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(54) Title: COMPOSITIONS FOR TREATING ECTOPIC CALCIFICATION DISORDERS, AND METHODS USING SAME

(57) Abstract: The present invention includes compositions and methods for treating disease and disorders associated with pathological calcification or pathological ossification.

TITLE OF THE INVENTION

Compositions for Treating Ectopic Calcification Disorders, 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 Application No. 62/257,883, filed November 20, 2015, which application is hereby incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

Calcification is the accumulation of calcium salts in a body tissue. It normally occurs during formation of bone, but calcium can also be deposited abnormally in soft tissues such as arteries, cartilage and heart valves. Vascular calcification frequently develops in patients with atherosclerosis, stroke, valvular disease and varicosis. Advanced age and metabolic disorders, including diabetes mellitus are contributing factors.

Ossification refers to the process of bone tissue formation or bone remodeling orchestrated by the osteoblasts. Ossification allows bones to form while a fetus is still in the womb, and also converts various types of connective tissue into bone. The two main processes of ossification are intra-membranous ossification and intra-cartilaginous ossification, which differ based on the area of the body in which the cartilage is located.

Abnormalities in the levels of calcification and ossification lead to a spectrum of diseases, a few examples of such as general arterial calcification of infancy (GACI), idiopathic infantile arterial calcification (IIAC), pseudoxanthoma elasticum (PXE), ossification of posterior longitudinal ligament (OPLL), medial wall vascular calcification (MWVC), autosomal recessive hypophosphatemia rickets type-2 (ARHR2), end state renal disease (ESRD), chronic kidney disease- bone/mineral disorder (CKD-MBD), X-linked hypophosphatemia (XLH), age related osteopenia, calcific uremic arteriolopathy (CUA) and hypophosphatemic rickets.

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 (such as etridonate) 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, and the ineffectiveness of bisphosphonates to prevent mortality in some patients even when instituted early. Further, 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. Interestingly, serum PPi levels appear to be significantly depleted in GACI patients.

Kidneys are integral to maintenance of normal bone and mineral metabolism, including excretion of phosphate. 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 (NKFK/DOQI). The prevalence of 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. Importantly, 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 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. Deposition of calcium into the small vessels of the skin causes an inflammatory vasculitis called calciphylaxis, which 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. It is thus important to regulate the amount of pyrophosphate in the system and reduce the occurrence of calciphylaxis in patients.

CUA is a fatal disease seen in patients with CKD on dialysis. Calcification of small arteries leads to tissue/skin ischemia, 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, exhibiting low levels of PPi

and high levels of fibroblast growth factor 23 (FGF23). In ESRD patients requiring dialysis, this calcification process is further accelerated, with an average life-expectancy of 5-6 years.

PXE is a heritable disorder characterized by mineralization of elastic fibers in skin, arteries and the retina, which results 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. 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. 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. 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.

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 hypercakiuria (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.

There is 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. Such compositions and methods should reduce the level of calcification and increasing PPi plasma levels in individuals who exhibit lower than normal plasma PPi levels. The present invention fulfills this need.

BRIEF DESCRIPTION OF THE DRAWINGS

The following detailed description of exemplary embodiments of the invention will be better understood when read in conjunction with the appended drawings. For the purpose of illustrating the invention, there are shown in the drawings exemplary 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-1C comprise graphs illustrating studies of human ENPP3 steady state ATP hydrolysis activity. FIG. 1A illustrates time courses of AMP product formation after addition of 50 nM hNPP3 with (from bottom to top) 0.98, 1.95, 3.9, 7.8, 15.6, 31.3, 62.5, 125, 250 and 500 μM ATP. The enzyme reaction was quenched by equal volume of 3 M formic acid at different times, and the reaction product AMP was quantified by HPLC analysis with an AMP standard curve. The smooth line though data points are best fits to a non-linear enzyme kinetic model with product inhibition and substrate depletion. FIG. 1B illustrates steady state ATPase cycling rate comparison. ENPP3 substrate concentration dependence of initial steady state enzyme cycling rate was compared with the previously measured values for human ENPP1. ATPase cycling reaction of both 50 nM hNPP3 and hNPP1 totally depleted ATP substrate in 1 minute for 0.98, 1.95 and 3.9 μM ATP, and thus these three rates were omitted from the plot because their rates could not be accurately determined. The hNPP3 steady state ATPase reaction reached the maximum (k_{cat}) of 2.59 (±0.04) s^{-1} $enzyme^{-1}$, from the weighted average of the measured rates with 7.8, 15.6, 31.3, 62.5, 125 μM substrate concentration, seeming slower than that for hNPP1 3.46 (±0.44) s^{-1} $enzyme^{-1}$. The K_M can be estimated < 8 μM. At substrate [ATP] > 125 μM, hNPP3 ATPase cycling rate gradually decreased. FIG. 1C illustrates substrate concentration dependent η. The decreasing η value with substrate concentration for both enzymes indicates that substrate depletion contributes to the non-linearity in the enzyme reaction time courses much more than product inhibition at the lower initial substrate concentration. The striking similarity with human ENPP3 vs. human ENPP1 η indicates the two enzymes have similar reaction rate and product inhibition. hNPP1 has slightly faster rate and thus depletes substrate ATP slightly faster than hNPP3 at low substrate concentration.

FIG. 2 illustrates a non-limiting purification profile of NPP3 fusion protein through a Coomasie stained sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel, wherein the purified NPP3 protein is shown in relation to certain size markers.

FIG. 3 illustrates a non-limiting plasmid construct map of human NPP121-NPP3-Fc in the plasmid, cloned using the indicated restriction endonuclease sites.

FIG. 4 illustrates a non-limiting plasmid construct map of human NPP121-NPP3-Fc in the plasmid pcDNA3, cloned using IN-FUSION® technology..

FIG. 5 illustrates a non-limiting plasmid construct map of human NPP121-NPP3-Albumin in the plasmid pcDNA3.

BRIEF SUMMARY OF THE INVENTION

The invention provides an isolated polypeptide, or a pharmaceutical 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 vascular calcification in a subject with low plasma pyrophosphate (PPi) or high serum phosphate (Pi). The invention further provides a method of treating of a subject having NPP1 deficiency or NPP1-associated disease. The invention further provides a kit comprising at least one isolated polypeptide of the invention and instructions reciting the use of the at least one polypeptide for treating a disease or disorder associated with pathological calcification or pathological ossification in a subject in need thereof, optionally further comprising an applicator.

In certain embodiments, the polypeptide of the invention has formula (I): EXPORT-PROTEIN-Z-DOMAIN-X-Y (I), wherein in (I): EXPORT is absent, or a signal export sequence or a biologically active fragment thereof; PROTEIN is the extracellular domain of ENPP3 (SEQ ID NO:1) or a biologically active fragment thereof; DOMAIN is selected from the group consisting of a human IgG Fc domain and human albumin domain; 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: $(DSS)_n$ (SEQ ID NO:6), $(ESS)_n$ (SEQ ID NO:7), $(RQQ)_n$ (SEQ ID NO:8), $(KR)_n$ (SEQ ID NO:9), R_n (SEQ ID NO:10), $(KR)_n$ (SEQ ID NO:11), DSSSEEKFLRRIGRFG (SEQ ID NO:12), EEEEEEEPRGDT (SEQ ID NO:13), APWHLSSQYSRT (SEQ ID NO:14), STLPIPHEFSRE (SEQ ID NO:15), VTKHLNQISQSY (SEQ ID NO:16), E_n (SEQ ID NO:17), and D_n (SEQ ID NO:18), wherein

each occurrence of n is independently an integer ranging from 1 to 20.

In certain embodiments, the nuclease domain of the PROTEIN or mutant thereof is absent. In other embodiments, EXPORT is absent or selected from the group consisting of SEQ ID NOs:2-5. In yet other embodiments, X is selected from the group consisting of: absent, a polypeptide consisting of 20 amino acids, a polypeptide consisting of 19 amino acids, a polypeptide consisting of 18 amino acids, a polypeptide consisting of 17 amino acids, a polypeptide consisting of 16 amino acids, a polypeptide consisting of 15 amino acids, a polypeptide consisting of 14 amino acids, a polypeptide consisting of 13 amino acids, a polypeptide consisting of 12 amino acids, a polypeptide consisting of 11 amino acids, a polypeptide consisting of 10 amino acids, a polypeptide consisting of 9 amino acids, a polypeptide consisting of 8 amino acids, a polypeptide consisting of 7 amino acids, a polypeptide consisting of 6 amino acids, a polypeptide consisting of 5 amino acids, a polypeptide consisting of 4 amino acids, a polypeptide consisting of 3 amino acids, a polypeptide consisting of 2 amino acids, and a polypeptide consisting of 1 amino acid. In yet other embodiments, Z is selected from the group consisting of: absent, a polypeptide consisting of 20 amino acids, a polypeptide consisting of 19 amino acids, a polypeptide consisting of 18 amino acids, a polypeptide consisting of 17 amino acids, a polypeptide consisting of 16 amino acids, a polypeptide consisting of 15 amino acids, a polypeptide consisting of 14 amino acids, a polypeptide consisting of 13 amino acids, a polypeptide consisting of 12 amino acids, a polypeptide consisting of 11 amino acids, a polypeptide consisting of 10 amino acids, a polypeptide consisting of 9 amino acids, a polypeptide consisting of 8 amino acids, a polypeptide consisting of 7 amino acids, a polypeptide consisting of 6 amino acids, a polypeptide consisting of 5 amino acids, a polypeptide consisting of 4 amino acids, a polypeptide consisting of 3 amino acids, a polypeptide consisting of 2 amino acids, and a polypeptide consisting of 1 amino acid.

In certain embodiments, DOMAIN is a human IgG Fc domain selected from the group consisting of IgG1, IgG2, IgG3 and IgG4. In other embodiments, the polypeptide is selected from the group consisting of SEQ ID NOs:19, 21 and 22. In yet other embodiments, DOMAIN is a human albumin domain. In yet other embodiments, the polypeptide is selected from the group consisting of SEQ ID NOs:24, 25 and 26.

In certain embodiments, the polypeptide comprises a soluble region of NPP3 and lacks a transmembrane domain and a signal peptide, or a fusion protein thereof, wherein the polypeptide reduces cellular calcification when administered to a subject suffering from diseases of calcification and ossification. In other embodiments, the polypeptide comprises a

soluble region of NPP3 and lacks a transmembrane domain and a signal peptide, wherein the polypeptide reduces cellular calcification when administered to a subject suffering from diseases of calcification and ossification.

In certain embodiments, the polypeptide comprises the extracellular domain of ENPP3 (SEQ ID NO:1) or a biologically active fragment thereof. In other embodiments, the polypeptide consists essentially of SEQ ID NO:1 or a biologically active fragment thereof. In yet other embodiments, the polypeptide consists of SEQ ID NO:1 or a biologically active fragment thereof.

In certain embodiments, the soluble ENPP3 fragment or fusion protein thereof comprises the extracellular domain of ENPP3 (SEQ ID NO:1) or a biologically active fragment thereof. In other embodiments, the soluble ENPP3 fragment consists essentially of SEQ ID NO:1 or a biologically active fragment thereof. In yet other embodiments, the soluble ENPP3 fragment consists of SEQ ID NO:1 or a biologically active fragment thereof. In yet other embodiments, the soluble ENPP3 fragment or fusion protein thereof lacks a transmembrane domain and a signal peptide.

In certain embodiments, the method comprises administering to the subject a therapeutically effective amount of at least one polypeptide the invention, or a pharmaceutical salt or solvate thereof. In other embodiments, the method comprises administering to the subject a therapeutically effective amount of an isolated recombinant human soluble ENPP3 fragment or fusion protein thereof.

In certain embodiments, the disease or disorder comprises at least one selected from the group consisting of GACI, IIAC, PXE, OPLL, hypophosphatemic rickets, osteoarthritis, calcification of atherosclerotic plaques, hereditary and non-hereditary forms of osteoarthritis, ankylosing spondylitis, hardening of the arteries occurring with aging, and calciphylaxis resulting from end stage renal disease (or mineral bone disorder of chronic kidney disease).

In certain embodiments, the disease or disorder comprises at least one selected from a group consisting of GACI, IIAC, PXE, OPLL, MWVC, ARHR2, ESRD, CKD-MBD, XLH, age related osteopenia, CUA and hypophosphatemic rickets.

In certain embodiments, the disease or disorder is GACI. In other embodiments, the disease or disorder is IIAC. In yet other embodiments, the disease or disorder is PXE. In yet other embodiments, the disease or disorder is OPLL. In yet other embodiments, the disease or disorder is hypophosphatemic rickets. In yet other embodiments, the disease or disorder is osteoarthritis. In yet other embodiments, the disease

or disorder is calcification of atherosclerotic plaques. In yet other embodiments, the disease or disorder is hereditary and non-hereditary forms of osteoarthritis. In yet other embodiments, the disease or disorder is ankylosing spondylitis. In yet other embodiments, the disease or disorder is hardening of the arteries occurring with aging. In yet other embodiments, the disease or disorder is calciphylaxis resulting from end stage renal disease (or mineral bone disorder of chronic kidney disease). In yet other embodiments, the disease or disorder is age related osteopenia. In yet other embodiments, the disease or disorder is CUA. In yet other embodiments, the disease or disorder is MWVC. In yet other embodiments, the disease or disorder is ARHR2. In yet other embodiments, the disease or disorder is ESRD.

In certain embodiments, the administered amount raises the level of plasma PPi in the subject to at least about 800 nM. In other embodiments, the administered amount raises the level of plasma PPi in the subject to at least about 1 μM. In yet other embodiments, the administered amount raises the level of plasma PPi in the subject to at least about 1.5 μM.

In certain embodiments, the at least one polypeptide is administered acutely or chronically to the subject. In other embodiments, the at least one polypeptide is administered locally, regionally or systemically to the subject. In yet other embodiments, the subject is a mammal. In yet other embodiments, the mammal is human.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the discovery that ENPP3 (also known as NPP3), which is a member of the ectonucleotide pyrophosphatase/phosphodiesterase (ENPP or NPP) family of enzymes, has potent ATP hydrolase activity. ENPP3 hydrolyzes ATP to AMP and PPi, as demonstrated herein.

In certain aspects, the present invention provides compositions, such as but not limited to fusion proteins, that elevate plasma PPi in physiologic states where plasma PPi is low (as determined, for example, by a medical professional or by consulting of a medical document or manual), placing the individual at risk of morbidity associated with low PPi states. In certain embodiments, these physiologic states are recognized disease conditions such as GACI, PXE, Hutchinson Gilford Progeria Syndrome, chronic kidney disease (CKD), X-linked hypophosphatemia, sickle cell anemia, and end stage renal disease. In other embodiments, these physiologic states occur in non-disease states, such as in elderly adults who are afflicted with chronic ailments known to occur in all aging adults such as "hardening of the arteries" and osteopenia.

In certain embodiments, low plasma PPi is defined as plasma PPi concentration lower than about 1.5 μM. These disease states may or may not be accompanied by pathologic calcification of the arteries and/or soft tissues, medial vascular wall calcifications, strokes or cerebrovascular accidents, decreased pulse wave velocity, calcifications of the soft tissues such as the skin, calcifications of the Bruchs membrane in the eye, calcifications of soft tissues surrounding tendons also known as entheses, calcifications of ligaments in the spine such as the posterior longitudinal ligament, and disease of ossification such as Rickets. In other embodiments, the invention contemplates treatment of low PPi physiologic states via administration of the fusion proteins described herein.

