr  
625 630 635 640

Gln Phe Asn Leu Thr Val Ala Glu Glu Lys Ile Ile Lys His Glu Thr  
645 650 655

Leu Pro Tyr Gly Arg Pro Arg Val Leu Gln Lys Glu Asn Thr Ile Cys  
660 665 670

Leu Leu Ser Gln His Gln Phe Met Ser Gly Tyr Ser Gln Asp Ile Leu  
675 680 685

Met Pro Leu Trp Thr Ser Tyr Thr Val Asp Arg Asn Asp Ser Phe Ser  
690 695 700

Thr Glu Asp Phe Ser Asn Cys Leu Tyr Gln Asp Phe Arg Ile Pro Leu  
705 710 715 720

Ser Pro Val His Lys Cys Ser Phe Tyr Lys Asn Asn Thr Lys Val Ser  
725 730 735

Tyr Gly Phe Leu Ser Pro Pro Gln Leu Asn Lys Asn Ser Ser Gly Ile  
740 745 750

Tyr Ser Glu Ala Leu Leu Thr Thr Asn Ile Val Pro Met Tyr Gln Ser  
755 760 765

Phe Gln Val Ile Trp Arg Tyr Phe His Asp Thr Leu Leu Arg Lys Tyr  
770 775 780

Ala Glu Glu Arg Asn Gly Val Asn Val Val Ser Gly Pro Val Phe Asp  
785 790 795 800

Phe Asp Tyr Asp Gly Arg Cys Asp Ser Leu Glu Asn Leu Arg Gln Lys  
805 810 815



047162-7065W01\_Seq\_Listing.txt

Arg Arg Val Ile Arg Asn Gln Glu Ile Leu Ile Pro Thr His Phe Phe  
820 825 830

Ile Val Leu Thr Ser Cys Lys Asp Thr Ser Gln Thr Pro Leu His Cys  
835 840 845

Glu Asn Leu Asp Thr Leu Ala Phe Ile Leu Pro His Arg Thr Asp Asn  
850 855 860

Ser Glu Ser Cys Val His Gly Lys His Asp Ser Ser Trp Val Glu Glu  
865 870 875 880

Leu Leu Met Leu His Arg Ala Arg Ile Thr Asp Val Glu His Ile Thr  
885 890 895

Gly Leu Ser Phe Tyr Gln Gln Arg Lys Glu Pro Val Ser Asp Ile Leu  
900 905 910

Lys Leu Lys Thr His Leu Pro Thr Phe Ser Gln Glu Asp  
915 920 925

<210> 16  
<211> 1155  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> chemically synthesized  
<400> 16

Met Glu Arg Asp Gly Cys Ala Gly Gly Gly Ser Arg Gly Gly Glu Gly  
1 5 10 15

Gly Arg Ala Pro Arg Glu Gly Pro Ala Gly Asn Gly Arg Asp Arg Gly  
20 25 30

Arg Ser His Ala Ala Glu Ala Pro Gly Asp Pro Gln Ala Ala Ala Ser  
35 40 45

Leu Leu Ala Pro Met Asp Val Gly Glu Glu Pro Leu Glu Lys Ala Ala  
50 55 60

Arg Ala Arg Thr Ala Lys Asp Pro Asn Thr Tyr Lys Ile Ile Ser Leu  
65 70 75 80

Phe Thr Phe Ala Val Gly Val Asn Ile Cys Leu Gly Phe Thr Ala Gly  
85 90 95

Leu Lys Pro Ser Cys Ala Lys Glu Val Lys Ser Cys Lys Gly Arg Cys  
100 105 110

Phe Glu Arg Thr Phe Gly Asn Cys Arg Cys Asp Ala Ala Cys Val Glu  
Page 16

115

120

125

Leu Gly Asn Cys Cys Leu Asp Tyr Gln Glu Thr Cys Ile Glu Pro Glu  
 130 135 140

His Ile Trp Thr Cys Asn Lys Phe Arg Cys Gly Glu Lys Arg Leu Thr  
 145 150 155 160

Arg Ser Leu Cys Ala Cys Ser Asp Asp Cys Lys Asp Lys Gly Asp Cys  
 165 170 175

Cys Ile Asn Tyr Ser Ser Val Cys Gln Gly Glu Lys Ser Trp Val Glu  
 180 185 190

Glu Pro Cys Glu Ser Ile Asn Glu Pro Gln Cys Pro Ala Gly Phe Glu  
 195 200 205

Thr Pro Pro Thr Leu Leu Phe Ser Leu Asp Gly Phe Arg Ala Glu Tyr  
 210 215 220

Leu His Thr Trp Gly Gly Leu Leu Pro Val Ile Ser Lys Leu Lys Lys  
 225 230 235 240

Cys Gly Thr Tyr Thr Lys Asn Met Arg Pro Val Tyr Pro Thr Lys Thr  
 245 250 255

Phe Pro Asn His Tyr Ser Ile Val Thr Gly Leu Tyr Pro Glu Ser His  
 260 265 270

Gly Ile Ile Asp Asn Lys Met Tyr Asp Pro Lys Met Asn Ala Ser Phe  
 275 280 285

Ser Leu Lys Ser Lys Glu Lys Phe Asn Pro Glu Trp Tyr Lys Gly Glu  
 290 295 300

Pro Ile Trp Val Thr Ala Lys Tyr Gln Gly Leu Lys Ser Gly Thr Phe  
 305 310 315 320

Phe Trp Pro Gly Ser Asp Val Glu Ile Asn Gly Ile Phe Pro Asp Ile  
 325 330 335

Tyr Lys Met Tyr Asn Gly Ser Val Pro Phe Glu Glu Arg Ile Leu Ala  
 340 345 350

Val Leu Gln Trp Leu Gln Leu Pro Lys Asp Glu Arg Pro His Phe Tyr  
 355 360 365

Thr Leu Tyr Leu Glu Glu Pro Asp Ser Ser Gly His Ser Tyr Gly Pro  
 370 375 380

Val Ser Ser Glu Val Ile Lys Ala Leu Gln Arg Val Asp Gly Met Val

385						390						395						400
Gly	Met	Leu	Met	Asp 405	Gly	Leu	Lys	Glu	Leu 410	Asn	Leu	His	Arg	Cys 415	Leu			
Asn	Leu	Ile	Leu 420	Ile	Ser	Asp	His	Gly 425	Met	Glu	Gln	Gly	Ser 430	Cys	Lys			
Lys	Tyr	Ile 435	Tyr	Leu	Asn	Lys	Tyr 440	Leu	Gly	Asp	Val	Lys 445	Asn	Ile	Lys			
Val	Ile 450	Tyr	Gly	Pro	Ala	Ala 455	Arg	Leu	Arg	Pro	Ser 460	Asp	Val	Pro	Asp			
Lys 465	Tyr	Tyr	Ser	Phe	Asn 470	Tyr	Glu	Gly	Ile	Ala 475	Arg	Asn	Leu	Ser	Cys 480			
Arg	Glu	Pro	Asn	Gln 485	His	Phe	Lys	Pro	Tyr 490	Leu	Lys	His	Phe	Leu 495	Pro			
Lys	Arg	Leu	His 500	Phe	Ala	Lys	Ser	Asp 505	Arg	Ile	Glu	Pro	Leu 510	Thr	Phe			
Tyr	Leu	Asp 515	Pro	Gln	Trp	Gln	Leu 520	Ala	Leu	Asn	Pro	Ser 525	Glu	Arg	Lys			
Tyr	Cys 530	Gly	Ser	Gly	Phe	His 535	Gly	Ser	Asp	Asn	Val 540	Phe	Ser	Asn	Met			
Gln 545	Ala	Leu	Phe	Val	Gly 550	Tyr	Gly	Pro	Gly	Phe 555	Lys	His	Gly	Ile	Glu 560			
Ala	Asp	Thr	Phe	Glu 565	Asn	Ile	Glu	Val	Tyr 570	Asn	Leu	Met	Cys	Asp 575	Leu			
Leu	Asn	Leu	Thr 580	Pro	Ala	Pro	Asn	Asn 585	Gly	Thr	His	Gly	Ser 590	Leu	Asn			
His	Leu	Leu 595	Lys	Asn	Pro	Val	Tyr 600	Thr	Pro	Lys	His	Pro 605	Lys	Glu	Val			
His	Pro 610	Leu	Val	Gln	Cys	Pro 615	Phe	Thr	Arg	Asn	Pro 620	Arg	Asp	Asn	Leu			
Gly 625	Cys	Ser	Cys	Asn	Pro 630	Ser	Ile	Leu	Pro	Ile 635	Glu	Asp	Phe	Gln	Thr 640			
Gln	Phe	Asn	Leu	Thr 645	Val	Ala	Glu	Glu	Lys 650	Ile	Ile	Lys	His	Glu 655	Thr			
Leu	Pro	Tyr	Gly	Arg	Pro	Arg	Val	Leu	Gln	Lys	Glu	Asn	Thr	Ile	Cys			

660

665

670

Leu Leu Ser Gln His Gln Phe Met Ser Gly Tyr Ser Gln Asp Ile Leu  
675 680 685

Met Pro Leu Trp Thr Ser Tyr Thr Val Asp Arg Asn Asp Ser Phe Ser  
690 695 700

Thr Glu Asp Phe Ser Asn Cys Leu Tyr Gln Asp Phe Arg Ile Pro Leu  
705 710 715 720

Ser Pro Val His Lys Cys Ser Phe Tyr Lys Asn Asn Thr Lys Val Ser  
725 730 735

Tyr Gly Phe Leu Ser Pro Pro Gln Leu Asn Lys Asn Ser Ser Gly Ile  
740 745 750

Tyr Ser Glu Ala Leu Leu Thr Thr Asn Ile Val Pro Met Tyr Gln Ser  
755 760 765

Phe Gln Val Ile Trp Arg Tyr Phe His Asp Thr Leu Leu Arg Lys Tyr  
770 775 780

Ala Glu Glu Arg Asn Gly Val Asn Val Val Ser Gly Pro Val Phe Asp  
785 790 795 800

Phe Asp Tyr Asp Gly Arg Cys Asp Ser Leu Glu Asn Leu Arg Gln Lys  
805 810 815

Arg Arg Val Ile Arg Asn Gln Glu Ile Leu Ile Pro Thr His Phe Phe  
820 825 830

Ile Val Leu Thr Ser Cys Lys Asp Thr Ser Gln Thr Pro Leu His Cys  
835 840 845

Glu Asn Leu Asp Thr Leu Ala Phe Ile Leu Pro His Arg Thr Asp Asn  
850 855 860

Ser Glu Ser Cys Val His Gly Lys His Asp Ser Ser Trp Val Glu Glu  
865 870 875 880

Leu Leu Met Leu His Arg Ala Arg Ile Thr Asp Val Glu His Ile Thr  
885 890 895

Gly Leu Ser Phe Tyr Gln Gln Arg Lys Glu Pro Val Ser Asp Ile Leu  
900 905 910

Lys Leu Lys Thr His Leu Pro Thr Phe Ser Gln Glu Asp Leu Ile Asn  
915 920 925

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly

930

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met  
945 950 955 960

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His  
965 970 975

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val  
980 985 990

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr  
995 1000 1005

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn  
1010 1015 1020

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala  
1025 1030 1035

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu  
1040 1045 1050

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys  
1055 1060 1065

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser  
1070 1075 1080

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn  
1085 1090 1095

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe  
1100 1105 1110

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly  
1115 1120 1125

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His  
1130 1135 1140

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys  
1145 1150 1155

<210> 17  
<211> 852  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> chemically synthesized

<400> 17

047162-7065W01\_Seq\_Listing.txt

Met Arg Gly Pro Ala Val Leu Leu Thr Val Ala Leu Ala Thr Leu Leu  
1 5 10 15

Ala Pro Gly Ala Gly Ala Gly Leu Lys Pro Ser Cys Ala Lys Glu Val  
20 25 30

Lys Ser Cys Lys Gly Arg Cys Phe Glu Arg Thr Phe Gly Asn Cys Arg  
35 40 45

Cys Asp Ala Ala Cys Val Glu Leu Gly Asn Cys Cys Leu Asp Tyr Gln  
50 55 60

Glu Thr Cys Ile Glu Pro Glu His Ile Trp Thr Cys Asn Lys Phe Arg  
65 70 75 80

Cys Gly Glu Lys Arg Leu Thr Arg Ser Leu Cys Ala Cys Ser Asp Asp  
85 90 95

Cys Lys Asp Lys Gly Asp Cys Cys Ile Asn Tyr Ser Ser Val Cys Gln  
100 105 110

Gly Glu Lys Ser Trp Val Glu Glu Pro Cys Glu Ser Ile Asn Glu Pro  
115 120 125

Gln Cys Pro Ala Gly Phe Glu Thr Pro Pro Thr Leu Leu Phe Ser Leu  
130 135 140

Asp Gly Phe Arg Ala Glu Tyr Leu His Thr Trp Gly Gly Leu Leu Pro  
145 150 155 160

Val Ile Ser Lys Leu Lys Lys Cys Gly Thr Tyr Thr Lys Asn Met Arg  
165 170 175

Pro Val Tyr Pro Thr Lys Thr Phe Pro Asn His Tyr Ser Ile Val Thr  
180 185 190

Gly Leu Tyr Pro Glu Ser His Gly Ile Ile Asp Asn Lys Met Tyr Asp  
195 200 205

Pro Lys Met Asn Ala Ser Phe Ser Leu Lys Ser Lys Glu Lys Phe Asn  
210 215 220

Pro Glu Trp Tyr Lys Gly Glu Pro Ile Trp Val Thr Ala Lys Tyr Gln  
225 230 235 240

Gly Leu Lys Ser Gly Thr Phe Phe Trp Pro Gly Ser Asp Val Glu Ile  
245 250 255

Asn Gly Ile Phe Pro Asp Ile Tyr Lys Met Tyr Asn Gly Ser Val Pro  
260 265 270

047162-7065W01\_Seq\_Listing.txt

Phe Glu Glu Arg Ile Leu Ala Val Leu Gln Trp Leu Gln Leu Pro Lys  
 275 280 285  
 Asp Glu Arg Pro His Phe Tyr Thr Leu Tyr Leu Glu Glu Pro Asp Ser  
 290 295 300  
 Ser Gly His Ser Tyr Gly Pro Val Ser Ser Glu Val Ile Lys Ala Leu  
 305 310 315 320  
 Gln Arg Val Asp Gly Met Val Gly Met Leu Met Asp Gly Leu Lys Glu  
 325 330 335  
 Leu Asn Leu His Arg Cys Leu Asn Leu Ile Leu Ile Ser Asp His Gly  
 340 345 350  
 Met Glu Gln Gly Ser Cys Lys Lys Tyr Ile Tyr Leu Asn Lys Tyr Leu  
 355 360 365  
 Gly Asp Val Lys Asn Ile Lys Val Ile Tyr Gly Pro Ala Ala Arg Leu  
 370 375 380  
 Arg Pro Ser Asp Val Pro Asp Lys Tyr Tyr Ser Phe Asn Tyr Glu Gly  
 385 390 395 400  
 Ile Ala Arg Asn Leu Ser Cys Arg Glu Pro Asn Gln His Phe Lys Pro  
 405 410 415  
 Tyr Leu Lys His Phe Leu Pro Lys Arg Leu His Phe Ala Lys Ser Asp  
 420 425 430  
 Arg Ile Glu Pro Leu Thr Phe Tyr Leu Asp Pro Gln Trp Gln Leu Ala  
 435 440 445  
 Leu Asn Pro Ser Glu Arg Lys Tyr Cys Gly Ser Gly Phe His Gly Ser  
 450 455 460  
 Asp Asn Val Phe Ser Asn Met Gln Ala Leu Phe Val Gly Tyr Gly Pro  
 465 470 475 480  
 Gly Phe Lys His Gly Ile Glu Ala Asp Thr Phe Glu Asn Ile Glu Val  
 485 490 495  
 Tyr Asn Leu Met Cys Asp Leu Leu Asn Leu Thr Pro Ala Pro Asn Asn  
 500 505 510  
 Gly Thr His Gly Ser Leu Asn His Leu Leu Lys Asn Pro Val Tyr Thr  
 515 520 525  
 Pro Lys His Pro Lys Glu Val His Pro Leu Val Gln Cys Pro Phe Thr  
 530 535 540

047162-7065W01\_Seq\_Listing.txt

Arg Asn Pro Arg Asp Asn Leu Gly Cys Ser Cys Asn Pro Ser Ile Leu  
 545 550 555 560  
 Pro Ile Glu Asp Phe Gln Thr Gln Phe Asn Leu Thr Val Ala Glu Glu  
 565 570 575  
 Lys Ile Ile Lys His Glu Thr Leu Pro Tyr Gly Arg Pro Arg Val Leu  
 580 585 590  
 Gln Lys Glu Asn Thr Ile Cys Leu Leu Ser Gln His Gln Phe Met Ser  
 595 600 605  
 Gly Tyr Ser Gln Asp Ile Leu Met Pro Leu Trp Thr Ser Tyr Thr Val  
 610 615 620  
 Asp Arg Asn Asp Ser Phe Ser Thr Glu Asp Phe Ser Asn Cys Leu Tyr  
 625 630 635 640  
 Gln Asp Phe Arg Ile Pro Leu Ser Pro Val His Lys Cys Ser Phe Tyr  
 645 650 655  
 Lys Asn Asn Thr Lys Val Ser Tyr Gly Phe Leu Ser Pro Pro Gln Leu  
 660 665 670  
 Asn Lys Asn Ser Ser Gly Ile Tyr Ser Glu Ala Leu Leu Thr Thr Asn  
 675 680 685  
 Ile Val Pro Met Tyr Gln Ser Phe Gln Val Ile Trp Arg Tyr Phe His  
 690 695 700  
 Asp Thr Leu Leu Arg Lys Tyr Ala Glu Glu Arg Asn Gly Val Asn Val  
 705 710 715 720  
 Val Ser Gly Pro Val Phe Asp Phe Asp Tyr Asp Gly Arg Cys Asp Ser  
 725 730 735  
 Leu Glu Asn Leu Arg Gln Lys Arg Arg Val Ile Arg Asn Gln Glu Ile  
 740 745 750  
 Leu Ile Pro Thr His Phe Phe Ile Val Leu Thr Ser Cys Lys Asp Thr  
 755 760 765  
 Ser Gln Thr Pro Leu His Cys Glu Asn Leu Asp Thr Leu Ala Phe Ile  
 770 775 780  
 Leu Pro His Arg Thr Asp Asn Ser Glu Ser Cys Val His Gly Lys His  
 785 790 795 800  
 Asp Ser Ser Trp Val Glu Glu Leu Leu Met Leu His Arg Ala Arg Ile  
 805 810 815



047162-7065W01\_Seq\_Listing.txt

Thr Asp Val Glu His Ile Thr Gly Leu Ser Phe Tyr Gln Gln Arg Lys  
820 825 830

Glu Pro Val Ser Asp Ile Leu Lys Leu Lys Thr His Leu Pro Thr Phe  
835 840 845

Ser Gln Glu Asp  
850

<210> 18  
<211> 1082  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> chemically synthesized

<400> 18

Met Arg Gly Pro Ala Val Leu Leu Thr Val Ala Leu Ala Thr Leu Leu  
1 5 10 15

Ala Pro Gly Ala Gly Ala Gly Leu Lys Pro Ser Cys Ala Lys Glu Val  
20 25 30

Lys Ser Cys Lys Gly Arg Cys Phe Glu Arg Thr Phe Gly Asn Cys Arg  
35 40 45

Cys Asp Ala Ala Cys Val Glu Leu Gly Asn Cys Cys Leu Asp Tyr Gln  
50 55 60

Glu Thr Cys Ile Glu Pro Glu His Ile Trp Thr Cys Asn Lys Phe Arg  
65 70 75 80

Cys Gly Glu Lys Arg Leu Thr Arg Ser Leu Cys Ala Cys Ser Asp Asp  
85 90 95

Cys Lys Asp Lys Gly Asp Cys Cys Ile Asn Tyr Ser Ser Val Cys Gln  
100 105 110

Gly Glu Lys Ser Trp Val Glu Glu Pro Cys Glu Ser Ile Asn Glu Pro  
115 120 125

Gln Cys Pro Ala Gly Phe Glu Thr Pro Pro Thr Leu Leu Phe Ser Leu  
130 135 140

Asp Gly Phe Arg Ala Glu Tyr Leu His Thr Trp Gly Gly Leu Leu Pro  
145 150 155 160

Val Ile Ser Lys Leu Lys Lys Cys Gly Thr Tyr Thr Lys Asn Met Arg  
165 170 175

Pro Val Tyr Pro Thr Lys Thr Phe Pro Asn His Tyr Ser Ile Val Thr

180

185

190

Gly Leu Tyr<sub>195</sub> Pro Glu Ser His Gly<sub>200</sub> Ile Ile Asp Asn Lys<sub>205</sub> Met Tyr Asp  
 Pro Lys<sub>210</sub> Met Asn Ala Ser Phe<sub>215</sub> Ser Leu Lys Ser Lys<sub>220</sub> Glu Lys Phe Asn  
 Pro Glu Trp Tyr Lys Gly<sub>230</sub> Glu Pro Ile Trp Val<sub>235</sub> Thr Ala Lys Tyr Gln<sub>240</sub>  
 Gly Leu Lys Ser Gly<sub>245</sub> Thr Phe Phe Trp Pro Gly Ser Asp Val Glu<sub>255</sub> Ile  
 Asn Gly Ile Phe<sub>260</sub> Pro Asp Ile Tyr Lys<sub>265</sub> Met Tyr Asn Gly Ser Val Pro  
 Phe Glu Glu<sub>275</sub> Arg Ile Leu Ala Val<sub>280</sub> Leu Gln Trp Leu Gln<sub>285</sub> Leu Pro Lys  
 Asp Glu<sub>290</sub> Arg Pro His Phe Tyr<sub>295</sub> Thr Leu Tyr Leu Glu<sub>300</sub> Glu Pro Asp Ser  
 Ser Gly His Ser Tyr Gly<sub>310</sub> Pro Val Ser Ser Glu<sub>315</sub> Val Ile Lys Ala Leu<sub>320</sub>  
 Gln Arg Val Asp Gly<sub>325</sub> Met Val Gly Met Leu<sub>330</sub> Met Asp Gly Leu Lys<sub>335</sub> Glu  
 Leu Asn Leu His<sub>340</sub> Arg Cys Leu Asn Leu<sub>345</sub> Ile Leu Ile Ser Asp<sub>350</sub> His Gly  
 Met Glu Gln Gly Ser Cys Lys Lys<sub>360</sub> Tyr Ile Tyr Leu Asn<sub>365</sub> Lys Tyr Leu  
 Gly Asp<sub>370</sub> Val Lys Asn Ile Lys<sub>375</sub> Val Ile Tyr Gly Pro Ala Ala Arg Leu  
 Arg<sub>385</sub> Pro Ser Asp Val Pro<sub>390</sub> Asp Lys Tyr Tyr Ser<sub>395</sub> Phe Asn Tyr Glu Gly<sub>400</sub>  
 Ile Ala Arg Asn Leu<sub>405</sub> Ser Cys Arg Glu Pro Asn Gln His Phe Lys<sub>415</sub> Pro  
 Tyr Leu Lys His<sub>420</sub> Phe Leu Pro Lys Arg<sub>425</sub> Leu His Phe Ala Lys<sub>430</sub> Ser Asp  
 Arg Ile Glu<sub>435</sub> Pro Leu Thr Phe Tyr<sub>440</sub> Leu Asp Pro Gln Trp Gln Leu Ala  
 Leu Asn Pro Ser Glu Arg Lys Tyr Cys Gly Ser Gly Phe His Gly Ser