In other aspects, the compositions and methods of the invention can be used to treat disease states known to occur in conditions where the expression or the activity of the enzyme ENPP1 is reduced. These recognized disease states include, in non-limited manner, osteoarthritis, GACI, and ARHR2. These states may also occur in other physiologic states in which ENPP1 protein levels are reduced, such as in individuals who have a common polymporphism in the ENPP1 coding region in which a Q residue is substituted for a K reside at position 121 of the secreted protein (or position 173 of the full length protein) (Eller, *et al.*, 2008, Nephrol. Dial. Transplant. 23(1):321-7; Flanagan, *et al.*, 2013, Blood 121(16):3237-45).

As demonstrated herein, the products of ATP hydrolysis by ENPP3, and the corresponding enzymatic constants, were analyzed in order to study the enzymatic activity of this enzyme. ENPP3 was found to be a potent ATP hydrolase, capable of generating PPi and AMP from ATP. In certain embodiments, ENPP3 has an ATP hydrolase activity that is comparable to that of ENPP1. As demonstrated herein, ENPP3 catalyzes the hydrolysis of ATP to PPi with nearly the same Michaelis-Menton kinetics as ENPP1, which is another member of the ENPP family of enzymes. In certain embodiments, soluble fusion constructs of ENPP3, including albumin fusion constructs thereof and/or IgG Fc domain constructs thereof, are efficacious in treating diseases of ectopic calcification. In yet other embodiments, the constructs described herein are efficacious in treating and/or preventing disorders of ectopic vascular calcification.

In one aspect, NPP3 is poorly exported to the cell surface. In certain embodiments, soluble ENPP3 protein is constructed by replacing the signal sequence of NPP3 with the native signal sequence of other ENPPs. In other embodiments, soluble ENPP3 constructs are prepared by using the signal export signal sequence of other ENPP enzymes, such as but not limited to ENPP7 and/or ENPP5. In yet other embodiments,

soluble ENPP3 constructs are prepared by using a signal sequence comprised of a combination of the signal sequences of ENPP1 and ENPP2 ("ENPP1-2-1" hereinafter). In yet other embodiments, signal sequences of any other known proteins may be used to target the extracellular domain of ENPP3 for secretion as well, such as but not limited to the signal sequence of the immunoglobulin kappa and lambda light chain proteins. Further, the invention should not be construed to be limited to the constructs described herein, but also includes constructs comprising any enzymatically active truncation of the ENPP3 extracellular domain.

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, Idiopathic Infantile Arterial Calcification (IIAC), Ossification of the Posterior Longitudinal Ligament (OPLL), hypophosphatemic rickets, osteoarthritis, calcification of atherosclerotic plaques, Pseudoxanthoma elasticum (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.

Definitions

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, the preferred methods and materials are described.

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.

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.

"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 ±20% or ±10%, more preferably ±5%, even more preferably ±1%, and still more preferably ±0.1% from the specified value, as such variations are appropriate to perform the disclosed methods.

As used herein, the term "ADHR" refers to autosomal dominant hypophosphatemic rickets.

As used herein, the term "albumin" refers to the blood plasma protein that is produced in the liver and forms a large proportion of all plasma protein. In certain embodiments, albumin refers to human serum albumin. Usage of other albumins such as bovine serum albumin, equine serum album and porcine serum albumin are also contemplated within the invention.

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).

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.

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

As used herein, the term "ARHR2" refers to autosomal recessive hypophosphatemic rickets type-2.

As used herein, the term "CKD" refers to chronic kidney disease.

As used herein, the term "CKD-MBD" refers to chronic kidney disease-bone/ mineral disorder.

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.

As used herein, the term "composition" or "pharmaceutical 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 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 substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine.

As used herein, the term "CUA" refers to calcific uremic arteriolopathy.

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.

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 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 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 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 "ESRD" refers to end-stage renal disease.

As used herein, the term "Fc" refers to a human IgG Fc domain. Subtypes of IgG such as IgG1, IgG2, IgG3, and IgG4 are all being contemplated for usage as Fc domains.

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).

As used herein, the term "HBD" refers to hypophosphatemic bone disease.

As used herein, the term "HHRH" refers to hereditary hypophosphatemic rickets with hypercakiuria.

"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, the term "IIAC" refers to idiopathic infantile arterial calcification.

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.

As used herein, the term "immunoglobulin" or "Ig" is defined as a class of proteins that 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 15 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. Alternatively, the kit comprises an applicator that can be used to administer the nucleic acid, peptide, and/or compound of the invention to the subject. The application may be for example a drop dispenser, a bottle, a pill dispenser, a syringe and so forth.

"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 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 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 gene encoding additional polypeptide sequence.

As used herein, the term "MWVC" refers to medial wall vascular calcification.

As used herein, the term "NPP" 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 polymer or oligomer of pyrimidine and purine bases, preferably cytosine, thymine, and uracil, and adenine and guanine, respectively (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 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.

An "oligonucleotide" or "polynucleotide" is a nucleic acid ranging from at least 2, preferably 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. 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 "OPLL" refers to ossification of posterior longitudinal ligament.

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. Exemplarily, the patient, individual or subject is human.

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. "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-hydroxyethane sulfonic, p-toluenesulfonic, sulfanilic, cyclohexylaminosulfonic, stearic, alginic, β-hydroxy butyric, 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, chloroprocaine, 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, the term "plasma pyrophosphate levels" or "plasma PPi" refers to the amount of pyrophosphate (PPi) present in plasma of animals. In certain embodiments, animals include mammals, such as but not limited to rat, mouse, cat, dog, human, cow and horse. In certain embodiments, PPi is measured in plasma rather than

serum, because of its release from platelets. There are several non-limiting ways to measure PPi, one of which is by enzymatic assay using uridine-diphosphoglucose (UDPG) pyrophosphorylase as described by Lust and Seegmiller (Lust, *et al.*, 1976, Clin. Chim. Acta 66:241-249; Cheung & Suhadolnik, 1977, Anal. Biochem. 83:61-63) with modifications. Typically healthy individuals exhibit a mean plasma level of about 3.0 μM. The levels of plasma PPi in subjects with aging and or with diseases of calcification or ossification are much lower than the normal levels. In certain embodiments, subjects exhibit a low plasma PPi level of about 1.5 μm. In other embodiments, for subjects with diseases of calcification the plasma PPi levels are about 500 nM, about 600 nM, about 700 nM, about 800 nM, about 900 nM, about 1 μM, about 1.1 μM, about 1.2 μM, about 1.3 μM, about 1.4 μM, about 1.5 μM, about 1.6 μM, about 1.7 μM, about 1.8 μM, about 1.9 μM, about 2 μM, about 2.2 μM, about 2.4 μM, and/or about 2.6 μM. In yet other embodiments, for subjects with diseases of calcification the plasma PPi levels range from about 500 nM to about 2.8 μM, about 600 nM to about 2.8 μM, about 700 nM to about 2.8 μM, about 800 nM to about 2.8 μM, about 900 nM to about 2.8 μM, about 1 μM to about 2.8 μM, about 1.1 μM to about 2.8 μM, about 1.2 μM to about 2.8 μM, about 1.3 μM to about 2.8 μM, about 1.4 μM to about 2.8 μM, about 1.5 μM to about 2.8 μM, about 1.6 μM to about 2.8 μM, about 1.7 μM to about 2.8 μM, about 1.8 μM to about 2.8 μM, about 1.9 μM to about 2.8 μM, about 2 μM to about 2.8 μM, about 2.2 μM to about 2.8 μM, about 2.4 μM to about 2.8 μM, and/or about 2.6 μM to about 2.8 μM.

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.

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 "PXE" refers to pseudoxanthoma elasticum.

"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.

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 "XLH" refers to X-linked hypophosphatemia, X-linked dominant hypophosphatemic rickets, X-linked vitamin D-resistant rickets, and/or X-linked hypophosphatemic rickets.

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.

Compositions

In certain embodiments, the polypeptide of the invention has formula (I): EXPORT-PROTEIN-Z-DOMAIN-X-Y (I), wherein in (I): EXPORT is absent, or a signal export sequence or a biologically active fragment thereof; PROTEIN is the extracellular domain of ENPP3 (SEQ ID NO:1) or a biologically active fragment thereof; DOMAIN is selected from the group consisting of a human IgG Fc domain and human albumin domain; 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: $(DSS)_n$ (SEQ ID NO:6), $(ESS)_n$ (SEQ ID NO:7), $(RQQ)_n$ (SEQ ID NO:8), $(KR)_n$ (SEQ ID NO:9), R_n (SEQ ID NO:10), $(KR)_n$ (SEQ ID NO:11), DSSSEEKFLRRIGRFG (SEQ ID NO:12), EEEEEEEPRGDT (SEQ ID NO:13), APWHLSSQYSRT (SEQ ID NO:14), STLPIPHEFSRE (SEQ ID NO:15), VTKHLNQISQSY (SEQ ID NO:16), E_n (SEQ ID NO:17), and D_n (SEQ ID NO:18), wherein each occurrence of n is independently an integer ranging from 1 to 20.

In certain embodiments, the polypeptide comprises the extracellular domain of ENPP3 (SEQ ID NO:1) or a biologically active fragment (or region) thereof.

In certain embodiments, the polypeptide is soluble. In other embodiments, the nuclease domain of the PROTEIN or mutant thereof is absent. In yet other embodiments, EXPORT is absent or selected from the group consisting of SEQ ID NOs:2-5. In yet other embodiments, X is selected from the group consisting of: absent, a polypeptide consisting of 20 amino acids, a polypeptide consisting of 19 amino acids, a polypeptide consisting of 18 amino acids, a polypeptide consisting of 17 amino acids, a polypeptide consisting of 16 amino acids, a polypeptide consisting of 15 amino acids, a polypeptide consisting of 14 amino acids, a polypeptide consisting of 13 amino acids, a polypeptide consisting of 12 amino acids, a polypeptide consisting of 11 amino acids, a polypeptide consisting of 10 amino acids, a polypeptide consisting of 9 amino acids, a polypeptide consisting of 8 amino acids, a polypeptide consisting of 7 amino acids, a polypeptide consisting of 6 amino acids, a polypeptide consisting of 5 amino acids, a polypeptide consisting of 4 amino acids, a polypeptide consisting of 3 amino acids, a polypeptide consisting of 2 amino acids, and a polypeptide consisting of 1 amino acid. In yet other embodiments, Z is selected from the group consisting of: absent, a polypeptide consisting of 20 amino acids, a polypeptide consisting of 19 amino acids, a polypeptide consisting of 18 amino acids, a polypeptide consisting of 17 amino acids, a polypeptide consisting of 16 amino acids, a polypeptide consisting of 15 amino acids, a polypeptide consisting of 14 amino acids, a polypeptide consisting of 13 amino acids, a polypeptide consisting of 12 amino acids, a polypeptide consisting of 11 amino acids, a polypeptide consisting of 10 amino acids, a polypeptide consisting of 9 amino acids, a polypeptide consisting of 8 amino acids, a polypeptide consisting of 7 amino acids, a polypeptide consisting of 6 amino acids, a polypeptide consisting of 5 amino acids, a polypeptide consisting of 4 amino acids, a polypeptide consisting of 3 amino acids, a polypeptide consisting of 2 amino acids, and a polypeptide consisting of 1 amino acid.

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, DOMAIN is a human IgG Fc domain selected from the group consisting of IgG1, IgG2, IgG3 and IgG4. In other embodiments, the polypeptide is selected from the group consisting of SEQ ID NOs: 19, 21 and 22. In yet other embodiments, DOMAIN is a human albumin domain. In yet other embodiments, the polypeptide is selected from the group consisting of SEQ ID NOs: 24, 25 and 26.

In certain embodiments, the soluble polypeptide lacks a transmembrane domain and/or signal peptide. In other embodiments, the soluble polypeptide lacks a transmembrane domain. In yet other embodiments, the soluble polypeptide lacks a signal peptide. In yet other embodiments, the soluble polypeptide lacks a transmembrane domain and signal peptide.

In certain embodiments, the polypeptide comprises a soluble region (or fragment) of NPP3 and lacks a transmembrane domain and a signal peptide, or a fusion protein thereof. In other embodiments, the polypeptide comprises a soluble region of NPP3 and lacks a transmembrane domain and/or a signal peptide. In yet other embodiments, the polypeptide comprises a soluble region of NPP3 and lacks a transmembrane domain. In yet other embodiments, the polypeptide comprises a soluble region of NPP3 and lacks a signal peptide. In yet other embodiments, the polypeptide reduces cellular calcification when administered to a subject suffering from diseases of calcification and ossification.

In certain embodiments, the polypeptide consists essentially of SEQ ID NO:1 or a biologically active fragment thereof. In other embodiments, the polypeptide consists of SEQ ID NO:1 or a biologically active fragment thereof.

In certain embodiments, the soluble ENPP3 fragment or fusion protein thereof comprises the extracellular domain of ENPP3 (SEQ ID NO:1) or a biologically active fragment thereof. In other embodiments, the soluble ENPP3 fragment consists essentially of SEQ ID NO:1 or a biologically active fragment thereof. In yet other embodiments, the soluble ENPP3 fragment consists of SEQ ID NO:1 or a biologically active fragment thereof. In yet other embodiments, the soluble ENPP3 fragment or fusion protein thereof lacks a transmembrane domain and a signal peptide.

In certain embodiments, the polypeptide of the invention is soluble. In other embodiments, the polypeptide of the invention is a recombinant polypeptide. In yet other embodiments, the polypeptide of the invention is further pegylated.

Methods

The invention 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 vascular calcification in a subject with low plasma pyrophosphate (PPi) or high serum phosphate (Pi). The invention further provides a method of treating of a subject having NPP1 deficiency or NPP1-associated disease. The invention further provides a method of treating or preventing disorders and diseases in a subject where an increased activity or level of ENPP3 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 polypeptide of the invention. In other embodiments, the method comprises administering to the subject a therapeutically effective amount of an isolated recombinant human soluble ENPP3 fragment or fusion protein thereof.

In certain embodiments, the disease or disorder comprises at least one selected from the group consisting of GACI, IIAC, PXE, OPLL, hypophosphatemic rickets, osteoarthritis, calcification of atherosclerotic plaques, hereditary and non-hereditary forms of osteoarthritis, ankylosing spondylitis, hardening of the arteries occurring with aging, and calciphylaxis resulting from end stage renal disease (or mineral bone disorder of chronic kidney disease).

In certain embodiments, the disease or disorder comprises at least one selected from a group consisting of GACI, IIAC, PXE, OPLL, MWVC, ARHR2, ESRD, CKD-MBD, XLH, age related osteopenia, CUA and hypophosphatemic rickets.