450

455

460

Asp Asn Val Phe Ser Asn Met Gl n Al a Leu Phe Val Gly Tyr Gly Pro  
 465 470 475 480

Gly Phe Lys His Gly Ile Gl u Al a Asp Thr Phe Gl u Asn Ile Gl u Val  
 485 490 495

Tyr Asn Leu Met Cys Asp Leu Leu Asn Leu Thr Pro Al a Pro Asn Asn  
 500 505 510

Gly Thr His Gly Ser Leu Asn His Leu Leu Lys Asn Pro Val Tyr Thr  
 515 520 525

Pro Lys His Pro Lys Gl u Val His Pro Leu Val Gl n Cys Pro Phe Thr  
 530 535 540

Arg Asn Pro Arg Asp Asn Leu Gly Cys Ser Cys Asn Pro Ser Ile Leu  
 545 550 555 560

Pro Ile Gl u Asp Phe Gl n Thr Gl n Phe Asn Leu Thr Val Al a Gl u Gl u  
 565 570 575

Lys Ile Ile Lys His Gl u Thr Leu Pro Tyr Gly Arg Pro Arg Val Leu  
 580 585 590

Gl n Lys Gl u Asn Thr Ile Cys Leu Leu Ser Gl n His Gl n Phe Met Ser  
 595 600 605

Gly Tyr Ser Gl n Asp Ile Leu Met Pro Leu Trp Thr Ser Tyr Thr Val  
 610 615 620

Asp Arg Asn Asp Ser Phe Ser Thr Gl u Asp Phe Ser Asn Cys Leu Tyr  
 625 630 635 640

Gl n Asp Phe Arg Ile Pro Leu Ser Pro Val His Lys Cys Ser Phe Tyr  
 645 650 655

Lys Asn Asn Thr Lys Val Ser Tyr Gly Phe Leu Ser Pro Pro Gl n Leu  
 660 665 670

Asn Lys Asn Ser Ser Gly Ile Tyr Ser Gl u Al a Leu Leu Thr Thr Asn  
 675 680 685

Ile Val Pro Met Tyr Gl n Ser Phe Gl n Val Ile Trp Arg Tyr Phe His  
 690 695 700

Asp Thr Leu Leu Arg Lys Tyr Al a Gl u Gl u Arg Asn Gly Val Asn Val  
 705 710 715 720

Val Ser Gly Pro Val Phe Asp Phe Asp Tyr Asp Gly Arg Cys Asp Ser  
 Page 26

725

730

735

Leu Glu Asn Leu Arg Gln Lys Arg Arg Val Ile Arg Asn Gln Glu Ile  
 740 745 750

Leu Ile Pro Thr His Phe Phe Ile Val Leu Thr Ser Cys Lys Asp Thr  
 755 760 765

Ser Gln Thr Pro Leu His Cys Glu Asn Leu Asp Thr Leu Ala Phe Ile  
 770 775 780

Leu Pro His Arg Thr Asp Asn Ser Glu Ser Cys Val His Gly Lys His  
 785 790 795 800

Asp Ser Ser Trp Val Glu Glu Leu Leu Met Leu His Arg Ala Arg Ile  
 805 810 815

Thr Asp Val Glu His Ile Thr Gly Leu Ser Phe Tyr Gln Gln Arg Lys  
 820 825 830

Glu Pro Val Ser Asp Ile Leu Lys Leu Lys Thr His Leu Pro Thr Phe  
 835 840 845

Ser Gln Glu Asp Leu Ile Asn Asp Lys Thr His Thr Cys Pro Pro Cys  
 850 855 860

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro  
 865 870 875 880

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys  
 885 890 895

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp  
 900 905 910

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu  
 915 920 925

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu  
 930 935 940

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn  
 945 950 955 960

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly  
 965 970 975

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu  
 980 985 990

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr

995

1000

1005

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu  
1010 1015 1020

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser  
1025 1030 1035

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln  
1040 1045 1050

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His  
1055 1060 1065

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys  
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Ala Pro Gly Ala Gly Ala Pro Ser Cys Ala Lys Glu Val Lys Ser Cys  
20 25 30

Lys Gly Arg Cys Phe Glu Arg Thr Phe Gly Asn Cys Arg Cys Asp Ala  
35 40 45

Ala Cys Val Glu Leu Gly Asn Cys Cys Leu Asp Tyr Gln Glu Thr Cys  
50 55 60

Ile Glu Pro Glu His Ile Trp Thr Cys Asn Lys Phe Arg Cys Gly Glu  
65 70 75 80

Lys Arg Leu Thr Arg Ser Leu Cys Ala Cys Ser Asp Asp Cys Lys Asp  
85 90 95

Lys Gly Asp Cys Cys Ile Asn Tyr Ser Ser Val Cys Gln Gly Glu Lys  
100 105 110

Ser Trp Val Glu Glu Pro Cys Glu Ser Ile Asn Glu Pro Gln Cys Pro  
115 120 125

Ala Gly Phe Glu Thr Pro Pro Thr Leu Leu Phe Ser Leu Asp Gly Phe  
130 135 140

047162-7065W01\_Seq\_Listing.txt

Arg Ala Glu Tyr Leu His Thr Trp Gly Gly Leu Leu Pro Val Ile Ser  
 145 150 155 160  
 Lys Leu Lys Lys Cys Gly Thr Tyr Thr Lys Asn Met Arg Pro Val Tyr  
 165 170 175  
 Pro Thr Lys Thr Phe Pro Asn His Tyr Ser Ile Val Thr Gly Leu Tyr  
 180 185 190  
 Pro Glu Ser His Gly Ile Ile Asp Asn Lys Met Tyr Asp Pro Lys Met  
 195 200 205  
 Asn Ala Ser Phe Ser Leu Lys Ser Lys Glu Lys Phe Asn Pro Glu Trp  
 210 215 220  
 Tyr Lys Gly Glu Pro Ile Trp Val Thr Ala Lys Tyr Gln Gly Leu Lys  
 225 230 235 240  
 Ser Gly Thr Phe Phe Trp Pro Gly Ser Asp Val Glu Ile Asn Gly Ile  
 245 250 255  
 Phe Pro Asp Ile Tyr Lys Met Tyr Asn Gly Ser Val Pro Phe Glu Glu  
 260 265 270  
 Arg Ile Leu Ala Val Leu Gln Trp Leu Gln Leu Pro Lys Asp Glu Arg  
 275 280 285  
 Pro His Phe Tyr Thr Leu Tyr Leu Glu Glu Pro Asp Ser Ser Gly His  
 290 295 300  
 Ser Tyr Gly Pro Val Ser Ser Glu Val Ile Lys Ala Leu Gln Arg Val  
 305 310 315 320  
 Asp Gly Met Val Gly Met Leu Met Asp Gly Leu Lys Glu Leu Asn Leu  
 325 330 335  
 His Arg Cys Leu Asn Leu Ile Leu Ile Ser Asp His Gly Met Glu Gln  
 340 345 350  
 Gly Ser Cys Lys Lys Tyr Ile Tyr Leu Asn Lys Tyr Leu Gly Asp Val  
 355 360 365  
 Lys Asn Ile Lys Val Ile Tyr Gly Pro Ala Ala Arg Leu Arg Pro Ser  
 370 375 380  
 Asp Val Pro Asp Lys Tyr Tyr Ser Phe Asn Tyr Glu Gly Ile Ala Arg  
 385 390 395 400  
 Asn Leu Ser Cys Arg Glu Pro Asn Gln His Phe Lys Pro Tyr Leu Lys  
 405 410 415

047162-7065W01\_Seq\_Listing.txt

His Phe Leu Pro Lys Arg Leu His Phe Ala Lys Ser Asp Arg Ile Glu  
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 Pro Leu Thr Phe Tyr Leu Asp Pro Gln Trp Gln Leu Ala Leu Asn Pro  
                     435                    440                    445  
 Ser Glu Arg Lys Tyr Cys Gly Ser Gly Phe His Gly Ser Asp Asn Val  
                     450                    455                    460  
 Phe Ser Asn Met Gln Ala Leu Phe Val Gly Tyr Gly Pro Gly Phe Lys  
                     465                    470                    475                    480  
 His Gly Ile Glu Ala Asp Thr Phe Glu Asn Ile Glu Val Tyr Asn Leu  
                     485                    490                    495  
 Met Cys Asp Leu Leu Asn Leu Thr Pro Ala Pro Asn Asn Gly Thr His  
                     500                    505                    510  
 Gly Ser Leu Asn His Leu Leu Lys Asn Pro Val Tyr Thr Pro Lys His  
                     515                    520                    525  
 Pro Lys Glu Val His Pro Leu Val Gln Cys Pro Phe Thr Arg Asn Pro  
                     530                    535                    540  
 Arg Asp Asn Leu Gly Cys Ser Cys Asn Pro Ser Ile Leu Pro Ile Glu  
                     545                    550                    555                    560  
 Asp Phe Gln Thr Gln Phe Asn Leu Thr Val Ala Glu Glu Lys Ile Ile  
                     565                    570                    575  
 Lys His Glu Thr Leu Pro Tyr Gly Arg Pro Arg Val Leu Gln Lys Glu  
                     580                    585                    590  
 Asn Thr Ile Cys Leu Leu Ser Gln His Gln Phe Met Ser Gly Tyr Ser  
                     595                    600                    605  
 Gln Asp Ile Leu Met Pro Leu Trp Thr Ser Tyr Thr Val Asp Arg Asn  
                     610                    615                    620  
 Asp Ser Phe Ser Thr Glu Asp Phe Ser Asn Cys Leu Tyr Gln Asp Phe  
                     625                    630                    635                    640  
 Arg Ile Pro Leu Ser Pro Val His Lys Cys Ser Phe Tyr Lys Asn Asn  
                     645                    650                    655  
 Thr Lys Val Ser Tyr Gly Phe Leu Ser Pro Pro Gln Leu Asn Lys Asn  
                     660                    665                    670  
 Ser Ser Gly Ile Tyr Ser Glu Ala Leu Leu Thr Thr Asn Ile Val Pro  
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047162-7065W01\_Seq\_Listing.txt

Met Tyr Gln Ser Phe Gln Val Ile Trp Arg Tyr Phe His Asp Thr Leu  
690 695 700

Leu Arg Lys Tyr Ala Glu Glu Arg Asn Gly Val Asn Val Val Ser Gly  
705 710 715 720

Pro Val Phe Asp Phe Asp Tyr Asp Gly Arg Cys Asp Ser Leu Glu Asn  
725 730 735

Leu Arg Gln Lys Arg Arg Val Ile Arg Asn Gln Glu Ile Leu Ile Pro  
740 745 750

Thr His Phe Phe Ile Val Leu Thr Ser Cys Lys Asp Thr Ser Gln Thr  
755 760 765

Pro Leu His Cys Glu Asn Leu Asp Thr Leu Ala Phe Ile Leu Pro His  
770 775 780

Arg Thr Asp Asn Ser Glu Ser Cys Val His Gly Lys His Asp Ser Ser  
785 790 795 800

Trp Val Glu Glu Leu Leu Met Leu His Arg Ala Arg Ile Thr Asp Val  
805 810 815

Glu His Ile Thr Gly Leu Ser Phe Tyr Gln Gln Arg Lys Glu Pro Val  
820 825 830

Ser Asp Ile Leu Lys Leu Lys Thr His Leu Pro Thr Phe Ser Gln Glu  
835 840 845

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Ala Pro Gly Ala Gly Ala Pro Ser Cys Ala Lys Glu Val Lys Ser Cys  
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Lys Gly Arg Cys Phe Glu Arg Thr Phe Gly Asn Cys Arg Cys Asp Ala  
35 40 45

Ala Cys Val Glu Leu Gly Asn Cys Cys Leu Asp Tyr Gln Glu Thr Cys  
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50

Ile Glu Pro Glu His Ile Trp Thr Cys Asn Lys Phe Arg Cys Gly Glu  
65 70 75 80

Lys Arg Leu Thr Arg Ser Leu Cys Ala Cys Ser Asp Asp Cys Lys Asp  
85 90 95

Lys Gly Asp Cys Cys Ile Asn Tyr Ser Ser Val Cys Gln Gly Glu Lys  
100 105 110

Ser Trp Val Glu Glu Pro Cys Glu Ser Ile Asn Glu Pro Gln Cys Pro  
115 120 125

Ala Gly Phe Glu Thr Pro Pro Thr Leu Leu Phe Ser Leu Asp Gly Phe  
130 135 140

Arg Ala Glu Tyr Leu His Thr Trp Gly Gly Leu Leu Pro Val Ile Ser  
145 150 155 160

Lys Leu Lys Lys Cys Gly Thr Tyr Thr Lys Asn Met Arg Pro Val Tyr  
165 170 175

Pro Thr Lys Thr Phe Pro Asn His Tyr Ser Ile Val Thr Gly Leu Tyr  
180 185 190

Pro Glu Ser His Gly Ile Ile Asp Asn Lys Met Tyr Asp Pro Lys Met  
195 200 205

Asn Ala Ser Phe Ser Leu Lys Ser Lys Glu Lys Phe Asn Pro Glu Trp  
210 215 220

Tyr Lys Gly Glu Pro Ile Trp Val Thr Ala Lys Tyr Gln Gly Leu Lys  
225 230 235 240

Ser Gly Thr Phe Phe Trp Pro Gly Ser Asp Val Glu Ile Asn Gly Ile  
245 250 255

Phe Pro Asp Ile Tyr Lys Met Tyr Asn Gly Ser Val Pro Phe Glu Glu  
260 265 270

Arg Ile Leu Ala Val Leu Gln Trp Leu Gln Leu Pro Lys Asp Glu Arg  
275 280 285

Pro His Phe Tyr Thr Leu Tyr Leu Glu Glu Pro Asp Ser Ser Gly His  
290 295 300

Ser Tyr Gly Pro Val Ser Ser Glu Val Ile Lys Ala Leu Gln Arg Val  
305 310 315 320

Asp Gly Met Val Gly Met Leu Met Asp Gly Leu Lys Glu Leu Asn Leu

325

330

335

His Arg Cys Leu Asn Leu Ile Leu Ile Ser Asp His Gly Met Glu Gln  
 340 345 350

Gly Ser Cys Lys Lys Tyr Ile Tyr Leu Asn Lys Tyr Leu Gly Asp Val  
 355 360 365

Lys Asn Ile Lys Val Ile Tyr Gly Pro Ala Ala Arg Leu Arg Pro Ser  
 370 375 380

Asp Val Pro Asp Lys Tyr Tyr Ser Phe Asn Tyr Glu Gly Ile Ala Arg  
 385 390 395 400

Asn Leu Ser Cys Arg Glu Pro Asn Gln His Phe Lys Pro Tyr Leu Lys  
 405 410 415

His Phe Leu Pro Lys Arg Leu His Phe Ala Lys Ser Asp Arg Ile Glu  
 420 425 430

Pro Leu Thr Phe Tyr Leu Asp Pro Gln Trp Gln Leu Ala Leu Asn Pro  
 435 440 445

Ser Glu Arg Lys Tyr Cys Gly Ser Gly Phe His Gly Ser Asp Asn Val  
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Phe Ser Asn Met Gln Ala Leu Phe Val Gly Tyr Gly Pro Gly Phe Lys  
 465 470 475 480

His Gly Ile Glu Ala Asp Thr Phe Glu Asn Ile Glu Val Tyr Asn Leu  
 485 490 495

Met Cys Asp Leu Leu Asn Leu Thr Pro Ala Pro Asn Asn Gly Thr His  
 500 505 510

Gly Ser Leu Asn His Leu Leu Lys Asn Pro Val Tyr Thr Pro Lys His  
 515 520 525

Pro Lys Glu Val His Pro Leu Val Gln Cys Pro Phe Thr Arg Asn Pro  
 530 535 540

Arg Asp Asn Leu Gly Cys Ser Cys Asn Pro Ser Ile Leu Pro Ile Glu  
 545 550 555 560

Asp Phe Gln Thr Gln Phe Asn Leu Thr Val Ala Glu Glu Lys Ile Ile  
 565 570 575

Lys His Glu Thr Leu Pro Tyr Gly Arg Pro Arg Val Leu Gln Lys Glu  
 580 585 590

Asn Thr Ile Cys Leu Leu Ser Gln His Gln Phe Met Ser Gly Tyr Ser

595

600

605

Gln Asp Ile Leu Met Pro Leu Trp Thr Ser Tyr Thr Val Asp Arg Asn  
610 615 620

Asp Ser Phe Ser Thr Glu Asp Phe Ser Asn Cys Leu Tyr Gln Asp Phe  
625 630 635 640

Arg Ile Pro Leu Ser Pro Val His Lys Cys Ser Phe Tyr Lys Asn Asn  
645 650 655

Thr Lys Val Ser Tyr Gly Phe Leu Ser Pro Pro Gln Leu Asn Lys Asn  
660 665 670

Ser Ser Gly Ile Tyr Ser Glu Ala Leu Leu Thr Thr Asn Ile Val Pro  
675 680 685

Met Tyr Gln Ser Phe Gln Val Ile Trp Arg Tyr Phe His Asp Thr Leu  
690 695 700

Leu Arg Lys Tyr Ala Glu Glu Arg Asn Gly Val Asn Val Val Ser Gly  
705 710 715 720

Pro Val Phe Asp Phe Asp Tyr Asp Gly Arg Cys Asp Ser Leu Glu Asn  
725 730 735

Leu Arg Gln Lys Arg Arg Val Ile Arg Asn Gln Glu Ile Leu Ile Pro  
740 745 750

Thr His Phe Phe Ile Val Leu Thr Ser Cys Lys Asp Thr Ser Gln Thr  
755 760 765

Pro Leu His Cys Glu Asn Leu Asp Thr Leu Ala Phe Ile Leu Pro His  
770 775 780

Arg Thr Asp Asn Ser Glu Ser Cys Val His Gly Lys His Asp Ser Ser  
785 790 795 800

Trp Val Glu Glu Leu Leu Met Leu His Arg Ala Arg Ile Thr Asp Val  
805 810 815

Glu His Ile Thr Gly Leu Ser Phe Tyr Gln Gln Arg Lys Glu Pro Val  
820 825 830

Ser Asp Ile Leu Lys Leu Lys Thr His Leu Pro Thr Phe Ser Gln Glu  
835 840 845

Asp Leu Ile Asn Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro  
850 855 860

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys

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865                               870                               875                               880
Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
      885                               890                               895

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
      900                               905                               910

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
      915                               920                               925

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
      930                               935                               940

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
      945                               950                               955                               960

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
      965                               970                               975

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys
      980                               985                               990

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
      995                               1000                               1005

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
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Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
      1025                               1030                               1035

Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
      1040                               1045                               1050

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
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Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
      1070                               1075

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 Arg Ser His Ala Ala Glu Ala Pro Gly Asp Pro Gln Ala Ala Ala Ser  
           35                                  40                                  45  
 Leu Leu Ala Pro Met Asp Val Gly Glu Glu Pro Leu Glu Lys Ala Ala  
       50                                  55  
 Arg Ala Arg Thr Ala Lys Asp Pro Asn Thr Tyr Lys Ile Ile Ser Leu  
   65                                  70                                  75                                  80  
 Phe Thr Phe Ala Val Gly Val Asn Ile Cys Leu Gly Phe Thr Ala Gly  
                   85                                  90                                  95  
 Leu Lys Pro Ser Cys Ala Lys Glu Val Lys Ser Cys Lys Gly Arg Cys  
                   100                                  105                                  110  
 Phe Glu Arg Thr Phe Gly Asn Cys Arg Cys Asp Ala Ala Cys Val Glu  
           115                                  120                                  125  
 Leu Gly Asn Cys Cys Leu Asp Tyr Gln Glu Thr Cys Ile Glu Pro Glu  
       130                                  135                                  140  
 His Ile Trp Thr Cys Asn Lys Phe Arg Cys Gly Glu Lys Arg Leu Thr  
   145                                  150                                  155                                  160  
 Arg Ser Leu Cys Ala Cys Ser Asp Asp Cys Lys Asp Lys Gly Asp Cys  
                   165                                  170                                  175  
 Cys Ile Asn Tyr Ser Ser Val Cys Gln Gly Glu Lys Ser Trp Val Glu  
                   180                                  185                                  190  
 Glu Pro Cys Glu Ser Ile Asn Glu Pro Gln Cys Pro Ala Gly Phe Glu  
                   195                                  200                                  205  
 Thr Pro Pro Thr Leu Leu Phe Ser Leu Asp Gly Phe Arg Ala Glu Tyr  
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 Leu His Thr Trp Gly Gly Leu Leu Pro Val Ile Ser Lys Leu Lys Lys  
   225                                  230                                  235                                  240  
 Cys Gly Thr Tyr Thr Lys Asn Met Arg Pro Val Tyr Pro Thr Lys Thr  
                   245                                  250                                  255  
 Phe Pro Asn His Tyr Ser Ile Val Thr Gly Leu Tyr Pro Glu Ser His  
                   260                                  265                                  270  
 Gly Ile Ile Asp Asn Lys Met Tyr Asp Pro Lys Met Asn Ala Ser Phe  
                   275                                  280                                  285

047162-7065W01\_Seq\_Listing.txt

Ser Leu Lys Ser Lys Glu Lys Phe Asn Pro Glu Trp Tyr Lys Gly Glu  
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Pro Ile Trp Val Thr Ala Lys Tyr Gln Gly Leu Lys Ser Gly Thr Phe  
305 310 315 320

Phe Trp Pro Gly Ser Asp Val Glu Ile Asn Gly Ile Phe Pro Asp Ile  
325 330 335

Tyr Lys Met Tyr Asn Gly Ser Val Pro Phe Glu Glu Arg Ile Leu Ala  
340 345 350

Val Leu Gln Trp Leu Gln Leu Pro Lys Asp Glu Arg Pro His Phe Tyr  
355 360 365

Thr Leu Tyr Leu Glu Glu Pro Asp Ser Ser Gly His Ser Tyr Gly Pro  
370 375 380

Val Ser Ser Glu Val Ile Lys Ala Leu Gln Arg Val Asp Gly Met Val  
385 390 395 400

Gly Met Leu Met Asp Gly Leu Lys Glu Leu Asn Leu His Arg Cys Leu  
405 410 415

Asn Leu Ile Leu Ile Ser Asp His Gly Met Glu Gln Gly Ser Cys Lys  
420 425 430

Lys Tyr Ile Tyr Leu Asn Lys Tyr Leu Gly Asp Val Lys Asn Ile Lys  
435 440 445

Val Ile Tyr Gly Pro Ala Ala Arg Leu Arg Pro Ser Asp Val Pro Asp  
450 455 460

Lys Tyr Tyr Ser Phe Asn Tyr Glu Gly Ile Ala Arg Asn Leu Ser Cys  
465 470 475 480