In certain embodiments, the disease or disorder is GACI. In other embodiments, the disease or disorder is IIAC. In yet other embodiments, the disease or disorder is PXE. In yet other embodiments, the disease or disorder is OPLL. In yet other embodiments, the disease or disorder is hypophosphatemic rickets. In yet other embodiments, the disease or disorder is osteoarthritis. In yet other embodiments, the disease or disorder is calcification of atherosclerotic plaques. In yet other embodiments, the disease or disorder is hereditary and non-hereditary forms of osteoarthritis. In yet other embodiments, the disease or disorder is ankylosing spondylitis. In yet other embodiments,

the disease or disorder is hardening of the arteries occurring with aging. In yet other embodiments, the disease or disorder is calciphylaxis resulting from end stage renal disease (or mineral bone disorder of chronic kidney disease). In yet other embodiments, the disease or disorder is age related osteopenia. In yet other embodiments, the disease or disorder is CUA. In yet other embodiments, the disease or disorder is MWVC. In yet other embodiments, the disease or disorder is ARHR2. In yet other embodiments, the disease or disorder is ESRD.

In certain embodiments, the at least one polypeptide is administered acutely or chronically to the subject. In other embodiments, the at least one polypeptide is administered locally, regionally or systemically to the subject. In yet other embodiments, the subject is a mammal. In yet other embodiments, the mammal is human.

In certain embodiments, the administered amount raises the level of plasma PPi in the subject to at least about 250 nM. In other embodiments, the administered amount raises the level of plasma PPi in the subject to at least about 500 nM. In yet other embodiments, the administered amount raises the level of plasma PPi in the subject to at least about 800 nM. In yet other embodiments, the administered amount raises the level of plasma PPi in the subject to at least about 900 nM. In yet other embodiments, the administered amount raises the level of plasma PPi in the subject to at least about 1 μM. In yet other embodiments, the administered amount raises the level of plasma PPi in the subject to at least about 1.2 μM. In yet other embodiments, the administered amount raises the level of plasma PPi in the subject to at least about 1.4 μM. In yet other embodiments, the administered amount raises the level of plasma PPi in the subject to at least about 1.5 μM. In certain embodiments, the administered amount raises the level of plasma PPi in the subject to at least about 2 μM. In certain embodiments, the administered amount raises the level of plasma PPi in the subject to at least about 4 μM.

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 polypeptide of the invention, 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 polypeptide of the invention, or a mutant thereof as a preventative measure against a disease or disorder.

The invention encompasses administration of a polypeptide of the invention, 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 polypeptide of the invention, 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 envisions the use of a pharmaceutical composition comprising a polypeptide of the invention 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 certain embodiments, 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, it is preferable to include isotonic agents, for example, sugars, sodium chloride, or polyalcohols such as mannitol and sorbitol, 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 particularly preferred preservative is a combination of about 0.5% to 2.0% benzyl alcohol and 0.05% to 0.5% sorbic acid.

The composition preferably includes an antioxidant and a chelating agent, which inhibit the degradation of the compound. Preferred antioxidants for some compounds are BHT, BHA, alpha-tocopherol and ascorbic acid in the preferred range of about 0.01% to 0.3% and more preferably BHT in the range of 0.03% to 0.1% by weight by total weight of the composition. Preferably, the chelating agent is present in an amount ranging from 0.01% to 0.5% by weight by total weight of the composition. Particularly preferred chelating agents include edetate salts (*e.g.* disodium edetate) and citric acid in the weight range of about 0.01% to 0.20% and more preferably 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 the particularly preferred 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.

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 indicated by the exigencies of the therapeutic or prophylactic situation.

Administration of the compositions of the present invention to a patient, preferably a mammal, more preferably 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 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 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 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, 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.

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 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 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 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.

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.

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 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 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 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 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 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 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 glycollate. 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. 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 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 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 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 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. 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.

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 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, 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 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.

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 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 that 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 a preferred embodiment 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 mat, 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 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: Sequences:

Extracellular domain of ENPP3 (SEQ ID NO:1)

EKQGSCRKKC FDASFRG

LENCRCDVAC KDRGDCCWDF EDTCVESTRI WMCNKFRCGE TRLEASLCSC SDDCLQRKDC

CADYKSVCQG ETSWLEENCD TAQQSQCPEG FDLPPVILFS MDGFRAEYLY TWDTLMPNIN
KLKTCGIHSK YMRAMYPTKT FPNHYTIVTG LYPESHGIID NNMYDVNLNK NFSLSSKEQN
NPAWWHGQPM WLTAMYQGLK AATYFWPGSE VAINGSFPSI YMPYNGSVPF EERISTLLKW
LDLPKAERPR FYTMYFEEPD SSGHAGGPVS ARVIKALQVV DHAFGMLMEG LKQRNLHNCV
NIILLADHGM DQTYCNKMEY MTDYFPRINF FYMYEGPAPR IRAHNIPHDF FSFNSEEIVR
NLSCRKPDQH FKPYLTPDLP KRLHYAKNVR IDKVHLFVDQ QWLAVRSKSN TNCGGGNHGY
NNEFRSMEAI FLAHGPSFKE KTEVEPFENI EVYNLMCDLL RIQPAPNNGT HGSLNHLLKV
PFYEPSHAEE VSKFSVCGFA NPLPTESLDC FCPHLQNSTQ LEQVNQMLNL TQEEITATVK
VNLPFGRPRV LQKNVDHCLL YHREYVSGFG KAMRMPMWSS YTVPQLGDTS PLPPTVPDCL
RADVRVPPSE SQKCSFYLAD KNITHGFLYP PASNRTSDSQ YDALITSNLV PMYEEFRKMW
DYFHSVLLIK HATERNGVNV VSGPIFDYNY DGHFDAPDEI TKHLANTDVP IPTHYFVVLT
SCKNKSHTPE NCPGWLDVLP FIIPHRPTNV ESCPEGKPEA LWVEERFTAH IARVRDVELL
TGLDFYQDKV QPVSEILQLK TYLPTFETTI

Signal sequence ENPP7 (SEQ ID NO:2)

MRGPAVLLTV ALATLLAPGA

Signal sequence ENPP7 (SEQ ID NO:3)

MRGPAVLLTV ALATLLAPGA GA

Signal Sequence ENPP5 (SEQ ID NO:4)

MTSKFLLVSF ILAALSLSTT FS

Signal Sequence ENPP1-2-1 (SEQ ID NO:5)

M E R D G C A G G G S R G G E G G R A P R E G
P A G N G R D R G R S H A A E A P G D P Q A A
A S L L A P M D V G E E P L E K A A R A R T A
K D P N T Y K I I S L F T F A V G V N I C L G
F T A

(singly underlined)-(doubly underlined): Swapped residues with NPP2 residues 1-27 to give cleavage at the singly underlined-doubly underlined transition

SEQ ID NO:6 $(DSS)_n$, wherein n is an integer ranging between 1 and 20.

SEQ ID NO:7 $(ESS)_n$, wherein n is an integer ranging between 1 and 20.

SEQ ID NO:8	$(RQQ)_n$, wherein n is an integer ranging between 1 and 20.
SEQ ID NO:9	$(KR)_n$, wherein n is an integer ranging between 1 and 20.
SEQ ID NO:10	R_n, wherein n is an integer ranging between 1 and 20.
SEQ ID NO:11	$(KR)_n$, wherein n is an integer ranging between 1 and 20.
SEQ ID NO:12	DSSSEEKFLRRIGRFG
SEQ ID NO:13	EEEEEEEPRGDT
SEQ ID NO:14	APWHLSSQYSRT
SEQ ID NO:15	STLPIPHEFSRE
SEQ ID NO:16	VTKHLNQISQSY
SEQ ID NO:17	E_n, wherein n is an integer ranging between 1 and 20.
SEQ ID NO:18	D_n, wherein n is an integer ranging between 1 and 20.

ENPP121-NPP3-Fc sequence (SEQ ID NO:19)

MERDGCAGGG SRGGEGGRAP REGPAGNGRD RGRSHAAEAP GDPQAAASLL APMDVGEEPL
EKAARARTAK DPNTYKIISL FTFAVGVNIC LGFTAKQGSC RKKCFDASFR GLENCRCDVA
CKDRGDCCWD FEDTCVESTR IWMCNKFRCG ERLEASLCSC SDDCLQRKDC CADYKSVCQG
ETSWLEENCD TAQQSQCPEG FDLPPVILFS MDGFRAEYLY TWDTLMPNIN KLKTCGIHSK
YMRAMYPTKT FPNHYTIVTG LYPESHGIID NNMYDVNLNK NFSLSSKEQN NPAWWHGQPM
WLTAMYQGLK AATYFWPGSE VAINGSFPSI YMPYNGSVPF EERISTLLKW LDLPKAERPR
FYTMYFEEPD SSGHAGGPVS ARVIKALQVV DHAFGMLMEG LKQRNLHNCV NIILLADHGM
DQTYCNKMEY MTDYFPRINF FYMYEGPAPR IRAHNIPHDF FSFNSEEIVR NLSCRKPDQH
FKPYLTPDLP KRLHYAKNVR IDKVHLFVDQ QWLAVRSKSN TNCGGGNHGY NNEFRSMEAI
FLAHGPSFKE KTEVEPFENI EVYNLMCDLL RIQPAPNNGT HGSLNHLLKV PFYEPSHAEE
VSKFSVCGFA NPLPTESLDC FCPHLQNSTQ LEQVNQMLNL TQEEITATVK VNLPFGRPRV
LQKNVDHCLL YHREYVSGFG KAMRMPMWSS YTVPQLGDTS PLPPTVPDCL RADVRVPPSE

SQKCSFYLAD KNITHGFLYP PASNRTSDSQ YDALITSNLV PMYEEFRKMW DYFHSVLLIK
HATERNGVNV VSGPIFDYNY DGHFDAPDEI TKHLANTDVP IPTHYFVVLT SCKNKSHTPE
NCPGWLDVLP FIIPHRPTNV ESCPEGKPEA LWVEERFTAH IARVRDVELL TGLDFYQDKV
QPVSEILQLK TYLPTFETTI DKTHTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT
CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK
CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE
WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS
LSLSPGK

Bold residues = amino acid sequence from NPP1; Single underlined residues = signal peptide sequence from NPP2; Double underlined residues = amino acid sequence of IgG Fc domain. In certain embodiments, the IgG Fc domain is selected from any of the subclasses IgG1, IgG2, IgG3 and IgG4. In other embodiments, instead of Fc domain, albumin domain is used.

In certain embodiments, the NPP3 C-terminus and the Fc/albumin domain are connected by a linker. In other embodiments, the linker comprises at least two amino acids. In yet other embodiments, the linker comprises 2-40 amino acids, 2-30 amino acids, 2-20 amino acids, 2-18 amino acids, 2-16 amino acids, 2-14 amino acids, 2-12 amino acids, 2-10 amino acids, 2-8 amino acids, 2-6 amino acids, 2-4 amino acids, or 2 amino acids. In yet other embodiments, the flexible linker comprises a polyethylene glycol chain and/or a hydrocarbon chain (such as an alkylene chain).

IgG Fc sequence (SEQ ID NO:20)

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGK

ENPP7-NPP3-Fc sequence (SEQ ID NO:21)

MRGPAVLLTV ALATLLAPGA KQGSC RKKCFDASFR GLENCRCDVA
CKDRGDCCWD FEDTCVESTR IWMCNKFRCG ERLEASLCSC SDDCLQRKDC CADYKSVCQG
ETSWLEENCD TAQQSQCPEG FDLPPVILFS MDGFRAEYLY TWDTLMPNIN KLKTCGIHSK
YMRAMYPTKT FPNHYTIVTG LYPESHGIID NNMYDVNLNK NFSLSSKEQN NPAWWHGQPM
WLTAMYQGLK AATYFWPGSE VAINGSFPSI YMPYNGSVPF EERISTLLKW LDLPKAERPR
FYTMYFEEPD SSGHAGGPVS ARVIKALQVV DHAFGMLMEG LKQRNLHNCV NIILLADHGM
DQTYCNKMEY MTDYFPRINF FYMYEGPAPR IRAHNIPHDF FSFNSEEIVR NLSCRKPDQH

FKPYLTPDLP KRLHYAKNVR IDKVHLFVDQ QWLAVRSKSN TNCGGGNHGY NNEFRSMEAI
FLAHGPSFKE KTEVEPFENI EVYNLMCDLL RIQPAPNNGT HGSLNHLLKV PFYEPSHAEE
VSKFSVCGFA NPLPTESLDC FCPHLQNSTQ LEQVNQMLNL TQEEITATVK VNLPFGRPRV
LQKNVDHCLL YHREYVSGFG KAMRMPMWSS YTVPQLGDTS PLPPTVPDCL RADVRVPPSE
SQKCSFYLAD KNITHGFLYP PASNRTSDSQ YDALITSNLV PMYEEFRKMW DYFHSVLLIK
HATERNGVNV VSGPIFDYNY DGHFDAPDEI TKHLANTDVP IPTHYFVVLT SCKNKSHTPE
NCPGWLDVLP FIIPHRPTNV ESCPEGKPEA LWVEERFTAH IARVRDVELL TGLDFYQDKV
QPVSEILQLK TYLPTFETTI DKTHTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT
CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK
CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE
WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS
LSLSPGK

Single underlined residues = signal peptide sequence from NPP7; Double underlined residues = amino acid sequence of IgG Fc domain. In certain embodiments, the IgG Fc domain is selected from any of the subclasses IgG1, IgG2, IgG3 and IgG4. In other embodiments, instead of Fc domain, albumin domain is used.

In certain embodiments, the NPP3 C-terminus and the Fc/albumin domain are connected by a linker. In other embodiments, the linker comprises at least two amino acids. In yet other embodiments, the linker comprises 2-40 amino acids, 2-30 amino acids, 2-20 amino acids, 2-18 amino acids, 2-16 amino acids, 2-14 amino acids, 2-12 amino acids, 2-10 amino acids, 2-8 amino acids, 2-6 amino acids, 2-4 amino acids, or 2 amino acids. In yet other embodiments, the flexible linker comprises a polyethylene glycol chain and/or a hydrocarbon chain (such as an alkylene chain).

ENPP5-NPP3-Fc sequence (SEQ ID NO:22)

MTSKFLLVSF ILAALSLSTT FSKQGSC RKKCFDASFR GLENCRCDVA
CKDRGDCCWD FEDTCVESTR IWMCNKFRCG ERLEASLCSC SDDCLQRKDC CADYKSVCQG
ETSWLEENCD TAQQSQCPEG FDLPPVILFS MDGFRAEYLY TWDTLMPNIN KLKTCGIHSK
YMRAMYPTKT FPNHYTIVTG LYPESHGIID NNMYDVNLNK NFSLSSKEQN NPAWWHGQPM
WLTAMYQGLK AATYFWPGSE VAINGSFPSI YMPYNGSVPF EERISTLLKW LDLPKAERPR
FYTMYFEEPD SSGHAGGPVS ARVIKALQVV DHAFGMLMEG LKQRNLHNCV NIILLADHGM
DQTYCNKMEY MTDYFPRINF FYMYEGPAPR IRAHNIPHDF FSFNSEEIVR NLSCRKPDQH
FKPYLTPDLP KRLHYAKNVR IDKVHLFVDQ QWLAVRSKSN TNCGGGNHGY NNEFRSMEAI
FLAHGPSFKE KTEVEPFENI EVYNLMCDLL RIQPAPNNGT HGSLNHLLKV PFYEPSHAEE
VSKFSVCGFA NPLPTESLDC FCPHLQNSTQ LEQVNQMLNL TQEEITATVK VNLPFGRPRV
LQKNVDHCLL YHREYVSGFG KAMRMPMWSS YTVPQLGDTS PLPPTVPDCL RADVRVPPSE

SQKCSFYLAD KNITHGFLYP PASNRTSDSQ YDALITSNLV PMYEEFRKMW DYFHSVLLIK
HATERNGVNV VSGPIFDYNY DGHFDAPDEI TKHLANTDVP IPTHYFVVLT SCKNKSHTPE
NCPGWLDVLP FIIPHRPTNV ESCPEGKPEA LWVEERFTAH IARVRDVELL TGLDFYQDKV
QPVSEILQLK TYLPTFETTI DKTHTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT
CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK
CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE
WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS
LSLSPGK

Single underlined residues = signal peptide sequence from NPP5; Double underlined residues = amino acid sequence of IgG Fc domain. In certain embodiments, the IgG Fc domain is selected from any of the subclasses IgG1, IgG2, IgG3 and IgG4. In other embodiments, instead of Fc domain, albumin domain is used.

In certain embodiments, the NPP3 C-terminus and the Fc/albumin domain are connected by a linker. In other embodiments, the linker comprises at least two amino acids. In yet other embodiments, the linker comprises 2-40 amino acids, 2-30 amino acids, 2-20 amino acids, 2-18 amino acids, 2-16 amino acids, 2-14 amino acids, 2-12 amino acids, 2-10 amino acids, 2-8 amino acids, 2-6 amino acids, 2-4 amino acids, or 2 amino acids. In yet other embodiments, the flexible linker comprises a polyethylene glycol chain and/or a hydrocarbon chain (such as an alkylene chain).

Albumin sequence (SEQ ID NO:23)

GGGGSGGGGSGGGGSMKWVTFLLLLFVSGSAFSRGVFRREAHKSEIAHRYNDLGEQHFKGLVLIAFSQ
YLQKCSYDEHAKLVQEVTDFAKTCVADESAANCDKSLHTLFGDKLCAIPNLRENYGELADCCTKQEPE
RNECFLQHKDDNPSLPPFERPEAEAMCTSFKENPTTFMGHYLHEVARRHPYFYAPELLYYAEQYNEIL
TQCCAEADKESCLTPKLDGVKEKALVSSVRQRMKCSSMQKFGERAFKAWAVARLSQTFPNADFAEITK
LATDLTKVNKECCHGDLLECADDRAELAKYMCENQATISSKLQTCCDKPLLKKAHCLSEVEHDTMPAD
LPAIAADFVEDQEVCKNYAEAKDVFLGTFLYEYSRRHPDYSVSLLLRLAKKYEATLEKCCAEANPPAC
YGTVLAEFQPLVEEPKNLVKTNCDLYEKLGEYGFQNAILVRYTQKAPQVSTPTLVEAARNLGRVGTKC
CTLPEDQRLPCVEDYLSAILNRVCLLHEKTPVSEHVTKCCSGSLVERRPCFSALTVDETYVPKEFKAE
TFTFHSDICTLPEKEKQIKKQTALAELVKHKPKATAEQLKTVMDDFAQFLDTCCKAADKDTCFSTEGP
NLVTRCKDALA

ENPP121-NPP3-Albumin sequence (SEQ ID NO:24)

**MERDGCAGGG SRGGEGGRAP REGPAGNGRD RGRSHAAEAP GDPQAAASLL APMDVGEEPL
EKAARARTAK DPNTYKIIS**L FTFAVGVNIC LGFTAKQGSC RKKCFDASFR GLENCRCDVA
CKDRGDCCWD FEDTCVESTR IWMCNKFRCG ERLEASLCSC SDDCLQRKDC CADYKSVCQG

ETSWLEENCD TAQQSQCPEG FDLPPVILFS MDGFRAEYLY TWDTLMPNIN KLKTCGIHSK
YMRAMYPTKT FPNHYTIVTG LYPESHGIID NNMYDVNLNK NFSLSSKEQN NPAWWHGQPM
WLTAMYQGLK AATYFWPGSE VAINGSFPSI YMPYNGSVPF EERISTLLKW LDLPKAERPR
FYTMYFEEPD SSGHAGGPVS ARVIKALQVV DHAFGMLMEG LKQRNLHNCV NIILLADHGM
DQTYCNKMEY MTDYFPRINF FYMYEGPAPR IRAHNIPHDF FSFNSEEIVR NLSCRKPDQH
FKPYLTPDLP KRLHYAKNVR IDKVHLFVDQ QWLAVRSKSN TNCGGGNHGY NNEFRSMEAI
FLAHGPSFKE KTEVEPFENI EVYNLMCDLL RIQPAPNNGT HGSLNHLLKV PFYEPSHAEE
VSKFSVCGFA NPLPTESLDC FCPHLQNSTQ LEQVNQMLNL TQEEITATVK VNLPFGRPRV
LQKNVDHCLL YHREYVSGFG KAMRMPMWSS YTVPQLGDTS PLPPTVPDCL RADVRVPPSE
SQKCSFYLAD KNITHGFLYP PASNRTSDSQ YDALITSNLV PMYEEFRKMW DYFHSVLLIK
HATERNGVNV VSGPIFDYNY DGHFDAPDEI TKHLANTDVP IPTHYFVVLT SCKNKSHTPE
NCPGWLDVLP FIIPHRPTNV ESCPEGKPEA LWVEERFTAH IARVRDVELL TGLDFYQDKV
QPVSEILQLK TYLPTFETTI
GGGSGGGGSG GGGSMKWVTF LLLLFVSGSA FSRGVFRREA HKSEIAHRYN DLGEQHFKGL
VLIAFSQYLQ KCSYDEHAKL VQEVTDFAKT CVADESAANC DKSLHTLFGD KLCAIPNLRE
NYGELADCCT KQEPERNECF LQHKDDNPSL PPFERPEAEA MCTSFKENPT TFMGHYLHEV
ARRHPYFYAP ELLYYAEQYN EILTQCCAEA DKESCLTPKL DGVKEKALVS SVRQRMKCSS
MQKFGERAFK AWAVARLSQT FPNADFAEIT KLATDLTKVN KECCHGDLLE CADDRAELAK
YMCENQATIS SKLQTCCDKP LLKKAHCLSE VEHDTMPADL PAIAADFVED QEVCKNYAEA
KDVFLGTFLY EYSRRHPDYS VSLLLRLAKK YEATLEKCCA EANPPACYGT VLAEFQPLVE
EPKNLVKTNC DLYEKLGEYG FQNAILVRYT QKAPQVSTPT LVEAARNLGR VGTKCCTLPE
DQRLPCVEDY LSAILNRVCL LHEKTPVSEH VTKCCSGSLV ERRPCFSALT VDETYVPKEF
KAETFTFHSD ICTLPEKEKQ IKKQTALAEL VKHKPKATAE QLKTVMDDFA QFLDTCCKAA
DKDTCFSTEG PNLVTRCKDA LA

Bold residues = amino acid sequence from NPP1; Single underlined residues = signal peptide sequence from NPP2; Double underlined residues = amino acid sequence of spacer sequence and albumin domain.

In certain embodiments, the NPP3 C-terminus and the albumin domain are connected by a linker. In other embodiments, the linker comprises at least two amino acids. In yet other embodiments, the linker comprises 2-40 amino acids, 2-30 amino acids, 2-20 amino acids, 2-18 amino acids, 2-16 amino acids, 2-14 amino acids, 2-12 amino acids, 2-10 amino acids, 2-8 amino acids, 2-6 amino acids, 2-4 amino acids, or 2 amino acids. In yet other embodiments, the flexible linker comprises a polyethylene glycol chain and/or a hydrocarbon chain (such as an alkylene chain).

ENPP7-NPP3-Albumin sequence (SEQ ID NO:25)

MRGPAVLLTV ALATLLAPGA KQGSC RKKCFDASFR GLENCRCDVA
CKDRGDCCWD FEDTCVESTR IWMCNKFRCG ERLEASLCSC SDDCLQRKDC CADYKSVCQG
ETSWLEENCD TAQQSQCPEG FDLPPVILFS MDGFRAEYLY TWDTLMPNIN KLKTCGIHSK
YMRAMYPTKT FPNHYTIVTG LYPESHGIID NNMYDVNLNK NFSLSSKEQN NPAWWHGQPM
WLTAMYQGLK AATYFWPGSE VAINGSFPSI YMPYNGSVPF EERISTLLKW LDLPKAERPR
FYTMYFEEPD SSGHAGGPVS ARVIKALQVV DHAFGMLMEG LKQRNLHNCV NIILLADHGM
DQTYCNKMEY MTDYFPRINF FYMYEGPAPR IRAHNIPHDF FSFNSEEIVR NLSCRKPDQH
FKPYLTPDLP KRLHYAKNVR IDKVHLFVDQ QWLAVRSKSN TNCGGGNHGY NNEFRSMEAI
FLAHGPSFKE KTEVEPFENI EVYNLMCDLL RIQPAPNNGT HGSLNHLLKV PFYEPSHAEE
VSKFSVCGFA NPLPTESLDC FCPHLQNSTQ LEQVNQMLNL TQEEITATVK VNLPFGRPRV
LQKNVDHCLL YHREYVSGFG KAMRMPMWSS YTVPQLGDTS PLPPTVPDCL RADVRVPPSE
SQKCSFYLAD KNITHGFLYP PASNRTSDSQ YDALITSNLV PMYEEFRKMW DYFHSVLLIK
HATERNGVNV VSGPIFDYNY DGHFDAPDEI TKHLANTDVP IPTHYFVVLT SCKNKSHTPE
NCPGWLDVLP FIIPHRPTNV ESCPEGKPEA LWVEERFTAH IARVRDVELL TGLDFYQDKV
QPVSEILQLK TYLPTFETTI
GGGSGGGGSG GGGSMKWVTF LLLLFVSGSA FSRGVFRREA HKSEIAHRYN DLGEQHFKGL
VLIAFSQYLQ KCSYDEHAKL VQEVTDFAKT CVADESAANC DKSLHTLFGD KLCAIPNLRE
NYGELADCCT KQEPERNECF LQHKDDNPSL PPFERPEAEA MCTSFKENPT TFMGHYLHEV
ARRHPYFYAP ELLYYAEQYN EILTQCCAEA DKESCLTPKL DGVKEKALVS SVRQRMKCSS
MQKFGERAFK AWAVARLSQT FPNADFAEIT KLATDLTKVN KECCHGDLLE CADDRAELAK
YMCENQATIS SKLQTCCDKP LLKKAHCLSE VEHDTMPADL PAIAADFVED QEVCKNYAEA
KDVFLGTFLY EYSRRHPDYS VSLLLRLAKK YEATLEKCCA EANPPACYGT VLAEFQPLVE
EPKNLVKTNC DLYEKLGEYG FQNAILVRYT QKAPQVSTPT LVEAARNLGR VGTKCCTLPE
DQRLPCVEDY LSAILNRVCL LHEKTPVSEH VTKCCSGSLV ERRPCFSALT VDETYVPKEF
KAETFTFHSD ICTLPEKEKQ IKKQTALAEL VKHKPKATAE QLKTVMDDFA QFLDTCCKAA
DKDTCFSTEG PNLVTRCKDA LA

Single underlined residues = signal peptide sequence from NPP7; Double underlined residues = amino acid sequence of spacer sequence and albumin domain.

In certain embodiments, the NPP3 C-terminus and the albumin domain are connected by a linker. In other embodiments, the linker comprises at least two amino acids. In yet other embodiments, the linker comprises 2-40 amino acids, 2-30 amino acids, 2-20 amino acids, 2-18 amino acids, 2-16 amino acids, 2-14 amino acids, 2-12 amino acids, 2-10 amino acids, 2-8 amino acids, 2-6 amino acids, 2-4 amino acids, or 2 amino acids. In yet other embodiments, the flexible linker comprises a polyethylene glycol chain and/or a hydrocarbon chain (such as an alkylene chain).

ENPP5-NPP3-albumin sequence (SEQ ID NO:26)

MTSKFLLVSF ILAALSLSTT FSKQGSC RKKCFDASFR GLENCRCDVA
CKDRGDCCWD FEDTCVESTR IWMCNKFRCG ERLEASLCSC SDDCLQRKDC CADYKSVCQG
ETSWLEENCD TAQQSQCPEG FDLPPVILFS MDGFRAEYLY TWDTLMPNIN KLKTCGIHSK
YMRAMYPTKT FPNHYTIVTG LYPESHGIID NNMYDVNLNK NFSLSSKEQN NPAWWHGQPM
WLTAMYQGLK AATYFWPGSE VAINGSFPSI YMPYNGSVPF EERISTLLKW LDLPKAERPR
FYTMYFEEPD SSGHAGGPVS ARVIKALQVV DHAFGMLMEG LKQRNLHNCV NIILLADHGM
DQTYCNKMEY MTDYFPRINF FYMYEGPAPR IRAHNIPHDF FSFNSEEIVR NLSCRKPDQH
FKPYLTPDLP KRLHYAKNVR IDKVHLFVDQ QWLAVRSKSN TNCGGGNHGY NNEFRSMEAI
FLAHGPSFKE KTEVEPFENI EVYNLMCDLL RIQPAPNNGT HGSLNHLLKV PFYEPSHAEE
VSKFSVCGFA NPLPTESLDC FCPHLQNSTQ LEQVNQMLNL TQEEITATVK VNLPFGRPRV
LQKNVDHCLL YHREYVSGFG KAMRMPMWSS YTVPQLGDTS PLPPTVPDCL RADVRVPPSE
SQKCSFYLAD KNITHGFLYP PASNRTSDSQ YDALITSNLV PMYEEFRKMW DYFHSVLLIK
HATERNGVNV VSGPIFDYNY DGHFDAPDEI TKHLANTDVP IPTHYFVVLT SCKNKSHTPE
NCPGWLDVLP FIIPHRPTNV ESCPEGKPEA LWVEERFTAH IARVRDVELL TGLDFYQDKV
QPVSEILQLK TYLPTFETTI
GGGSGGGGSG GGGSMKWVTF LLLLFVSGSA FSRGVFRREA HKSEIAHRYN DLGEQHFKGL
VLIAFSQYLQ KCSYDEHAKL VQEVTDFAKT CVADESAANC DKSLHTLFGD KLCAIPNLRE
NYGELADCCT KQEPERNECF LQHKDDNPSL PPFERPEAEA MCTSFKENPT TFMGHYLHEV
ARRHPYFYAP ELLYYAEQYN EILTQCCAEA DKESCLTPKL DGVKEKALVS SVRQRMKCSS
MQKFGERAFK AWAVARLSQT FPNADFAEIT KLATDLTKVN KECCHGDLLE CADDRAELAK
YMCENQATIS SKLQTCCDKP LLKKAHCLSE VEHDTMPADL PAIAADFVED QEVCKNYAEA
KDVFLGTFLY EYSRRHPDYS VSLLLRLAKK YEATLEKCCA EANPPACYGT VLAEFQPLVE
EPKNLVKTNC DLYEKLGEYG FQNAILVRYT QKAPQVSTPT LVEAARNLGR VGTKCCTLPE
DQRLPCVEDY LSAILNRVCL LHEKTPVSEH VTKCCSGSLV ERRPCFSALT VDETYVPKEF
KAETFTFHSD ICTLPEKEKQ IKKQTALAEL VKHKPKATAE QLKTVMDDFA QFLDTCCKAA
DKDTCFSTEG PNLVTRCKDA LA

Single underlined residues = signal peptide sequence from NPP5; Double underlined residues = amino acid sequence of spacer sequence and albumin domain.