Arg Glu Pro Asn Gln His Phe Lys Pro Tyr Leu Lys His Phe Leu Pro  
485 490 495

Lys Arg Leu His Phe Ala Lys Ser Asp Arg Ile Glu Pro Leu Thr Phe  
500 505 510

Tyr Leu Asp Pro Gln Trp Gln Leu Ala Leu Asn Pro Ser Glu Arg Lys  
515 520 525

Tyr Cys Gly Ser Gly Phe His Gly Ser Asp Asn Val Phe Ser Asn Met  
530 535 540

Gln Ala Leu Phe Val Gly Tyr Gly Pro Gly Phe Lys His Gly Ile Glu  
545 550 555 560

047162-7065W01\_Seq\_Listing.txt

Ala Asp Thr Phe Glu Asn Ile Glu Val Tyr Asn Leu Met Cys Asp Leu  
565 570 575

Leu Asn Leu Thr Pro Ala Pro Asn Asn Gly Thr His Gly Ser Leu Asn  
580 585 590

His Leu Leu Lys Asn Pro Val Tyr Thr Pro Lys His Pro Lys Glu Val  
595 600 605

His Pro Leu Val Gln Cys Pro Phe Thr Arg Asn Pro Arg Asp Asn Leu  
610 615 620

Gly Cys Ser Cys Asn Pro Ser Ile Leu Pro Ile Glu Asp Phe Gln Thr  
625 630 635 640

Gln Phe Asn Leu Thr Val Ala Glu Glu Lys Ile Ile Lys His Glu Thr  
645 650 655

Leu Pro Tyr Gly Arg Pro Arg Val Leu Gln Lys Glu Asn Thr Ile Cys  
660 665 670

Leu Leu Ser Gln His Gln Phe Met Ser Gly Tyr Ser Gln Asp Ile Leu  
675 680 685

Met Pro Leu Trp Thr Ser Tyr Thr Val Asp Arg Asn Asp Ser Phe Ser  
690 695 700

Thr Glu Asp Phe Ser Asn Cys Leu Tyr Gln Asp Phe Arg Ile Pro Leu  
705 710 715 720

Ser Pro Val His Lys Cys Ser Phe Tyr Lys Asn Asn Thr Lys Val Ser  
725 730 735

Tyr Gly Phe Leu Ser Pro Pro Gln Leu Asn Lys Asn Ser Ser Gly Ile  
740 745 750

Tyr Ser Glu Ala Leu Leu Thr Thr Asn Ile Val Pro Met Tyr Gln Ser  
755 760 765

Phe Gln Val Ile Trp Arg Tyr Phe His Asp Thr Leu Leu Arg Lys Tyr  
770 775 780

Ala Glu Glu Arg Asn Gly Val Asn Val Val Ser Gly Pro Val Phe Asp  
785 790 795 800

Phe Asp Tyr Asp Gly Arg Cys Asp Ser Leu Glu Asn Leu Arg Gln Lys  
805 810 815

Arg Arg Val Ile Arg Asn Gln Glu Ile Leu Ile Pro Thr His Phe Phe  
820 825 830

047162-7065W01\_Seq\_Listing.txt

Ile Val Leu Thr Ser Cys Lys Asp Thr Ser Gln Thr Pro Leu His Cys  
835 840 845

Glu Asn Leu Asp Thr Leu Ala Phe Ile Leu Pro His Arg Thr Asp Asn  
850 855 860

Ser Glu Ser Cys Val His Gly Lys His Asp Ser Ser Trp Val Glu Glu  
865 870 875 880

Leu Leu Met Leu His Arg Ala Arg Ile Thr Asp Val Glu His Ile Thr  
885 890 895

Gly Leu Ser Phe Tyr Gln Gln Arg Lys Glu Pro Val Ser Asp Ile Leu  
900 905 910

Lys Leu Lys Thr His Leu Pro Thr Phe Ser Gln Glu Asp Arg Ser Gly  
915 920 925

Ser Gly Gly Ser Met Lys Trp Val Thr Phe Leu Leu Leu Phe Val  
930 935 940

Ser Gly Ser Ala Phe Ser Arg Gly Val Phe Arg Arg Glu Ala His Lys  
945 950 955 960

Ser Glu Ile Ala His Arg Tyr Asn Asp Leu Gly Glu Gln His Phe Lys  
965 970 975

Gly Leu Val Leu Ile Ala Phe Ser Gln Tyr Leu Gln Lys Cys Ser Tyr  
980 985 990

Asp Glu His Ala Lys Leu Val Gln Glu Val Thr Asp Phe Ala Lys Thr  
995 1000 1005

Cys Val Ala Asp Glu Ser Ala Ala Asn Cys Asp Lys Ser Leu His  
1010 1015 1020

Thr Leu Phe Gly Asp Lys Leu Cys Ala Ile Pro Asn Leu Arg Glu  
1025 1030 1035

Asn Tyr Gly Glu Leu Ala Asp Cys Cys Thr Lys Gln Glu Pro Glu  
1040 1045 1050

Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Ser Leu  
1055 1060 1065

Pro Pro Phe Glu Arg Pro Glu Ala Glu Ala Met Cys Thr Ser Phe  
1070 1075 1080

Lys Glu Asn Pro Thr Thr Phe Met Gly His Tyr Leu His Glu Val  
1085 1090 1095



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Ala	Glu	Gln	Tyr	Asn	Glu	Ile	Leu	Thr	Gln	Cys	Cys	Ala	Glu	Ala
	1115					1120					1125			
Asp	Lys	Glu	Ser	Cys	Leu	Thr	Pro	Lys	Leu	Asp	Gly	Val	Lys	Glu
	1130					1135					1140			
Lys	Ala	Leu	Val	Ser	Ser	Val	Arg	Gln	Arg	Met	Lys	Cys	Ser	Ser
	1145					1150					1155			
Met	Gln	Lys	Phe	Gly	Glu	Arg	Ala	Phe	Lys	Ala	Trp	Ala	Val	Ala
	1160					1165					1170			
Arg	Leu	Ser	Gln	Thr	Phe	Pro	Asn	Ala	Asp	Phe	Ala	Glu	Ile	Thr
	1175					1180					1185			
Lys	Leu	Ala	Thr	Asp	Leu	Thr	Lys	Val	Asn	Lys	Glu	Cys	Cys	His
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Gly	Asp	Leu	Leu	Glu	Cys	Ala	Asp	Asp	Arg	Ala	Glu	Leu	Ala	Lys
	1205					1210					1215			
Tyr	Met	Cys	Glu	Asn	Gln	Ala	Thr	Ile	Ser	Ser	Lys	Leu	Gln	Thr
	1220					1225					1230			
Cys	Cys	Asp	Lys	Pro	Leu	Leu	Lys	Lys	Ala	His	Cys	Leu	Ser	Glu
	1235					1240					1245			
Val	Glu	His	Asp	Thr	Met	Pro	Ala	Asp	Leu	Pro	Ala	Ile	Ala	Ala
	1250					1255					1260			
Asp	Phe	Val	Glu	Asp	Gln	Glu	Val	Cys	Lys	Asn	Tyr	Ala	Glu	Ala
	1265					1270					1275			
Lys	Asp	Val	Phe	Leu	Gly	Thr	Phe	Leu	Tyr	Glu	Tyr	Ser	Arg	Arg
	1280					1285					1290			
His	Pro	Asp	Tyr	Ser	Val	Ser	Leu	Leu	Leu	Arg	Leu	Ala	Lys	Lys
	1295					1300					1305			
Tyr	Glu	Ala	Thr	Leu	Glu	Lys	Cys	Cys	Ala	Glu	Ala	Asn	Pro	Pro
	1310					1315					1320			
Ala	Cys	Tyr	Gly	Thr	Val	Leu	Ala	Glu	Phe	Gln	Pro	Leu	Val	Glu
	1325					1330					1335			
Glu	Pro	Lys	Asn	Leu	Val	Lys	Thr	Asn	Cys	Asp	Leu	Tyr	Glu	Lys
	1340					1345					1350			

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Leu Gly Glu Tyr Gly Phe Gln Asn Ala Ile Leu Val Arg Tyr Thr  
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Gln Lys Ala Pro Gln Val Ser Thr Pro Thr Leu Val Glu Ala Ala  
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Arg Asn Leu Gly Arg Val Gly Thr Lys Cys Cys Thr Leu Pro Glu  
1385 1390 1395

Asp Gln Arg Leu Pro Cys Val Glu Asp Tyr Leu Ser Ala Ile Leu  
1400 1405 1410

Asn Arg Val Cys Leu Leu His Glu Lys Thr Pro Val Ser Glu His  
1415 1420 1425

Val Thr Lys Cys Cys Ser Gly Ser Leu Val Glu Arg Arg Pro Cys  
1430 1435 1440

Phe Ser Ala Leu Thr Val Asp Glu Thr Tyr Val Pro Lys Glu Phe  
1445 1450 1455

Lys Ala Glu Thr Phe Thr Phe His Ser Asp Ile Cys Thr Leu Pro  
1460 1465 1470

Glu Lys Glu Lys Gln Ile Lys Lys Gln Thr Ala Leu Ala Glu Leu  
1475 1480 1485

Val Lys His Lys Pro Lys Ala Thr Ala Glu Gln Leu Lys Thr Val  
1490 1495 1500

Met Asp Asp Phe Ala Gln Phe Leu Asp Thr Cys Cys Lys Ala Ala  
1505 1510 1515

Asp Lys Asp Thr Cys Phe Ser Thr Glu Gly Pro Asn Leu Val Thr  
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Glu Lys  
1550

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<223> chemically synthesized

<400> 22

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Page 41

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Lys Gly Arg Cys Phe Glu Arg Thr Phe Gly Asn Cys Arg Cys Asp Ala
35          40          45
Ala Cys Val Glu Leu Gly Asn Cys Cys Leu Asp Tyr Gln Glu Thr Cys
50          55          60
Ile Glu Pro Glu His Ile Trp Thr Cys Asn Lys Phe Arg Cys Gly Glu
65          70          75          80
Lys Arg Leu Thr Arg Ser Leu Cys Ala Cys Ser Asp Asp Cys Lys Asp
85          90          95
Lys Gly Asp Cys Cys Ile Asn Tyr Ser Ser Val Cys Gln Gly Glu Lys
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Ser Trp Val Glu Glu Pro Cys Glu Ser Ile Asn Glu Pro Gln Cys Pro
115         120         125
Ala Gly Phe Glu Thr Pro Pro Thr Leu Leu Phe Ser Leu Asp Gly Phe
130         135         140
Arg Ala Glu Tyr Leu His Thr Trp Gly Gly Leu Leu Pro Val Ile Ser
145         150         155         160
Lys Leu Lys Lys Cys Gly Thr Tyr Thr Lys Asn Met Arg Pro Val Tyr
165         170         175
Pro Thr Lys Thr Phe Pro Asn His Tyr Ser Ile Val Thr Gly Leu Tyr
180         185         190
Pro Glu Ser His Gly Ile Ile Asp Asn Lys Met Tyr Asp Pro Lys Met
195         200         205
Asn Ala Ser Phe Ser Leu Lys Ser Lys Glu Lys Phe Asn Pro Glu Trp
210         215         220
Tyr Lys Gly Glu Pro Ile Trp Val Thr Ala Lys Tyr Gln Gly Leu Lys
225         230         235         240
Ser Gly Thr Phe Phe Trp Pro Gly Ser Asp Val Glu Ile Asn Gly Ile
245         250         255
Phe Pro Asp Ile Tyr Lys Met Tyr Asn Gly Ser Val Pro Phe Glu Glu
260         265         270
Arg Ile Leu Ala Val Leu Gln Trp Leu Gln Leu Pro Lys Asp Glu Arg

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275

280

285

Pro His Phe Tyr Thr Leu Tyr Leu Glu Glu Pro Asp Ser Ser Gly His  
 290 295 300  
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 Asp Gly Met Val Gly Met Leu Met Asp Gly Leu Lys Glu Leu Asn Leu  
 325 330 335  
 His Arg Cys Leu Asn Leu Ile Leu Ile Ser Asp His Gly Met Glu Gln  
 340 345 350  
 Gly Ser Cys Lys Lys Tyr Ile Tyr Leu Asn Lys Tyr Leu Gly Asp Val  
 355 360 365  
 Lys Asn Ile Lys Val Ile Tyr Gly Pro Ala Ala Arg Leu Arg Pro Ser  
 370 375 380  
 Asp Val Pro Asp Lys Tyr Tyr Ser Phe Asn Tyr Glu Gly Ile Ala Arg  
 385 390 395 400  
 Asn Leu Ser Cys Arg Glu Pro Asn Gln His Phe Lys Pro Tyr Leu Lys  
 405 410 415  
 His Phe Leu Pro Lys Arg Leu His Phe Ala Lys Ser Asp Arg Ile Glu  
 420 425 430  
 Pro Leu Thr Phe Tyr Leu Asp Pro Gln Trp Gln Leu Ala Leu Asn Pro  
 435 440 445  
 Ser Glu Arg Lys Tyr Cys Gly Ser Gly Phe His Gly Ser Asp Asn Val  
 450 455 460  
 Phe Ser Asn Met Gln Ala Leu Phe Val Gly Tyr Gly Pro Gly Phe Lys  
 465 470 475 480  
 His Gly Ile Glu Ala Asp Thr Phe Glu Asn Ile Glu Val Tyr Asn Leu  
 485 490 495  
 Met Cys Asp Leu Leu Asn Leu Thr Pro Ala Pro Asn Asn Gly Thr His  
 500 505 510  
 Gly Ser Leu Asn His Leu Leu Lys Asn Pro Val Tyr Thr Pro Lys His  
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 Pro Lys Glu Val His Pro Leu Val Gln Cys Pro Phe Thr Arg Asn Pro  
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 Arg Asp Asn Leu Gly Cys Ser Cys Asn Pro Ser Ile Leu Pro Ile Glu

545 550 555 560

Asp Phe Gln Thr Gln Phe Asn Leu Thr Val Ala Glu Glu Lys Ile Ile  
565 570 575

Lys His Glu Thr Leu Pro Tyr Gly Arg Pro Arg Val Leu Gln Lys Glu  
580 585 590

Asn Thr Ile Cys Leu Leu Ser Gln His Gln Phe Met Ser Gly Tyr Ser  
595 600 605

Gln Asp Ile Leu Met Pro Leu Trp Thr Ser Tyr Thr Val Asp Arg Asn  
610 615 620

Asp 625 Ser Phe Ser Thr Glu 630 Asp Phe Ser Asn Cys 635 Leu Tyr Gl n Asp Phe 640

Arg Ile Pro Leu Ser Pro Val His Lys Cys Ser Phe Tyr Lys Asn Asn  
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Thr Lys Val Ser Tyr Gly Phe Leu Ser Pro Pro Gln Leu Asn Lys Asn  
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Ser Ser Gly Ile Tyr Ser Glu Ala Leu Leu Thr Thr Asn Ile Val Pro  
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Met Tyr Gln Ser Phe Gln Val Ile Trp Arg Tyr Phe His Asp Thr Leu  
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Leu Arg Lys Tyr Ala Glu Glu Arg Asn Gly Val Asn Val Val Ser Gly  
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Pro Val Phe Asp Phe Asp Tyr Asp Gly Arg Cys Asp Ser Leu Glu Asn  
725 730 735

Leu Arg Gln Lys Arg Arg Val Ile Arg Asn Gln Glu Ile Leu Ile Pro  
740 745 750

Thr His Phe Phe Ile Val Leu Thr Ser Cys Lys Asp Thr Ser Gln Thr  
755 760 765

Pro Leu His Cys Glu Asn Leu Asp Thr Leu Ala Phe Ile Leu Pro His  
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Arg Thr Asp Asn Ser Glu Ser Cys Val His Gly Lys His Asp Ser Ser  
785 790 795 800

Trp Val Glu Glu Leu Met Leu His Arg Ala Arg Ile Thr Asp Val  
805 810 815

Glu His Ile Thr Gly Leu Ser Phe Tyr Gln Gln Arg Lys Glu Pro Val

820

825

830

Ser Asp Ile Leu Lys Leu Lys Thr His Leu Pro Thr Phe Ser Gln Glu  
835 840 845

Asp Arg Ser Gly Ser Gly Gly Ser Met Lys Trp Val Thr Phe Leu Leu  
850 855 860

Leu Leu Phe Val Ser Gly Ser Ala Phe Ser Arg Gly Val Phe Arg Arg  
865 870 875 880

Glu Ala His Lys Ser Glu Ile Ala His Arg Tyr Asn Asp Leu Gly Glu  
885 890 895

Gln His Phe Lys Gly Leu Val Leu Ile Ala Phe Ser Gln Tyr Leu Gln  
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Lys Cys Ser Tyr Asp Glu His Ala Lys Leu Val Gln Glu Val Thr Asp  
915 920 925

Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Ala Asn Cys Asp Lys  
930 935 940

Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Ala Ile Pro Asn Leu  
945 950 955 960

Arg Glu Asn Tyr Gly Glu Leu Ala Asp Cys Cys Thr Lys Gln Glu Pro  
965 970 975

Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Ser Leu  
980 985 990

Pro Pro Phe Glu Arg Pro Glu Ala Glu Ala Met Cys Thr Ser Phe Lys  
995 1000 1005

Glu Asn Pro Thr Thr Phe Met Gly His Tyr Leu His Glu Val Ala  
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Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Tyr Tyr Ala  
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Glu Gln Tyr Asn Glu Ile Leu Thr Gln Cys Cys Ala Glu Ala Asp  
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Lys Glu Ser Cys Leu Thr Pro Lys Leu Asp Gly Val Lys Glu Lys  
1055 1060 1065

Ala Leu Val Ser Ser Val Arg Gln Arg Met Lys Cys Ser Ser Met  
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Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg

1085

1090

1095

Leu	Ser	Gln	Thr	Phe	Pro	Asn	Ala	Asp	Phe	Ala	Glu	Ile	Thr	Lys
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Leu	Ala	Thr	Asp	Leu	Thr	Lys	Val	Asn	Lys	Glu	Cys	Cys	His	Gly
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Asp	Leu	Leu	Glu	Cys	Ala	Asp	Asp	Arg	Ala	Glu	Leu	Ala	Lys	Tyr
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Met	Cys	Glu	Asn	Gln	Ala	Thr	Ile	Ser	Ser	Lys	Leu	Gln	Thr	Cys
1145						1150					1155			
Cys	Asp	Lys	Pro	Leu	Leu	Lys	Lys	Ala	His	Cys	Leu	Ser	Glu	Val
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Glu	His	Asp	Thr	Met	Pro	Ala	Asp	Leu	Pro	Ala	Ile	Ala	Ala	Asp
1175						1180					1185			
Phe	Val	Glu	Asp	Gln	Glu	Val	Cys	Lys	Asn	Tyr	Ala	Glu	Ala	Lys
1190						1195					1200			
Asp	Val	Phe	Leu	Gly	Thr	Phe	Leu	Tyr	Glu	Tyr	Ser	Arg	Arg	His
1205						1210					1215			
Pro	Asp	Tyr	Ser	Val	Ser	Leu	Leu	Leu	Arg	Leu	Ala	Lys	Lys	Tyr
1220						1225					1230			
Glu	Ala	Thr	Leu	Glu	Lys	Cys	Cys	Ala	Glu	Ala	Asn	Pro	Pro	Ala
1235						1240					1245			
Cys	Tyr	Gly	Thr	Val	Leu	Ala	Glu	Phe	Gln	Pro	Leu	Val	Glu	Glu
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Pro	Lys	Asn	Leu	Val	Lys	Thr	Asn	Cys	Asp	Leu	Tyr	Glu	Lys	Leu
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Gly	Glu	Tyr	Gly	Phe	Gln	Asn	Ala	Ile	Leu	Val	Arg	Tyr	Thr	Gln
1280						1285					1290			
Lys	Ala	Pro	Gln	Val	Ser	Thr	Pro	Thr	Leu	Val	Glu	Ala	Ala	Arg
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Asn	Leu	Gly	Arg	Val	Gly	Thr	Lys	Cys	Cys	Thr	Leu	Pro	Glu	Asp
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Gln	Arg	Leu	Pro	Cys	Val	Glu	Asp	Tyr	Leu	Ser	Ala	Ile	Leu	Asn
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Arg	Val	Cys	Leu	Leu	His	Glu	Lys	Thr	Pro	Val	Ser	Glu	His	Val

1340

1345

1350

Thr Lys Cys Cys Ser Gly Ser Leu Val Glu Arg Arg Pro Cys Phe  
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Ser Ala Leu Thr Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Lys  
 1370 1375 1380

Ala Glu Thr Phe Thr Phe His Ser Asp Ile Cys Thr Leu Pro Glu  
 1385 1390 1395

Lys Glu Lys Gln Ile Lys Lys Gln Thr Ala Leu Ala Glu Leu Val  
 1400 1405 1410

Lys His Lys Pro Lys Ala Thr Ala Glu Gln Leu Lys Thr Val Met  
 1415 1420 1425

Asp Asp Phe Ala Gln Phe Leu Asp Thr Cys Cys Lys Ala Ala Asp  
 1430 1435 1440

Lys Asp Thr Cys Phe Ser Thr Glu Gly Pro Asn Leu Val Thr Arg  
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Cys Lys Asp Ala Leu Ala Arg Ser Trp Ser His Pro Gln Phe Glu  
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Lys

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Phe Thr Ala

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 <211> 850  
 <212> PRT  
 <213> Artificial Sequence

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<400> 24

Met Thr Ser Lys Phe Leu Leu Val Ser Phe Ile Leu Ala Ala Leu Ser  
 Page 47



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1              5              10              15
Leu Ser Thr Thr Phe Ser Leu Gl n Pro Ser Cys Al a Lys Gl u Val Lys
      20      25      30
Ser Cys Lys Gly Arg Cys Phe Gl u Arg Thr Phe Ser Asn Cys Arg Cys
      35      40      45
Asp Al a Al a Cys Val Ser Leu Gly Asn Cys Cys Leu Asp Phe Gl n Gl u
      50      55      60
Thr Cys Val Gl u Pro Thr Hi s Ile Trp Thr Cys Asn Lys Phe Arg Cys
      65      70      75      80
Gly Gl u Lys Arg Leu Ser Arg Phe Val Cys Ser Cys Al a Asp Asp Cys
      85      90      95
Lys Thr Hi s Asn Asp Cys Cys Ile Asn Tyr Ser Ser Val Cys Gl n Asp
      100      105      110
Lys Lys Ser Trp Val Gl u Gl u Thr Cys Gl u Ser Ile Asp Thr Pro Gl u
      115      120      125
Cys Pro Al a Gl u Phe Gl u Ser Pro Pro Thr Leu Leu Phe Ser Leu Asp
      130      135      140
Gly Phe Arg Al a Gl u Tyr Leu Hi s Thr Trp Gly Gly Leu Leu Pro Val
      145      150      155      160
Ile Ser Lys Leu Lys Asn Cys Gly Thr Tyr Thr Lys Asn Met Arg Pro
      165      170      175
Met Tyr Pro Thr Lys Thr Phe Pro Asn Hi s Tyr Ser Ile Val Thr Gly
      180      185      190
Leu Tyr Pro Gl u Ser Hi s Gly Ile Ile Asp Asn Lys Met Tyr Asp Pro
      195      200      205
Lys Met Asn Al a Ser Phe Ser Leu Lys Ser Lys Gl u Lys Phe Asn Pro
      210      215      220
Leu Trp Tyr Lys Gly Gl n Pro Ile Trp Val Thr Al a Asn Hi s Gl n Gl u
      225      230      235      240
Val Lys Ser Gly Thr Tyr Phe Trp Pro Gly Ser Asp Val Gl u Ile Asp
      245      250      255
Gly Ile Leu Pro Asp Ile Tyr Lys Val Tyr Asn Gly Ser Val Pro Phe
      260      265      270
Glu Gl u Arg Ile Leu Al a Val Leu Gl u Trp Leu Gl n Leu Pro Ser Hi s