In certain embodiments, the NPP3 C-terminus and the albumin domain are connected by a linker. In other embodiments, the linker comprises at least two amino acids. In yet other embodiments, the linker comprises 2-40 amino acids, 2-30 amino acids, 2-20 amino acids, 2-18 amino acids, 2-16 amino acids, 2-14 amino acids, 2-12 amino acids, 2-10 amino acids, 2-8 amino acids, 2-6 amino acids, 2-4 amino acids, or 2 amino acids. In yet other embodiments, the flexible linker comprises a polyethylene glycol chain and/or a hydrocarbon chain (such as an alkylene chain).

Nucleotide sequence of NPP121-NPP3-Fc (SEQ ID NO:27)

ATGGAAAGGGACGGATGCGCCGGTGGTGGATCTCG
CGGAGGCGAAGGTGGAAGGGCCCCTAGGGAAGGACCTGCCGGAAACGGAAGGGACAGGGG
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TATGGACGTCGGAGAAGAACCCCTGGAAAAGGCCGCCAGGGCCAGGACTGCCAAGGACCC
CAACACCTACAAGATCATCTCCCTCTTCACTTTCGCCGTCGGAGTCAACATCTGCCTGGG
ATTCACCGCCGAAAAGCAAGGCAGCTGCAGGAAGAAGTGCTTTGATGCATCATTTAGAGG
ACTGGAGAACTGCCGGTGTGATGTGGCATGTAAAGACCGAGGTGATTGCTGCTGGGATTT
TGAAGACACCTGTGTGGAATCAACTCGAATATGGATGTGCAATAAATTTCGTTGTGGAGA
GACCAGATTAGAGGCCAGCCTTTGCTCTTGTTCAGATGACTGTTTGCAGAGGAAAGATTG
CTGTGCTGACTATAAGAGTGTTTGCCAAGGAGAAACCTCATGGCTGGAAGAAAACTGTGA
CACAGCCCAGCAGTCTCAGTGCCCAGAAGGGTTTGACCTGCCACCAGTTATCTTGTTTTC
TATGGATGGATTTAGAGCTGAATATTTATACACATGGGATACTTTAATGCCAAATATCAA
TAAACTGAAAACATGTGGAATTCATTCAAAATACATGAGAGCTATGTATCCTACCAAAAC
CTTCCCAAATCATTACACCATTGTCACGGGCTTGTATCCAGAGTCACATGGCATCATTGA
CAATAATATGTATGATGTAAATCTCAACAAGAATTTTTCACTTTCTTCAAAGGAACAAAA
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CAATATCATCCTTCTGGCTGACCATGGAATGGACCAGACTTATTGTAACAAGATGGAATA
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AGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAA
CTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTATAGCAAGCT
CACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGA
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Nucleotide sequence of NPP121-NPP3-Fc (SEQ ID NO:28)

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Nucleotide sequence of hNPP3-hFc-pcDNA3 (SEQ ID NO:29)

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CCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCC
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TGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCT
GCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCAC
TGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGT
TCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTC
TGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCA
CCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGAT
CTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCAC
GTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATT
AAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACC
AATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTG
CCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTG
CTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGC
CAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTA
TTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTG
TTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCT
CCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTA
GCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGG
TTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGA
CTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTT
GCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCA
TTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTT
CGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTT
CTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGA
AATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATT
GTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGC
GCACATTTCCCCGAAAAGTGCCACCTGACGTC

Example 1:

FIGs. 1A-1C comprise graphs illustrating studies of hNPP3 steady state ATP hydrolysis activity.

As illustrated in FIG. 1A, time courses of AMP product formation after addition of 50 nM hNPP3 with (from bottom to top) 0.98, 1.95, 3.9, 7.8, 15.6, 31.3, 62.5, 125,

250 or 500 μM ATP were analyzed. The enzyme reaction was quenched with equal volume of 3 M formic acid at different times and the reaction product, AMP, was quantified by HPLC analysis with an AMP standard curve. The smooth line through the data points were best fits to a non-linear enzyme kinetic model with product inhibition and substrate depletion.

FIG. 1B illustrates steady state ATPase cycling rate comparison. hNPP3 substrate concentration dependence of initial steady state enzyme cycling rate was compared with that measured for hNPP1. ATPase cycling reaction of both 50nM hNPP3 and hNPP1 depleted ATP substrate within 1 minute at 0.98, 1.95 and 3.9 μM ATP. The uncertainty at these low ATP concentrations was significant, and thus these three rates were omitted from the data set during fitting. The hNPP3 steady state ATPase reaction reached the maximum (k_{cat}) of 2.59 (±0.04) s^{-1} enzyme^{-1}, from the weighted average of the measured rates at 7.8, 15.6, 31.3, 62.5, 125 μM substrate. The turnover rate of hNPP1 was 3.46 (±0.44) s^{-1} enzyme^{-1}. The K_M for ATP substrate was estimated to be < 8 μM.

FIG. 1C illustrates substrate concentration dependence of the η value. The decreasing η value with substrate concentration for both enzymes indicates that substrate depletion contributes to the non-linearity in the enzyme reaction time courses much more than product inhibition at lower initial substrate concentrations. The similarity of hNPP3 and hNPP1 η values was consistent with the two enzymes having similar reaction rates and product inhibition.

Example 2: Animal Models

The following non-limiting animal models can be used to test the efficacy of the presently claimed compositions on human disease resulting from low pyrophosphate (PPi):

1. enpp1asj/asj model of Generalized Arterial Calcification of Infancy (GACI); Li, *et al.*, 2013, Disease Models & Mech. 6(5):1227-35.
2. enpp12asj/2asj model of Generalized Arterial Calcification of Infancy (GACI); Li, *et al.*, 2014, PloS one 9(12):e113542.
3. ABCC6-/- mouse model of Pseudoxanthoma Elasticum (PXE); Jiang, *et al.*, 2007, J. Invest. Derm. 127(6):1392-402.
4. HYP mouse model of X-linked hypophosphatasia (XLH); Liang, *et al.*, 2009, Calcif. Tissue Int. 85(3):235-46.
5. LmnaG609G/+ mouse model of Hutchison-Gilford Progeria Syndrome; Villa-

Bellosta, *et al.*, 2013, Circulation 127(24):2442-51.

6. Tip toe walking (ttw) mouse model of Ossification of the Posterior Longitudinal Ligament (OPLL) (Okawa, *et al.*, 1998, Nature Genetics 19(3):271-3; Nakamura, *et al.*, 1999, Human Genetics 104(6):492-7) and osteoarthritis (Bertrand, *et al.*, 2012, Annals Rheum. Diseases 71(7):1249-53).

7. Rat model of chronic kidney disease (CKD) on the adenine diet; Schibler, *et al.*, 1968, Clin. Sci. 35(2):363-72; O'Neill, *et al.*, 2011, Kidney Int. 79(5):512-7.

8. Mouse model of chronic kidney disease (CKD) on the adenine diet; Jia, *et al.*, 2013, BMC Nephrol. 14:116.

9. 5/6th nephrectomy rat model of CKD; Morrison, 1962, Lab Invest. 11:321-32; Shimamura & Morrison, 1975, Am. J. Pathol. 79(1):95-106.

10. ENPP1 knockout mouse model of GACI and osteopenia; Mackenzie, *et al.*, 2012, PloS one 7(2):e32177.

In certain embodiments, there is no rodent model that recapitulates the adult form of the human disease GACI, also referred to in the literature as Autosomal Recessive Hypohposphatemic Rickets type 2 (ARHR2) (Levy-Litan, *et al.*, 2010, Am. J. Human Gen. 86(2):273-8.

Experimental details on enzymatic activity, quantification of plasma PPi, micro-CT scans, quantification of plasma pyrophosphate uptake and mouse models of calcification are described in detail in the patent applications and/or publications PCT/US2016/33236, WO2014126965 (relating to PCT Patent Application No. PCT/US2014/015945), and US 20150359858, each of which is herein incorporated in its entirety by reference.

Example 3: Production and Purification of ENPP3 fusion proteins

ENPP3 is produced by establishing stable transfections in either CHO or HEK293 mammalian cells. The protein can be produced in either adherent or suspension cells. To establish stable cell lines the nucleic acid sequence encoding NPP3 fusion proteins (FIGs. 3-5 & SEQ ID NO:s 1-29) into an appropriate vector for large scale protein production. There are a variety of these vectors available from commercial sources and any of those can be used.

For example, FIG. 3 illustrates a plasmid map of ENPP1-2-1-exENPP3-Fc cloned into the pcDNA3 plasmid with appropriate endonuclease restriction sites. The protein subdomains are color coded to illustrate the signal sequence, extracellular domain of ENPP3,

and Fc domains of the fusion protein. The amino acid sequence of the cloned protein is also displayed below the plasmid map and also color coded to illustrate the domains of the fusion protein. The pcDNA3 plasmid containing the desired protein constructs can be stably transfected into expression plasmid using established techniques such as electroporation or lipofectamine, and the cells can be grown under antibiotic selection to enhance for stably transfected cells.

Clones of single, stably transfected cells are then established and screened for high expressing clones of the desired fusion protein. Screening of the single cell clones for ENPP3 protein expression can be accomplished in a high-throughput manner in 96 well plates using the synthetic enzymatic substrate pNP-TMP as previously described for ENPP1 (Saunders, *et al.*, 2008, Mol. Cancer Therap. 7(10):3352-62; Albright, *et al.*, 2015, Nat Commun. 6:10006). Upon identification of high expressing clones through screening, protein production can be accomplished in shaking flasks or bio-reactors previously described for ENPP1 (Albright, *et al.*, 2015, Nat Commun. 6:10006).

Purification of ENPP3 can be accomplished using a combination of standard purification techniques known in the art. These techniques are well known in art and are selected from techniques such as column chromatograph, ultracentrifugation, filtration, and precipitation. Column chromatographic purification is accomplished using affinity chromatography such as protein-A and protein-G resins, metal affinity resins such as nickel or copper, hydrophobic exchange chromatography, and reverse-phase high-pressure chromatography (HPLC) using C8-C14 resins. Ion exchange may also be employed, such as anion and cation exchange chromatography using commercially available resins such as Q-sepharose (anion exchange) and SP-sepharose (cation exchange), blue sepharose resin and blue-sephadex resin, and hydroxyapatite resins. Size exclusion chromatography using commercially available S-75 and S200 Superdex resins can also be employed, as known in the art. Buffers used to solubilize the protein, and provide the selection media for the above described chromatographic steps, are standard biological buffers known to practitioners of the art and science of protein chemistry.

Some examples of buffers that are used in preparation include citrate, phosphate, acetate, tris(hydroxymethyl)aminomethane, saline buffers, glycine-HCL buffers, Cacodylate buffers, and sodium barbital buffers, which are well known in art. Using a single techniques, or a series of techniques in combination, and the appropriate buffer systems adjusted to the appropriate pH, one can purify the fusion proteins described to greater than 99% purity from crude material (see, for example, FIG. 2). This figure compares partially

purified ENPP3 and the crude starting material side by side on a Coomasie stained polyacrylamide gel after a single purification step. As demonstrated in FIG. 2, a protein of molecular weight slightly greater than 105 kD corresponding to the appropriate molecular weight of ENPP3 was enriched from the crude starting material displayed in the right lane after a single purification step. This material can then be additionally purified using additional techniques and/or chromatographic steps as described above, to reach substantially higher purity such as ~99% purity. In certain embodiments, the purified protein has enzymatic activity comparable to the enzymatic activity described and demonstrated in FIGs. 1A-1C.

Example 4: Usage of Plasma PPi as a Biomarker

Certain embodiments of the invention contemplate the usage of plasma pyrophosphate as a biomarker to determine which individuals are at risk for diseases of ectopic calcification of the soft tissues, calcification of the medial vascular wall, low bone mineral density, osteopenia, stroke, arthritis, and/or hereditary forms of rickets. Plasma PPi has not been clinically used to predict individuals at risk for the above disorders, as demonstrated by the lack of a plasma PPi test in catalogs of laboratory tests offered by leading clinical laboratories, such as Mayo Medical Laboratory (www *dot* mayomedicallaboratories *dot* com/test-catalog/alphabetical/P) or Yale University, or leading commercial reference laboratories such as ARUP (ltd *dot* aruplab *dot* com/Search/Browse/P) or The Quest Diagnostics Nichols Institute (www *dot* specialtylabs *dot* com/about_us/).

In certain embodiments, plasma PPi has clinical utility as a predictive and diagnostic agent to identify individuals at risk for the above disorders of calcification, ossification, stroke, osteopenia, low bone mineral density, and/or arthritis.

The measurement of plasma PPi can be accomplished by several published methods including radio-isotopic (Cheung, *et al.*, 1977, Anal. Biochem. 83(1):61-3) and fluorescent (Jansen, *et al.*, 2013, PNAS U S A 110(50):20206-11; Jansen, *et al.*, 2014, Arterioscler. Thromb. Vasc. Biol. 34(9):1985-9). Correct measurement of plasma PPi requires that platelets are removed from the plasma and that the whole blood, when collected, is not hemolyzed. Platelets can be removed from the blood either by high speed centrifugation or by ultrafiltration. Removal of platelets is required to prevent platelets from releasing PPi and ATP into the plasma upon activation and degranulation, which will artificially elevate the plasma PPi levels. Hemolysis of whole blood also releases ATP into the plasma and falsely elevate the measurement of plasma PPi. Plasma that has been

collected from non-hemolyzed blood and removed of platelets can be used to reliable measure PPi concentrations, and can provide clinical utility as predictive diagnostic identifying patients at risk for the above mentioned disorders.

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 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. An isolated polypeptide of formula (I), or a pharmaceutical salt or solvate thereof:

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

EXPORT is absent, or a signal export sequence or a biologically active fragment thereof;

PROTEIN is the extracellular domain of ENPP3 (SEQ ID NO:1) or a biologically active fragment thereof;

DOMAIN is selected from the group consisting of a human IgG Fc domain and human albumin domain;

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: $(DSS)_n$ (SEQ ID NO:6), $(ESS)_n$ (SEQ ID NO:7), $(RQQ)_n$ (SEQ ID NO:8), $(KR)_n$ (SEQ ID NO:9), R_n (SEQ ID NO:10), $(KR)_n$ (SEQ ID NO:11), DSSSEEKFLRRIGRFG (SEQ ID NO:12), EEEEEEEPRGDT (SEQ ID NO:13), APWHLSSQYSRT (SEQ ID NO:14), STLPIPHEFSRE (SEQ ID NO:15), VTKHLNQISQSY (SEQ ID NO:16), E_n (SEQ ID NO:17), and D_n (SEQ ID NO:18), wherein each occurrence of n is independently an integer ranging from 1 to 20.