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275

280

285

Glu Arg Pro His Phe Tyr Thr Leu Tyr Leu Glu Glu Pro Asp Ser Ser  
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Gly His Ser His Gly Pro Val Ser Ser Glu Val Ile Lys Ala Leu Gln  
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Lys Val Asp Arg Leu Val Gly Met Leu Met Asp Gly Leu Lys Asp Leu  
 325 330 335

Gly Leu Asp Lys Cys Leu Asn Leu Ile Leu Ile Ser Asp His Gly Met  
 340 345 350

Glu Gln Gly Ser Cys Lys Lys Tyr Val Tyr Leu Asn Lys Tyr Leu Gly  
 355 360 365

Asp Val Asn Asn Val Lys Val Val Tyr Gly Pro Ala Ala Arg Leu Arg  
 370 375 380

Pro Thr Asp Val Pro Glu Thr Tyr Tyr Ser Phe Asn Tyr Glu Ala Leu  
 385 390 395 400

Ala Lys Asn Leu Ser Cys Arg Glu Pro Asn Gln His Phe Arg Pro Tyr  
 405 410 415

Leu Lys Pro Phe Leu Pro Lys Arg Leu His Phe Ala Lys Ser Asp Arg  
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Ile Glu Pro Leu Thr Phe Tyr Leu Asp Pro Gln Trp Gln Leu Ala Leu  
 435 440 445

Asn Pro Ser Glu Arg Lys Tyr Cys Gly Ser Gly Phe His Gly Ser Asp  
 450 455 460

Asn Leu Phe Ser Asn Met Gln Ala Leu Phe Ile Gly Tyr Gly Pro Ala  
 465 470 475 480

Phe Lys His Gly Ala Glu Val Asp Ser Phe Glu Asn Ile Glu Val Tyr  
 485 490 495

Asn Leu Met Cys Asp Leu Leu Gly Leu Ile Pro Ala Pro Asn Asn Gly  
 500 505 510

Ser His Gly Ser Leu Asn His Leu Leu Lys Lys Pro Ile Tyr Asn Pro  
 515 520 525

Ser His Pro Lys Glu Glu Gly Phe Leu Ser Gln Cys Pro Ile Lys Ser  
 530 535 540

Thr Ser Asn Asp Leu Gly Cys Thr Cys Asp Pro Trp Ile Val Pro Ile  
 Page 49

545 550 555 560  
 Lys Asp Phe Glu Lys Gln Leu Asn Leu Thr Thr Glu Asp Val Asp Asp  
 565 570 575  
 Ile Tyr His Met Thr Val Pro Tyr Gly Arg Pro Arg Ile Leu Leu Lys  
 580 585 590  
 Gln His Arg Val Cys Leu Leu Gln Gln Gln Gln Phe Leu Thr Gly Tyr  
 595 600 605  
 Ser Leu Asp Leu Leu Met Pro Leu Trp Ala Ser Tyr Thr Phe Leu Ser  
 610 615 620  
 Asn Asp Gln Phe Ser Arg Asp Asp Phe Ser Asn Cys Leu Tyr Gln Asp  
 625 630 635 640  
 Leu Arg Ile Pro Leu Ser Pro Val His Lys Cys Ser Tyr Tyr Lys Ser  
 645 650 655  
 Asn Ser Lys Leu Ser Tyr Gly Phe Leu Thr Pro Pro Arg Leu Asn Arg  
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 Val Ser Asn His Ile Tyr Ser Glu Ala Leu Leu Thr Ser Asn Ile Val  
 675 680 685  
 Pro Met Tyr Gln Ser Phe Gln Val Ile Trp His Tyr Leu His Asp Thr  
 690 695 700  
 Leu Leu Gln Arg Tyr Ala His Glu Arg Asn Gly Ile Asn Val Val Ser  
 705 710 715 720  
 Gly Pro Val Phe Asp Phe Asp Tyr Asp Gly Arg Tyr Asp Ser Leu Glu  
 725 730 735  
 Ile Leu Lys Gln Asn Ser Arg Val Ile Arg Ser Gln Glu Ile Leu Ile  
 740 745 750  
 Pro Thr His Phe Phe Ile Val Leu Thr Ser Cys Lys Gln Leu Ser Glu  
 755 760 765  
 Thr Pro Leu Glu Cys Ser Ala Leu Glu Ser Ser Ala Tyr Ile Leu Pro  
 770 775 780  
 His Arg Pro Asp Asn Ile Glu Ser Cys Thr His Gly Lys Arg Glu Ser  
 785 790 795 800  
 Ser Trp Val Glu Glu Leu Leu Thr Leu His Arg Ala Arg Val Thr Asp  
 805 810 815  
 Val Glu Leu Ile Thr Gly Leu Ser Phe Tyr Gln Asp Arg Gln Glu Ser  
 Page 50

820

825

830

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Glu Asp  
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Ser Cys Lys Gly Arg Cys Phe Glu Arg Thr Phe Ser Asn Cys Arg Cys  
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Asp Ala Ala Cys Val Ser Leu Gly Asn Cys Cys Leu Asp Phe Gln Glu  
   50                  55                  60

Thr Cys Val Glu Pro Thr His Ile Trp Thr Cys Asn Lys Phe Arg Cys  
   65                  70                  75                  80

Gly Glu Lys Arg Leu Ser Arg Phe Val Cys Ser Cys Ala Asp Asp Cys  
           85                  90                  95

Lys Thr His Asn Asp Cys Cys Ile Asn Tyr Ser Ser Val Cys Gln Asp  
           100                  105                  110

Lys Lys Ser Trp Val Glu Glu Thr Cys Glu Ser Ile Asp Thr Pro Glu  
           115                  120                  125

Cys Pro Ala Glu Phe Glu Ser Pro Pro Thr Leu Leu Phe Ser Leu Asp  
   130                  135                  140

Gly Phe Arg Ala Glu Tyr Leu His Thr Trp Gly Gly Leu Leu Pro Val  
   145                  150                  155                  160

Ile Ser Lys Leu Lys Asn Cys Gly Thr Tyr Thr Lys Asn Met Arg Pro  
           165                  170                  175

Met Tyr Pro Thr Lys Thr Phe Pro Asn His Tyr Ser Ile Val Thr Gly  
           180                  185                  190

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Leu Tyr Pro Glu Ser His Gly Ile Ile Asp Asn Lys Met Tyr Asp Pro  
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 Lys Met Asn Ala Ser Phe Ser Leu Lys Ser Lys Glu Lys Phe Asn Pro  
 210 215 220  
 Leu Trp Tyr Lys Gly Gln Pro Ile Trp Val Thr Ala Asn His Gln Glu  
 225 230 235 240  
 Val Lys Ser Gly Thr Tyr Phe Trp Pro Gly Ser Asp Val Glu Ile Asp  
 245 250 255  
 Gly Ile Leu Pro Asp Ile Tyr Lys Val Tyr Asn Gly Ser Val Pro Phe  
 260 265 270  
 Glu Glu Arg Ile Leu Ala Val Leu Glu Trp Leu Gln Leu Pro Ser His  
 275 280 285  
 Glu Arg Pro His Phe Tyr Thr Leu Tyr Leu Glu Glu Pro Asp Ser Ser  
 290 295 300  
 Gly His Ser His Gly Pro Val Ser Ser Glu Val Ile Lys Ala Leu Gln  
 305 310 315 320  
 Lys Val Asp Arg Leu Val Gly Met Leu Met Asp Gly Leu Lys Asp Leu  
 325 330 335  
 Gly Leu Asp Lys Cys Leu Asn Leu Ile Leu Ile Ser Asp His Gly Met  
 340 345 350  
 Glu Gln Gly Ser Cys Lys Lys Tyr Val Tyr Leu Asn Lys Tyr Leu Gly  
 355 360 365  
 Asp Val Asn Asn Val Lys Val Val Tyr Gly Pro Ala Ala Arg Leu Arg  
 370 375 380  
 Pro Thr Asp Val Pro Glu Thr Tyr Tyr Ser Phe Asn Tyr Glu Ala Leu  
 385 390 395 400  
 Ala Lys Asn Leu Ser Cys Arg Glu Pro Asn Gln His Phe Arg Pro Tyr  
 405 410 415  
 Leu Lys Pro Phe Leu Pro Lys Arg Leu His Phe Ala Lys Ser Asp Arg  
 420 425 430  
 Ile Glu Pro Leu Thr Phe Tyr Leu Asp Pro Gln Trp Gln Leu Ala Leu  
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 485 490 495  
 Asn Leu Met Cys Asp Leu Leu Gly Leu Ile Pro Ala Pro Asn Asn Gly  
 500 505 510  
 Ser His Gly Ser Leu Asn His Leu Leu Lys Lys Pro Ile Tyr Asn Pro  
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 Ser His Pro Lys Glu Glu Gly Phe Leu Ser Gln Cys Pro Ile Lys Ser  
 530 535 540  
 Thr Ser Asn Asp Leu Gly Cys Thr Cys Asp Pro Trp Ile Val Pro Ile  
 545 550 555 560  
 Lys Asp Phe Glu Lys Gln Leu Asn Leu Thr Thr Glu Asp Val Asp Asp  
 565 570 575  
 Ile Tyr His Met Thr Val Pro Tyr Gly Arg Pro Arg Ile Leu Leu Lys  
 580 585 590  
 Gln His Arg Val Cys Leu Leu Gln Gln Gln Gln Phe Leu Thr Gly Tyr  
 595 600 605  
 Ser Leu Asp Leu Leu Met Pro Leu Trp Ala Ser Tyr Thr Phe Leu Ser  
 610 615 620  
 Asn Asp Gln Phe Ser Arg Asp Asp Phe Ser Asn Cys Leu Tyr Gln Asp  
 625 630 635 640  
 Leu Arg Ile Pro Leu Ser Pro Val His Lys Cys Ser Tyr Tyr Lys Ser  
 645 650 655  
 Asn Ser Lys Leu Ser Tyr Gly Phe Leu Thr Pro Pro Arg Leu Asn Arg  
 660 665 670  
 Val Ser Asn His Ile Tyr Ser Glu Ala Leu Leu Thr Ser Asn Ile Val  
 675 680 685  
 Pro Met Tyr Gln Ser Phe Gln Val Ile Trp His Tyr Leu His Asp Thr  
 690 695 700  
 Leu Leu Gln Arg Tyr Ala His Glu Arg Asn Gly Ile Asn Val Val Ser  
 705 710 715 720  
 Gly Pro Val Phe Asp Phe Asp Tyr Asp Gly Arg Tyr Asp Ser Leu Glu  
 725 730 735

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Ile Leu Lys Gln Asn Ser Arg Val Ile Arg Ser Gln Glu Ile Leu Ile  
740 745 750

Pro Thr His Phe Phe Ile Val Leu Thr Ser Cys Lys Gln Leu Ser Glu  
755 760 765

Thr Pro Leu Glu Cys Ser Ala Leu Glu Ser Ser Ala Tyr Ile Leu Pro  
770 775 780

His Arg Pro Asp Asn Ile Glu Ser Cys Thr His Gly Lys Arg Glu Ser  
785 790 795 800

Ser Trp Val Glu Glu Leu Leu Thr Leu His Arg Ala Arg Val Thr Asp  
805 810 815

Val Glu Leu Ile Thr Gly Leu Ser Phe Tyr Gln Asp Arg Gln Glu Ser  
820 825 830

Val Ser Glu Leu Leu Arg Leu Lys Thr His Leu Pro Ile Phe Ser Gln  
835 840 845

Glu Asp Gly Gly Ser Gly Gly Ser Met Lys Trp Val Thr Phe Leu Leu  
850 855 860

Leu Leu Phe Val Ser Gly Ser Ala Phe Ser Arg Gly Val Phe Arg Arg  
865 870 875 880