2. The polypeptide of claim 1, wherein the nuclease domain of the PROTEIN or mutant thereof is absent.

3. The polypeptide of claim 1, wherein EXPORT is absent or selected from the group consisting of SEQ ID NOs:2-5.

4. The polypeptide of claim 1, wherein X and Z are independently selected from the group consisting of: absent, a polypeptide consisting of 20 amino acids, a polypeptide consisting of 19 amino acids, a polypeptide consisting of 18 amino acids, a polypeptide consisting of 17 amino acids, a polypeptide consisting of 16 amino acids, a polypeptide consisting of 15 amino acids, a polypeptide consisting of 14 amino acids, a polypeptide consisting of 13 amino acids, a polypeptide consisting of 12 amino acids, a polypeptide consisting of 11 amino acids, a polypeptide consisting of 10 amino acids, a

polypeptide consisting of 9 amino acids, a polypeptide consisting of 8 amino acids, a polypeptide consisting of 7 amino acids, a polypeptide consisting of 6 amino acids, a polypeptide consisting of 5 amino acids, a polypeptide consisting of 4 amino acids, a polypeptide consisting of 3 amino acids, a polypeptide consisting of 2 amino acids, and a polypeptide consisting of 1 amino acid.

5. The polypeptide of claim 1, wherein DOMAIN is a human IgG Fc domain selected from the group consisting of IgG1, IgG2, IgG3 and IgG4.

6. The polypeptide of claim 5, which is selected from the group consisting of SEQ ID NOs:19, 21 and 22.

7. The polypeptide of claim 1, wherein DOMAIN is a human albumin domain.

8. The polypeptide of claim 7, which is selected from the group consisting of SEQ ID NOs:24, 25 and 26.

9. An isolated polypeptide comprising a soluble region of NPP3 and lacking a transmembrane domain and a signal peptide, or a fusion protein thereof, wherein the polypeptide reduces cellular calcification when administered to a subject suffering from diseases of calcification and ossification.

10. The polypeptide of claim 9, which comprises the extracellular domain of ENPP3 (SEQ ID NO:1) or a biologically active fragment thereof.

11. The polypeptide of claim 10, which consists essentially of SEQ ID NO:1 or a biologically active fragment thereof.

12. 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 isolated polypeptide of claim 1.

13. The method of claim 12, wherein the disease or disorder comprises at least one selected from the group consisting of general arterial calcification of infancy (GACI), idiopathic infantile arterial calcification (IIAC), pseudoxanthoma elasticum (PXE), OPLL, hypophosphatemic rickets, osteoarthritis, calcification of atherosclerotic plaques, pseudoxanthoma elasticum, hereditary and non-hereditary forms of osteoarthritis, ankylosing spondylitis, hardening of the arteries occurring with aging, and calciphylaxis resulting from end stage renal disease (or mineral bone disorder of chronic kidney disease).

14. The method of claim 12, wherein the nuclease domain of the PROTEIN or mutant thereof is absent.

15. The method of claim 12, wherein EXPORT is absent or selected from the group consisting of SEQ ID Nos:2-5.

16. The method of claim 12, wherein X and Z are independently selected from the group consisting of: absent, a polypeptide consisting of 20 amino acids, a polypeptide consisting of 19 amino acids, a polypeptide consisting of 18 amino acids, a polypeptide consisting of 17 amino acids, a polypeptide consisting of 16 amino acids, a polypeptide consisting of 15 amino acids, a polypeptide consisting of 14 amino acids, a polypeptide consisting of 13 amino acids, a polypeptide consisting of 12 amino acids, a polypeptide consisting of 11 amino acids, a polypeptide consisting of 10 amino acids, a polypeptide consisting of 9 amino acids, a polypeptide consisting of 8 amino acids, a polypeptide consisting of 7 amino acids, a polypeptide consisting of 6 amino acids, a polypeptide consisting of 5 amino acids, a polypeptide consisting of 4 amino acids, a polypeptide consisting of 3 amino acids, a polypeptide consisting of 2 amino acids, and a polypeptide consisting of 1 amino acid.

17. The method of claim 12, wherein the at least one polypeptide is administered acutely or chronically to the subject.

18. The method of claim 12, wherein the at least one polypeptide is administered locally, regionally or systemically to the subject.

19. The method of claim 12, wherein DOMAIN is a human IgG Fc domain

selected from the group consisting of IgG1, IgG2, IgG3 and IgG4.

20. The method of claim 19, wherein the at least one polypeptide is selected from the group consisting of SEQ ID NOs:19, 21 and 22.

21. The method of claim 12, wherein DOMAIN is a human albumin domain.

22. The method of claim 21, wherein the at least one polypeptide is selected from the group consisting of SEQ ID NOs:24, 25 and 26.

23. The method of claim 12, wherein the subject is a mammal.

24. The method of claim 23, wherein the mammal is human.

25. A method of reducing or preventing vascular calcification in a subject with low plasma pyrophosphate (PPi) or high serum phosphate (Pi), the method comprising administering to the subject a therapeutically effective amount of an isolated recombinant human soluble ENPP3 fragment or fusion protein thereof, wherein the administered amount raises the level of plasma PPi in the subject to at least about 800 nM.

26. The method of claim 25, wherein the administered amount raises the level of plasma PPi in the subject to at least about 1 μM.

27. The method of claim 26, wherein the administered amount raises the level of plasma PPi in the subject to at least about 1.5 μM.

28. The method of claim 25, wherein the subject has at least one disease selected from a group consisting of GACI, IIAC, PXE, OPLL, MWVC, ARHR2, ESRD, CKD-MBD, XLH, age related osteopenia, CUA and hypophosphatemic rickets.

29. The method of claim 25, wherein the soluble ENPP3 fragment or fusion protein thereof comprises the extracellular domain of ENPP3 (SEQ ID NO:1) or a biologically active fragment thereof.

30. The method of claim 25, wherein the soluble ENPP3 fragment consists essentially of SEQ ID NO:1 or a biologically active fragment thereof.

31. The method of claim 25, wherein the soluble ENPP3 fragment or fusion protein thereof lacks a transmembrane domain and a signal peptide.

32. A method of treating of a subject having NPP1 deficiency or NPP1-associated disease, the method comprising administering to the subject a therapeutically effective amount of an isolated recombinant human soluble ENPP3 fragment or fusion protein thereof.

33. The method of claim 32, wherein the subject has at least one disease selected from a group consisting of GACI, IIAC, PXE, OPLL, MWVC, ARHR2, ESRD, CKD-MBD, XLH, age related osteopenia, CUA and hypophosphatemic rickets.

34. The method of claim 32, wherein the soluble ENPP3 fragment or fusion protein thereof comprises the extracellular domain of ENPP3 (SEQ ID NO:1) or a biologically active fragment thereof.

35. The method of claim 32, wherein the soluble ENPP3 fragment consists essentially of SEQ ID NO:1 or a biologically active fragment thereof.

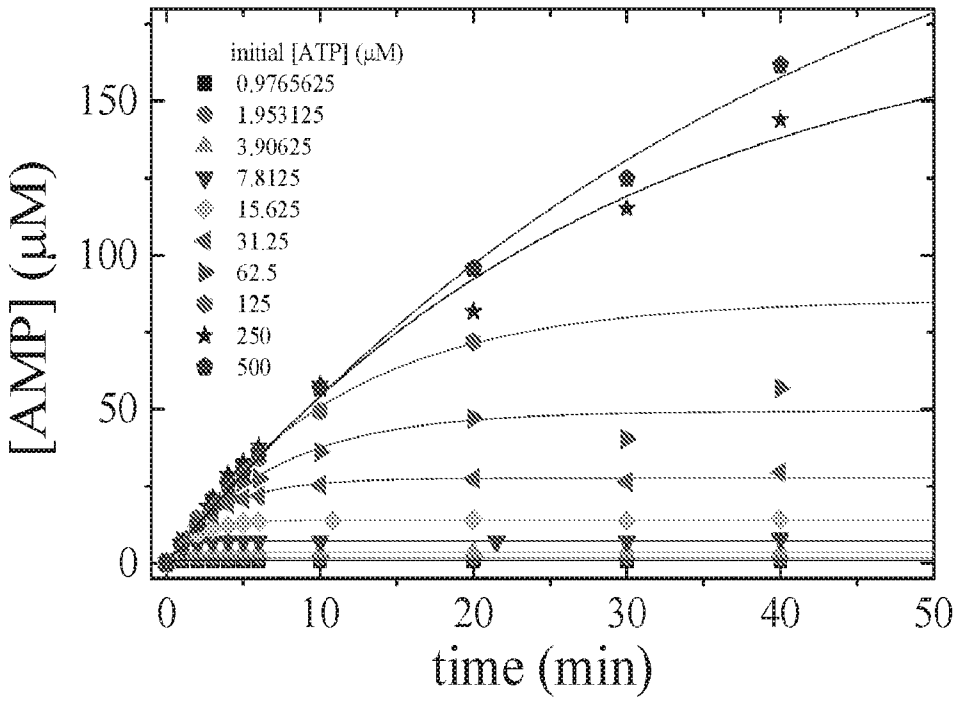
36. The method of claim 32, wherein the soluble ENPP3 fragment or fusion protein thereof lacks a transmembrane domain and a signal peptide.

37. A kit comprising at least one isolated polypeptide of any of claims 1-11 and instructions reciting the use of the at least one polypeptide for treating a disease or disorder associated with pathological calcification or pathological ossification in a subject in need thereof.

38. The kit of claim 37, wherein the disease or disorder comprises at least one selected from the group consisting of GACI, IIAC, OPLL, XLH, osteoarthritis, calcification of atherosclerotic plaques, pseudoxanthoma elasticum, hereditary and non-

hereditary forms of osteoarthritis, ankylosing spondylitis, hardening of the arteries occurring with aging, calciphylaxis resulting from end stage renal disease (or CKD-MBD), MWVC, ARHR2, ESRD, age related osteopenia, and CUA.

FIG. 1A


initial [ATP] (μM)
0.9765625
1.953125
3.90625
7.8125
15.625
31.25
62.5
125
250
500
[AMP] (μM)
time (min)
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50
100
150
0
10
20
30
40
50

FIG. 1B

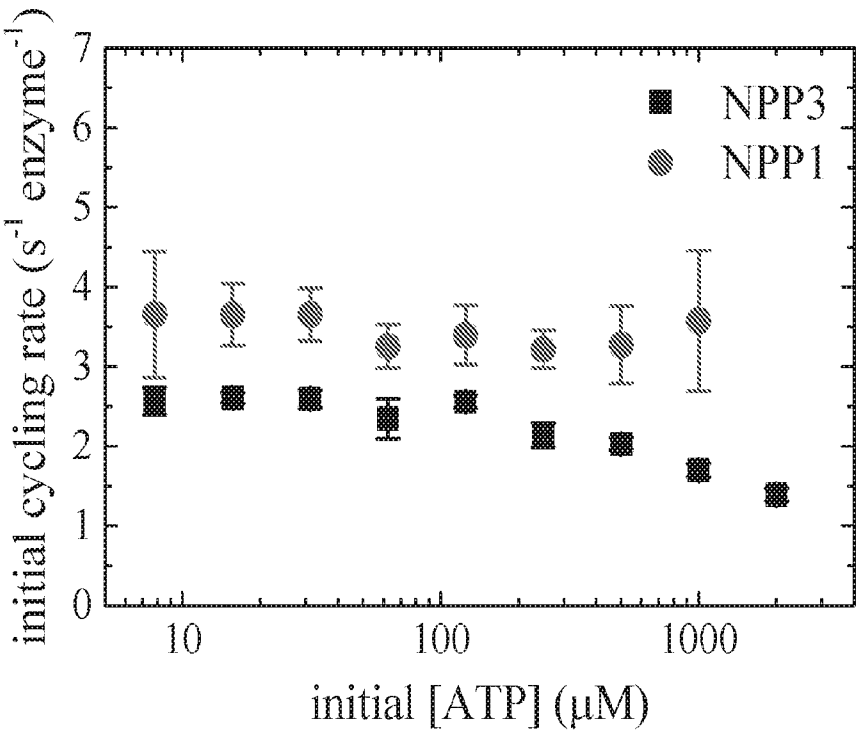

NPP3
NPP1
initial cycling rate (s^{-1} enzyme^{-1})
initial [ATP] (μM)
0
1
2
3
4
5
6
7
10
100
1000

FIG. 1C

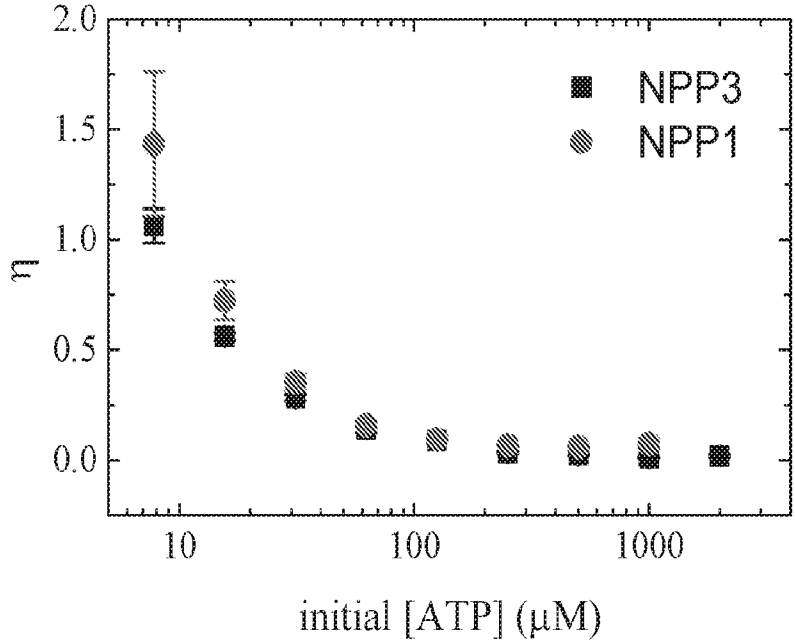
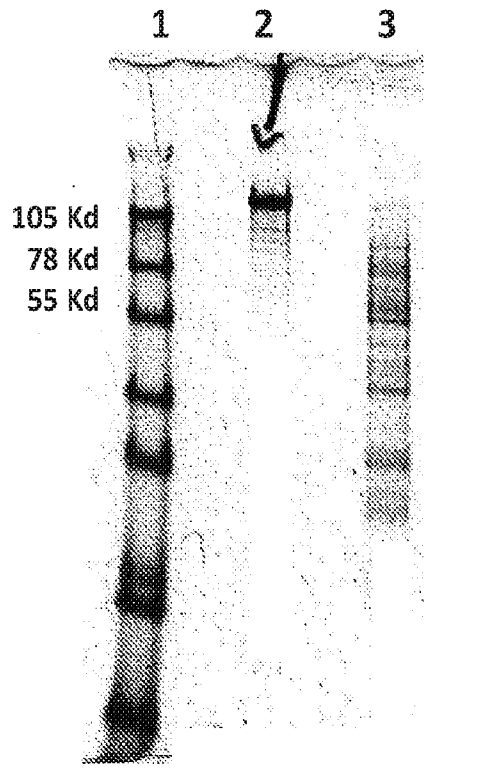


FIG. 2



1 M.W. Markers
2 Partially Purified ENPP3
3 Starting Crude Material

FIG. 3

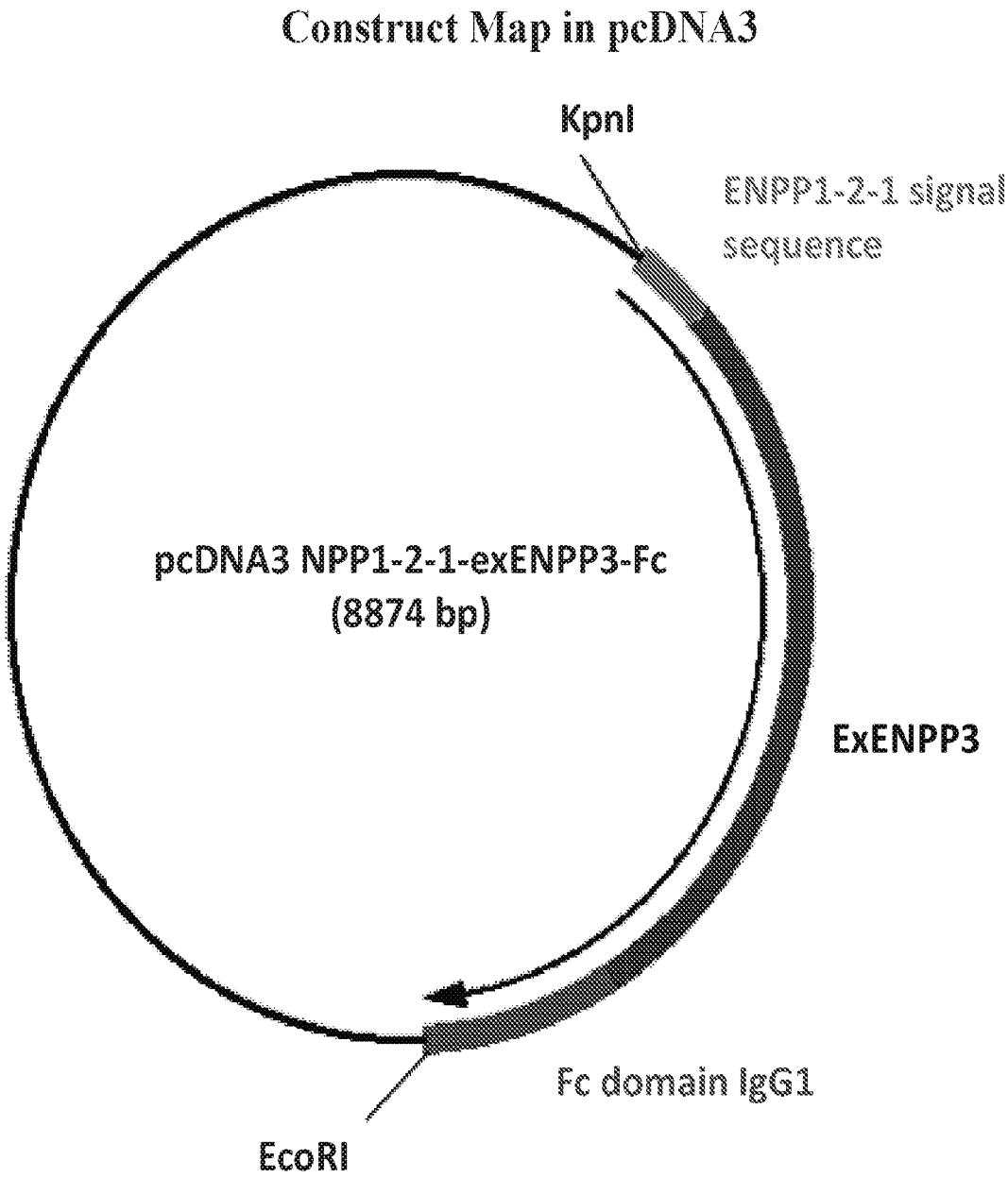
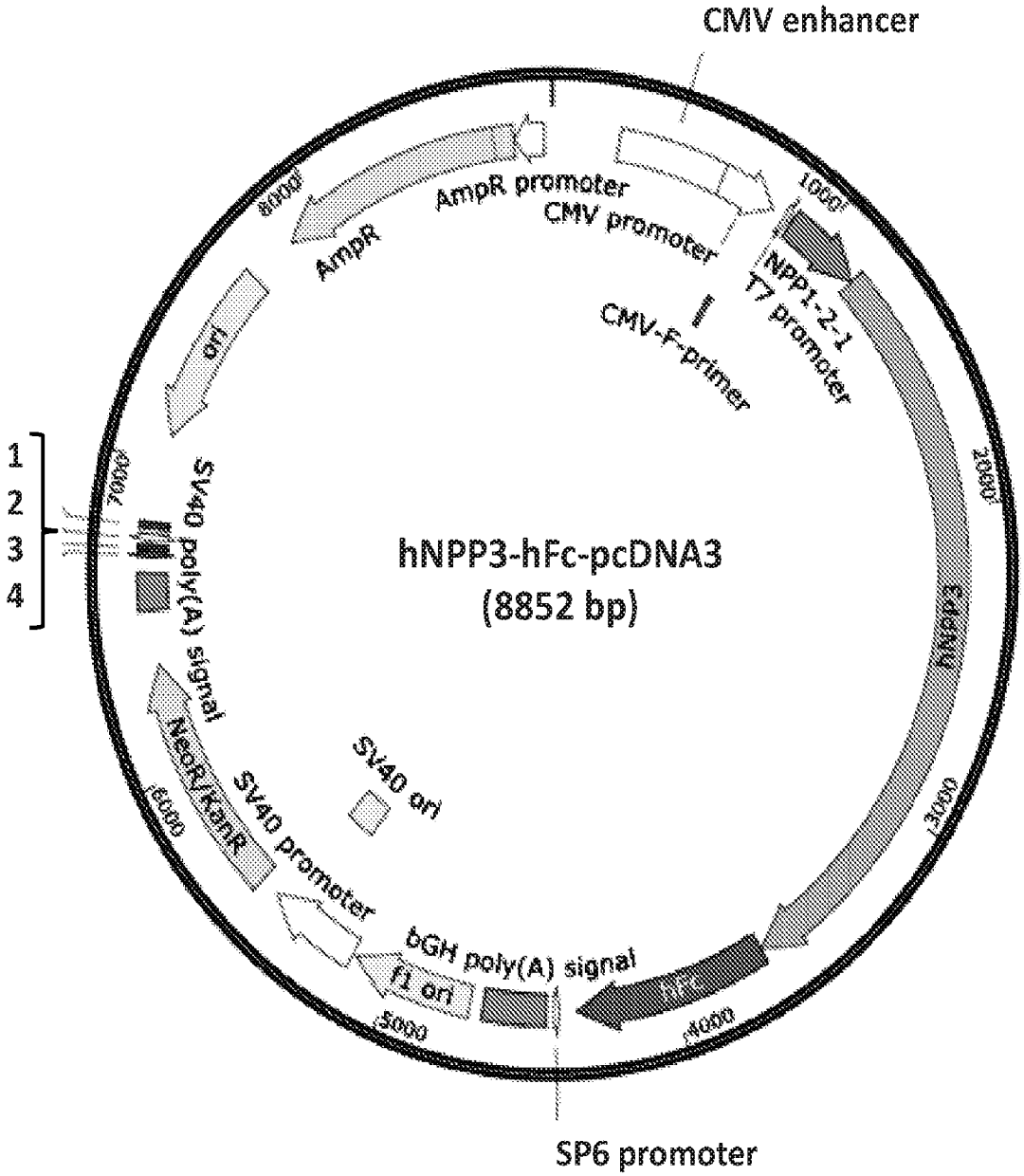

Construct Map in pcDNA3
KpnI
ENPP1-2-1 signal sequence
pcDNA3 NPP1-2-1-exENPP3-Fc
(8874 bp)
ExENPP3
Fc domain IgG1
EcoRI

FIG. 4



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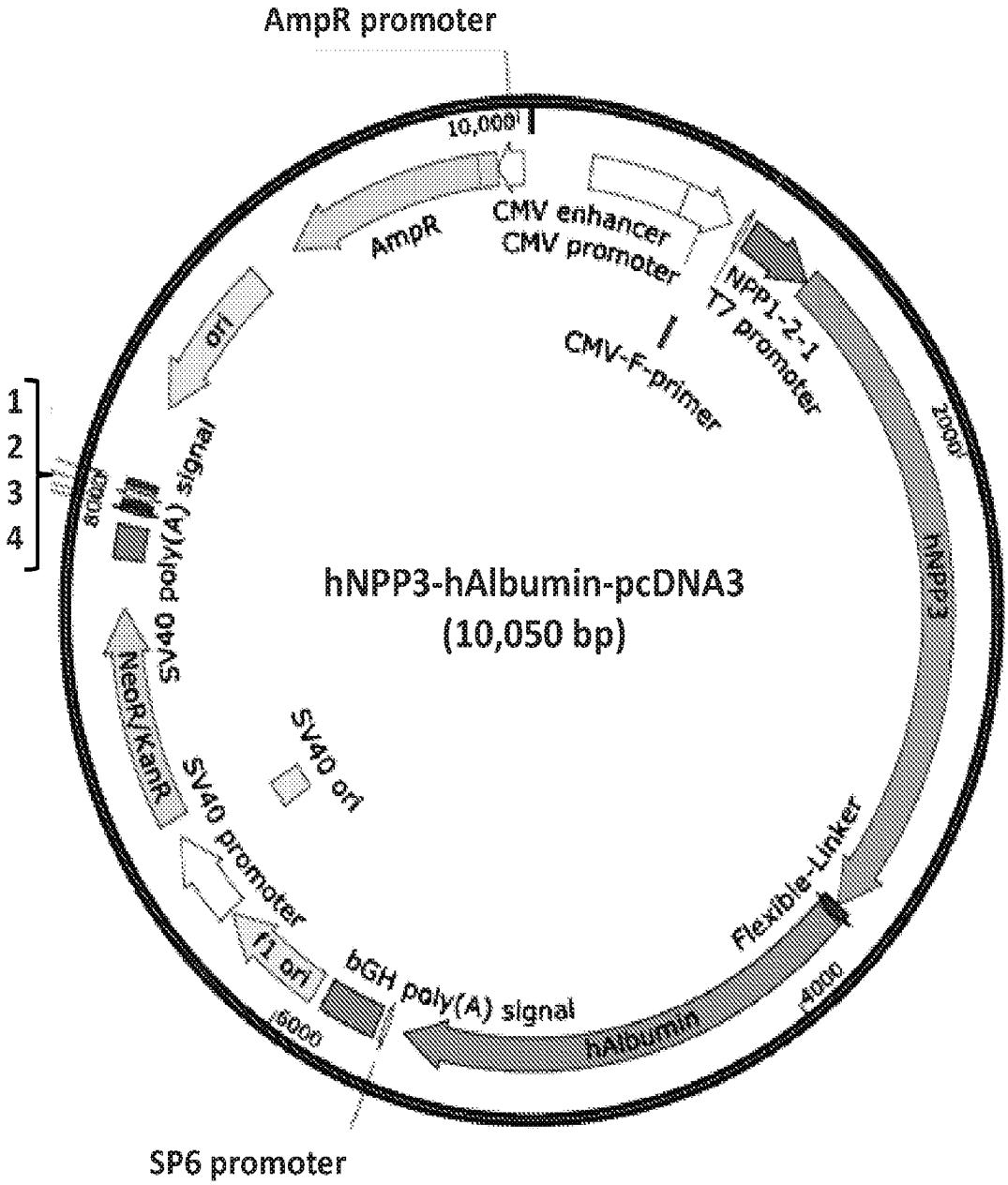
lac promoter — 2

lac operator — 3

M13 rev — 4

FIG. 5



CAP binding site — 1

lac promoter — 2

lac operator — 3

M13 rev — 4

SEQUENCE LISTING

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<120> Compositions for Treating Ectopic Calcification Disorders, and Methods Using Same

<130> 047162-7077WO1(00479)

<150> US 62/257,883
<151> 2015-11-20

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<170> PatentIn version 3.5

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<220>
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<400> 1