Glu Ala His Lys Ser Glu Ile Ala His Arg Tyr Asn Asp Leu Gly Glu  
885 890 895

Gln His Phe Lys Gly Leu Val Leu Ile Ala Phe Ser Gln Tyr Leu Gln  
900 905 910

Lys Cys Ser Tyr Asp Glu His Ala Lys Leu Val Gln Glu Val Thr Asp  
915 920 925

Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Ala Asn Cys Asp Lys  
930 935 940

Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Ala Ile Pro Asn Leu  
945 950 955 960

Arg Glu Asn Tyr Gly Glu Leu Ala Asp Cys Cys Thr Lys Gln Glu Pro  
965 970 975

Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Ser Leu  
980 985 990

Pro Pro Phe Glu Arg Pro Glu Ala Glu Ala Met Cys Thr Ser Phe Lys  
995 1000 1005

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	1025					1030					1035			
Glu	Gln	Tyr	Asn	Glu	Ile	Leu	Thr	Gln	Cys	Cys	Ala	Glu	Ala	Asp
	1040					1045					1050			
Lys	Glu	Ser	Cys	Leu	Thr	Pro	Lys	Leu	Asp	Gly	Val	Lys	Glu	Lys
	1055					1060					1065			
Ala	Leu	Val	Ser	Ser	Val	Arg	Gln	Arg	Met	Lys	Cys	Ser	Ser	Met
	1070					1075					1080			
Gln	Lys	Phe	Gly	Glu	Arg	Ala	Phe	Lys	Ala	Trp	Ala	Val	Ala	Arg
	1085					1090					1095			
Leu	Ser	Gln	Thr	Phe	Pro	Asn	Ala	Asp	Phe	Ala	Glu	Ile	Thr	Lys
	1100					1105					1110			
Leu	Ala	Thr	Asp	Leu	Thr	Lys	Val	Asn	Lys	Glu	Cys	Cys	His	Gly
	1115					1120					1125			
Asp	Leu	Leu	Glu	Cys	Ala	Asp	Asp	Arg	Ala	Glu	Leu	Ala	Lys	Tyr
	1130					1135					1140			
Met	Cys	Glu	Asn	Gln	Ala	Thr	Ile	Ser	Ser	Lys	Leu	Gln	Thr	Cys
	1145					1150					1155			
Cys	Asp	Lys	Pro	Leu	Leu	Lys	Lys	Ala	His	Cys	Leu	Ser	Glu	Val
	1160					1165					1170			
Glu	His	Asp	Thr	Met	Pro	Ala	Asp	Leu	Pro	Ala	Ile	Ala	Ala	Asp
	1175					1180					1185			
Phe	Val	Glu	Asp	Gln	Glu	Val	Cys	Lys	Asn	Tyr	Ala	Glu	Ala	Lys
	1190					1195					1200			
Asp	Val	Phe	Leu	Gly	Thr	Phe	Leu	Tyr	Glu	Tyr	Ser	Arg	Arg	His
	1205					1210					1215			
Pro	Asp	Tyr	Ser	Val	Ser	Leu	Leu	Leu	Arg	Leu	Ala	Lys	Lys	Tyr
	1220					1225					1230			
Glu	Ala	Thr	Leu	Glu	Lys	Cys	Cys	Ala	Glu	Ala	Asn	Pro	Pro	Ala
	1235					1240					1245			
Cys	Tyr	Gly	Thr	Val	Leu	Ala	Glu	Phe	Gln	Pro	Leu	Val	Glu	Glu
	1250					1255					1260			



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Pro Lys Asn Leu Val Lys Thr Asn Cys Asp Leu Tyr Glu Lys Leu  
1265 1270 1275

Gly Glu Tyr Gly Phe Gln Asn Ala Ile Leu Val Arg Tyr Thr Gln  
1280 1285 1290

Lys Ala Pro Gln Val Ser Thr Pro Thr Leu Val Glu Ala Ala Arg  
1295 1300 1305

Asn Leu Gly Arg Val Gly Thr Lys Cys Cys Thr Leu Pro Glu Asp  
1310 1315 1320

Gln Arg Leu Pro Cys Val Glu Asp Tyr Leu Ser Ala Ile Leu Asn  
1325 1330 1335

Arg Val Cys Leu Leu His Glu Lys Thr Pro Val Ser Glu His Val  
1340 1345 1350

Thr Lys Cys Cys Ser Gly Ser Leu Val Glu Arg Arg Pro Cys Phe  
1355 1360 1365

Ser Ala Leu Thr Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Lys  
1370 1375 1380

Ala Glu Thr Phe Thr Phe His Ser Asp Ile Cys Thr Leu Pro Glu  
1385 1390 1395

Lys Glu Lys Gln Ile Lys Lys Gln Thr Ala Leu Ala Glu Leu Val  
1400 1405 1410

Lys His Lys Pro Lys Ala Thr Ala Glu Gln Leu Lys Thr Val Met  
1415 1420 1425

Asp Asp Phe Ala Gln Phe Leu Asp Thr Cys Cys Lys Ala Ala Asp  
1430 1435 1440

Lys Asp Thr Cys Phe Ser Thr Glu Gly Pro Asn Leu Val Thr Arg  
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Cys Lys Asp Ala Leu Ala Arg Ser Trp Ser His Pro Gln Phe Glu  
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 35 40 45  
 Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val  
 50 55 60  
 His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr  
 65 70 75 80  
 Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly  
 85 90 95  
 Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile  
 100 105 110  
 Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val  
 115 120 125  
 Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser  
 130 135 140  
 Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu  
 145 150 155 160  
 Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro  
 165 170 175  
 Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val  
 180 185 190  
 Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met  
 195 200 205  
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 Pro Gly Lys  
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&lt;212&gt; PRT

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      20      25      30

His  Arg  Tyr  Asn  Asp  Leu  Gly  Glu  Gln  His  Phe  Lys  Gly  Leu  Val  Leu
      35      40      45

Ile  Ala  Phe  Ser  Gln  Tyr  Leu  Gln  Lys  Cys  Ser  Tyr  Asp  Glu  His  Ala
      50      55      60

Lys  Leu  Val  Gln  Glu  Val  Thr  Asp  Phe  Ala  Lys  Thr  Cys  Val  Ala  Asp
65      70      75      80

Glu  Ser  Ala  Ala  Asn  Cys  Asp  Lys  Ser  Leu  His  Thr  Leu  Phe  Gly  Asp
      85      90      95

Lys  Leu  Cys  Ala  Ile  Pro  Asn  Leu  Arg  Glu  Asn  Tyr  Gly  Glu  Leu  Ala
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Asp  Cys  Cys  Thr  Lys  Gln  Glu  Pro  Glu  Arg  Asn  Glu  Cys  Phe  Leu  Gln
      115     120     125

His  Lys  Asp  Asp  Asn  Pro  Ser  Leu  Pro  Pro  Phe  Glu  Arg  Pro  Glu  Ala
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Glu  Ala  Met  Cys  Thr  Ser  Phe  Lys  Glu  Asn  Pro  Thr  Thr  Phe  Met  Gly
145     150     155     160

His  Tyr  Leu  His  Glu  Val  Ala  Arg  Arg  His  Pro  Tyr  Phe  Tyr  Ala  Pro
      165     170     175

Glu  Leu  Leu  Tyr  Tyr  Ala  Glu  Gln  Tyr  Asn  Glu  Ile  Leu  Thr  Gln  Cys
      180     185     190

Cys  Ala  Glu  Ala  Asp  Lys  Glu  Ser  Cys  Leu  Thr  Pro  Lys  Leu  Asp  Gly
      195     200     205

Val  Lys  Glu  Lys  Ala  Leu  Val  Ser  Ser  Val  Arg  Gln  Arg  Met  Lys  Cys
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Ser  Ser  Met  Gln  Lys  Phe  Gly  Glu  Arg  Ala  Phe  Lys  Ala  Trp  Ala  Val
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Ala  Arg  Leu  Ser  Gln  Thr  Phe  Pro  Asn  Ala  Asp  Phe  Ala  Glu  Ile  Thr
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 Cys Glu<sub>290</sub> Asn Gln Ala Thr Ile<sub>295</sub> Ser Ser Lys Leu Gln<sub>300</sub> Thr Cys Cys Asp  
 Lys<sub>305</sub> Pro Leu Leu Lys Lys<sub>310</sub> Ala His Cys Leu Ser<sub>315</sub> Glu Val Glu His Asp<sub>320</sub>  
 Thr Met Pro Ala Asp<sub>325</sub> Leu Pro Ala Ile<sub>330</sub> Ala Ala Asp Phe Val Glu<sub>335</sub> Asp  
 Gln Glu Val Cys<sub>340</sub> Lys Asn Tyr Ala Glu<sub>345</sub> Ala Lys Asp Val Phe<sub>350</sub> Leu Gly  
 Thr Phe Leu<sub>355</sub> Tyr Glu Tyr Ser Arg<sub>360</sub> Arg His Pro Asp Tyr<sub>365</sub> Ser Val Ser  
 Leu Leu<sub>370</sub> Leu Arg Leu Ala Lys<sub>375</sub> Lys Tyr Glu Ala Thr<sub>380</sub> Leu Glu Lys Cys  
 Cys<sub>385</sub> Ala Glu Ala Asn Pro<sub>390</sub> Pro Ala Cys Tyr Gly<sub>395</sub> Thr Val Leu Ala Glu<sub>400</sub>  
 Phe Gln Pro Leu Val<sub>405</sub> Glu Glu Pro Lys Asn<sub>410</sub> Leu Val Lys Thr Asn<sub>415</sub> Cys  
 Asp Leu Tyr Glu<sub>420</sub> Lys Leu Gly Glu Tyr<sub>425</sub> Gly Phe Gln Asn Ala<sub>430</sub> Ile Leu  
 Val Arg Tyr<sub>435</sub> Thr Gln Lys Ala Pro<sub>440</sub> Gln Val Ser Thr Pro<sub>445</sub> Thr Leu Val  
 Glu Ala<sub>450</sub> Ala Arg Asn Leu Gly<sub>455</sub> Arg Val Gly Thr Lys<sub>460</sub> Cys Cys Thr Leu  
 Pro<sub>465</sub> Glu Asp Gln Arg Leu<sub>470</sub> Pro Cys Val Glu Asp<sub>475</sub> Tyr Leu Ser Ala Ile<sub>480</sub>  
 Leu Asn Arg Val Cys<sub>485</sub> Leu Leu His Glu Lys<sub>490</sub> Thr Pro Val Ser Glu<sub>495</sub> His  
 Val Thr Lys Cys<sub>500</sub> Cys Ser Gly Ser Leu<sub>505</sub> Val Glu Arg Arg Pro<sub>510</sub> Cys Phe  
 Ser Ala Leu<sub>515</sub> Thr Val Asp Glu Thr<sub>520</sub> Tyr Val Pro Lys Glu<sub>525</sub> Phe Lys Ala

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Glu Thr Phe Thr Phe His Ser Asp Ile Cys Thr Leu Pro Glu Lys Glu  
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Lys Gln Ile Lys Lys Gln Thr Ala Leu Ala Glu Leu Val Lys His Lys  
545 550 555 560

Pro Lys Ala Thr Ala Glu Gln Leu Lys Thr Val Met Asp Asp Phe Ala  
565 570 575

Gln Phe Leu Asp Thr Cys Cys Lys Ala Ala Asp Lys Asp Thr Cys Phe  
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Ser Thr Glu Gly Pro Asn Leu Val Thr Arg Cys Lys Asp Ala Leu Ala  
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Arg Ser Trp Ser His Pro Gln Phe Glu Lys  
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