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20 25 30

Cys Cys Trp Asp Phe Glu Asp Thr Cys Val Glu Ser Thr Arg Ile Trp
35 40 45

Met Cys Asn Lys Phe Arg Cys Gly Glu Thr Arg Leu Glu Ala Ser Leu
50 55 60

Cys Ser Cys Ser Asp Asp Cys Leu Gln Arg Lys Asp Cys Cys Ala Asp
65 70 75 80

Tyr Lys Ser Val Cys Gln Gly Glu Thr Ser Trp Leu Glu Glu Asn Cys
85 90 95

Asp Thr Ala Gln Gln Ser Gln Cys Pro Glu Gly Phe Asp Leu Pro Pro
100 105 110

Val Ile Leu Phe Ser Met Asp Gly Phe Arg Ala Glu Tyr Leu Tyr Thr
115 120 125

Trp Asp Thr Leu Met Pro Asn Ile Asn Lys Leu Lys Thr Cys Gly Ile
130 135 140

His Ser Lys Tyr Met Arg Ala Met Tyr Pro Thr Lys Thr Phe Pro Asn
145 150 155 160

His Tyr Thr Ile Val Thr Gly Leu Tyr Pro Glu Ser His Gly Ile Ile

165 170 175

Asp Asn Asn Met Tyr Asp Val Asn Leu Asn Lys Asn Phe Ser Leu Ser
180 185 190

Ser Lys Glu Gln Asn Asn Pro Ala Trp Trp His Gly Gln Pro Met Trp
195 200 205

Leu Thr Ala Met Tyr Gln Gly Leu Lys Ala Ala Thr Tyr Phe Trp Pro
210 215 220

Gly Ser Glu Val Ala Ile Asn Gly Ser Phe Pro Ser Ile Tyr Met Pro
225 230 235 240

Tyr Asn Gly Ser Val Pro Phe Glu Glu Arg Ile Ser Thr Leu Leu Lys
245 250 255

Trp Leu Asp Leu Pro Lys Ala Glu Arg Pro Arg Phe Tyr Thr Met Tyr
260 265 270

Phe Glu Glu Pro Asp Ser Ser Gly His Ala Gly Gly Pro Val Ser Ala
275 280 285

Arg Val Ile Lys Ala Leu Gln Val Val Asp His Ala Phe Gly Met Leu
290 295 300

Met Glu Gly Leu Lys Gln Arg Asn Leu His Asn Cys Val Asn Ile Ile
305 310 315 320

Leu Leu Ala Asp His Gly Met Asp Gln Thr Tyr Cys Asn Lys Met Glu
325 330 335

Tyr Met Thr Asp Tyr Phe Pro Arg Ile Asn Phe Phe Tyr Met Tyr Glu
340 345 350

Gly Pro Ala Pro Arg Ile Arg Ala His Asn Ile Pro His Asp Phe Phe
355 360 365

Ser Phe Asn Ser Glu Glu Ile Val Arg Asn Leu Ser Cys Arg Lys Pro
370 375 380

Asp Gln His Phe Lys Pro Tyr Leu Thr Pro Asp Leu Pro Lys Arg Leu
385 390 395 400

His Tyr Ala Lys Asn Val Arg Ile Asp Lys Val His Leu Phe Val Asp
405 410 415

Gln Gln Trp Leu Ala Val Arg Ser Lys Ser Asn Thr Asn Cys Gly Gly
420 425 430

Gly Asn His Gly Tyr Asn Asn Glu Phe Arg Ser Met Glu Ala Ile Phe

435 440 445

Leu Ala His Gly Pro Ser Phe Lys Glu Lys Thr Glu Val Glu Pro Phe
450 455 460

Glu Asn Ile Glu Val Tyr Asn Leu Met Cys Asp Leu Leu Arg Ile Gln
465 470 475 480

Pro Ala Pro Asn Asn Gly Thr His Gly Ser Leu Asn His Leu Leu Lys
485 490 495

Val Pro Phe Tyr Glu Pro Ser His Ala Glu Glu Val Ser Lys Phe Ser
500 505 510

Val Cys Gly Phe Ala Asn Pro Leu Pro Thr Glu Ser Leu Asp Cys Phe
515 520 525

Cys Pro His Leu Gln Asn Ser Thr Gln Leu Glu Gln Val Asn Gln Met
530 535 540

Leu Asn Leu Thr Gln Glu Glu Ile Thr Ala Thr Val Lys Val Asn Leu
545 550 555 560

Pro Phe Gly Arg Pro Arg Val Leu Gln Lys Asn Val Asp His Cys Leu
565 570 575

Leu Tyr His Arg Glu Tyr Val Ser Gly Phe Gly Lys Ala Met Arg Met
580 585 590

Pro Met Trp Ser Ser Tyr Thr Val Pro Gln Leu Gly Asp Thr Ser Pro
595 600 605

Leu Pro Pro Thr Val Pro Asp Cys Leu Arg Ala Asp Val Arg Val Pro
610 615 620

Pro Ser Glu Ser Gln Lys Cys Ser Phe Tyr Leu Ala Asp Lys Asn Ile
625 630 635 640

Thr His Gly Phe Leu Tyr Pro Pro Ala Ser Asn Arg Thr Ser Asp Ser
645 650 655

Gln Tyr Asp Ala Leu Ile Thr Ser Asn Leu Val Pro Met Tyr Glu Glu
660 665 670

Phe Arg Lys Met Trp Asp Tyr Phe His Ser Val Leu Leu Ile Lys His
675 680 685

Ala Thr Glu Arg Asn Gly Val Asn Val Val Ser Gly Pro Ile Phe Asp
690 695 700

Tyr Asn Tyr Asp Gly His Phe Asp Ala Pro Asp Glu Ile Thr Lys His

705 710 715 720

Leu Ala Asn Thr Asp Val Pro Ile Pro Thr His Tyr Phe Val Val Leu
725 730 735

Thr Ser Cys Lys Asn Lys Ser His Thr Pro Glu Asn Cys Pro Gly Trp
740 745 750

Leu Asp Val Leu Pro Phe Ile Ile Pro His Arg Pro Thr Asn Val Glu
755 760 765

Ser Cys Pro Glu Gly Lys Pro Glu Ala Leu Trp Val Glu Glu Arg Phe
770 775 780

Thr Ala His Ile Ala Arg Val Arg Asp Val Glu Leu Leu Thr Gly Leu
785 790 795 800

Asp Phe Tyr Gln Asp Lys Val Gln Pro Val Ser Glu Ile Leu Gln Leu
805 810 815

Lys Thr Tyr Leu Pro Thr Phe Glu Thr Thr Ile
820 825

<210> 2
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> Signal sequence ENPP7

<400> 2

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

Ala Pro Gly Ala
20

<210> 3
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Signal sequence ENPP7

<400> 3

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
20

<210> 4

<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Signal Sequence ENPP5

<400> 4

Met Thr Ser Lys Phe Leu Leu Val Ser Phe Ile Leu Ala Ala Leu Ser
1 5 10 15

Leu Ser Thr Thr Phe Ser
20

<210> 5
<211> 95
<212> PRT
<213> Artificial Sequence

<220>
<223> Signal Sequence ENPP1-2-1

<400> 5

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
85 90 95

<210> 6
<211> 3
<212> PRT
<213> Artificial Sequence

<220>
<223> Chemically synthesized

<220>
<221> REPEAT
<222> (1)..(3)
<223> (DSS)n, wherein n is an integer ranging between 1 and 20

<400> 6

Asp Ser Ser

1

<210> 7
<211> 3
<212> PRT
<213> Artificial Sequence

<220>
<223> Chemically synthesized

<220>
<221> REPEAT
<222> (1)..(3)
<223> (ESS)n, wherein n is an integer ranging between 1 and 20

<400> 7

Glu Ser Ser
1

<210> 8
<211> 3
<212> PRT
<213> Artificial Sequence

<220>
<223> Chemically synthesized

<220>
<221> REPEAT
<222> (1)..(3)
<223> (RQQ)n, wherein n is an integer ranging between 1 and 20

<400> 8

Arg Gln Gln
1

<210> 9
<211> 2
<212> PRT
<213> Artificial Sequence

<220>
<223> Chemically synthesized

<220>
<221> REPEAT
<222> (1)..(2)
<223> (KR)n, wherein n is an integer ranging between 1 and 20

<400> 9

Lys Arg
1

<210> 10
<211> 1
<212> PRT
<213> Artificial Sequence

<220>
<223> Chemically synthesized

<220>
<221> REPEAT
<222> (1)..(1)
<223> (R)n, wherein n is an integer ranging between 1 and 20

<400> 10

Arg
1

<210> 11
<211> 2
<212> PRT
<213> Artificial Sequence

<220>
<223> Chemically synthesized

<220>
<221> REPEAT
<222> (1)..(2)
<223> (KR)n, wherein n is an integer ranging between 1 and 20

<400> 11

Lys Arg
1

<210> 12
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Chemically synthesized

<400> 12

Asp Ser Ser Ser Glu Glu Lys Phe Leu Arg Arg Ile Gly Arg Phe Gly
1 5 10 15

<210> 13
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Chemically synthesized

<400> 13

Glu Glu Glu Glu Glu Glu Glu Pro Arg Gly Asp Thr
1 5 10

<210> 14
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Chemically synthesized

<400> 14

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

<210> 15
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Chemically synthesized

<400> 15

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

<210> 16
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Chemically synthesized

<400> 16

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

<210> 17
<211> 1
<212> PRT
<213> Artificial Sequence

<220>
<223> Chemically synthesized

<220>
<221> REPEAT
<222> (1)..(1)
<223> (E)n, wherein n is an integer ranging between 1 and 20

<400> 17

Glu
1

<210> 18
<211> 1
<212> PRT
<213> Artificial Sequence

<220>
<223> Chemically synthesized

<220>
<221> REPEAT

<222> (1)..(1)
<223> (D)n, wherein n is an integer ranging between 1 and 20

<400> 18

Asp
1

<210> 19
<211> 1147
<212> PRT
<213> Artificial Sequence

<220>
<223> ENPP121-NPP3-Fc sequence

<400> 19

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 Lys
85 90 95

Gln Gly Ser Cys Arg Lys Lys Cys Phe Asp Ala Ser Phe Arg Gly Leu
100 105 110

Glu Asn Cys Arg Cys Asp Val Ala Cys Lys Asp Arg Gly Asp Cys Cys
115 120 125

Trp Asp Phe Glu Asp Thr Cys Val Glu Ser Thr Arg Ile Trp Met Cys
130 135 140

Asn Lys Phe Arg Cys Gly Glu Arg Leu Glu Ala Ser Leu Cys Ser Cys
145 150 155 160

Ser Asp Asp Cys Leu Gln Arg Lys Asp Cys Cys Ala Asp Tyr Lys Ser
165 170 175

Val Cys Gln Gly Glu Thr Ser Trp Leu Glu Glu Asn Cys Asp Thr Ala
180 185 190

Gln Gln Ser Gln Cys Pro Glu Gly Phe Asp Leu Pro Pro Val Ile Leu

195 200 205

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

Leu Met Pro Asn Ile Asn Lys Leu Lys Thr Cys Gly Ile His Ser Lys
225 230 235 240

Tyr Met Arg Ala Met Tyr Pro Thr Lys Thr Phe Pro Asn His Tyr Thr
245 250 255

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

Met Tyr Asp Val Asn Leu Asn Lys Asn Phe Ser Leu Ser Ser Lys Glu
275 280 285

Gln Asn Asn Pro Ala Trp Trp His Gly Gln Pro Met Trp Leu Thr Ala
290 295 300

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

Val Ala Ile Asn Gly Ser Phe Pro Ser Ile Tyr Met Pro Tyr Asn Gly
325 330 335

Ser Val Pro Phe Glu Glu Arg Ile Ser Thr Leu Leu Lys Trp Leu Asp
340 345 350

Leu Pro Lys Ala Glu Arg Pro Arg Phe Tyr Thr Met Tyr Phe Glu Glu
355 360 365

Pro Asp Ser Ser Gly His Ala Gly Gly Pro Val Ser Ala Arg Val Ile
370 375 380

Lys Ala Leu Gln Val Val Asp His Ala Phe Gly Met Leu Met Glu Gly
385 390 395 400

Leu Lys Gln Arg Asn Leu His Asn Cys Val Asn Ile Ile Leu Leu Ala
405 410 415

Asp His Gly Met Asp Gln Thr Tyr Cys Asn Lys Met Glu Tyr Met Thr
420 425 430

Asp Tyr Phe Pro Arg Ile Asn Phe Phe Tyr Met Tyr Glu Gly Pro Ala
435 440 445

Pro Arg Ile Arg Ala His Asn Ile Pro His Asp Phe Phe Ser Phe Asn
450 455 460

Ser Glu Glu Ile Val Arg Asn Leu Ser Cys Arg Lys Pro Asp Gln His

465 470 475 480

Phe Lys Pro Tyr Leu Thr Pro Asp Leu Pro Lys Arg Leu His Tyr Ala
485 490 495

Lys Asn Val Arg Ile Asp Lys Val His Leu Phe Val Asp Gln Gln Trp
500 505 510

Leu Ala Val Arg Ser Lys Ser Asn Thr Asn Cys Gly Gly Gly Asn His
515 520 525

Gly Tyr Asn Asn Glu Phe Arg Ser Met Glu Ala Ile Phe Leu Ala His
530 535 540

Gly Pro Ser Phe Lys Glu Lys Thr Glu Val Glu Pro Phe Glu Asn Ile
545 550 555 560

Glu Val Tyr Asn Leu Met Cys Asp Leu Leu Arg Ile Gln Pro Ala Pro
565 570 575

Asn Asn Gly Thr His Gly Ser Leu Asn His Leu Leu Lys Val Pro Phe
580 585 590

Tyr Glu Pro Ser His Ala Glu Glu Val Ser Lys Phe Ser Val Cys Gly
595 600 605

Phe Ala Asn Pro Leu Pro Thr Glu Ser Leu Asp Cys Phe Cys Pro His
610 615 620

Leu Gln Asn Ser Thr Gln Leu Glu Gln Val Asn Gln Met Leu Asn Leu
625 630 635 640

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

Arg Pro Arg Val Leu Gln Lys Asn Val Asp His Cys Leu Leu Tyr His
660 665 670

Arg Glu Tyr Val Ser Gly Phe Gly Lys Ala Met Arg Met Pro Met Trp
675 680 685

Ser Ser Tyr Thr Val Pro Gln Leu Gly Asp Thr Ser Pro Leu Pro Pro
690 695 700

Thr Val Pro Asp Cys Leu Arg Ala Asp Val Arg Val Pro Pro Ser Glu
705 710 715 720

Ser Gln Lys Cys Ser Phe Tyr Leu Ala Asp Lys Asn Ile Thr His Gly
725 730 735

Phe Leu Tyr Pro Pro Ala Ser Asn Arg Thr Ser Asp Ser Gln Tyr Asp

740 745 750

Ala Leu Ile Thr Ser Asn Leu Val Pro Met Tyr Glu Glu Phe Arg Lys
755 760 765

Met Trp Asp Tyr Phe His Ser Val Leu Leu Ile Lys His Ala Thr Glu
770 775 780

Arg Asn Gly Val Asn Val Val Ser Gly Pro Ile Phe Asp Tyr Asn Tyr
785 790 795 800

Asp Gly His Phe Asp Ala Pro Asp Glu Ile Thr Lys His Leu Ala Asn
805 810 815

Thr Asp Val Pro Ile Pro Thr His Tyr Phe Val Val Leu Thr Ser Cys
820 825 830

Lys Asn Lys Ser His Thr Pro Glu Asn Cys Pro Gly Trp Leu Asp Val
835 840 845

Leu Pro Phe Ile Ile Pro His Arg Pro Thr Asn Val Glu Ser Cys Pro
850 855 860

Glu Gly Lys Pro Glu Ala Leu Trp Val Glu Glu Arg Phe Thr Ala His
865 870 875 880

Ile Ala Arg Val Arg Asp Val Glu Leu Leu Thr Gly Leu Asp Phe Tyr
885 890 895

Gln Asp Lys Val Gln Pro Val Ser Glu Ile Leu Gln Leu Lys Thr Tyr
900 905 910

Leu Pro Thr Phe Glu Thr Thr Ile Asp Lys Thr His Thr Cys Pro Pro
915 920 925

Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
930 935 940

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
945 950 955 960

Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
965 970 975

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
980 985 990

Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
995 1000 1005

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val

1010 1015 1020

Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
1025 1030 1035

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
1040 1045 1050

Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
1055 1060 1065

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
1070 1075 1080

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
1085 1090 1095

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

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
1115 1120 1125

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
1130 1135 1140

Ser Pro Gly Lys
1145

<210> 20
<211> 227
<212> PRT
<213> Artificial Sequence

<220>
<223> IgG Fc sequence

<400> 20

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
1 5 10 15

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
20 25 30

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
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

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
    210                 215                 220

Pro Gly Lys
225
```

<210> 21
<211> 1072
<212> PRT
<213> Artificial Sequence

<220>
<223> ENPP7-NPP3-Fc sequence

<400> 21

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

Ala Pro Gly Ala Lys Gln Gly Ser Cys Arg Lys Lys Cys Phe Asp Ala
            20                  25                  30

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

Arg Gly Asp Cys Cys Trp Asp Phe Glu Asp Thr Cys Val Glu Ser Thr
    50                  55                  60

Arg Ile Trp Met Cys Asn Lys Phe Arg Cys Gly Glu Arg Leu Glu Ala
```

65 70 75 80

Ser Leu Cys Ser Cys Ser Asp Asp Cys Leu Gln Arg Lys Asp Cys Cys
85 90 95

Ala Asp Tyr Lys Ser Val Cys Gln Gly Glu Thr Ser Trp Leu Glu Glu
100 105 110

Asn Cys Asp Thr Ala Gln Gln Ser Gln Cys Pro Glu Gly Phe Asp Leu
115 120 125

Pro Pro Val Ile Leu Phe Ser Met Asp Gly Phe Arg Ala Glu Tyr Leu
130 135 140

Tyr Thr Trp Asp Thr Leu Met Pro Asn Ile Asn Lys Leu Lys Thr Cys
145 150 155 160

Gly Ile His Ser Lys Tyr Met Arg Ala Met Tyr Pro Thr Lys Thr Phe
165 170 175

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

Ile Ile Asp Asn Asn Met Tyr Asp Val Asn Leu Asn Lys Asn Phe Ser
195 200 205

Leu Ser Ser Lys Glu Gln Asn Asn Pro Ala Trp Trp His Gly Gln Pro
210 215 220

Met Trp Leu Thr Ala Met Tyr Gln Gly Leu Lys Ala Ala Thr Tyr Phe
225 230 235 240

Trp Pro Gly Ser Glu Val Ala Ile Asn Gly Ser Phe Pro Ser Ile Tyr
245 250 255

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

Leu Lys Trp Leu Asp Leu Pro Lys Ala Glu Arg Pro Arg Phe Tyr Thr
275 280 285

Met Tyr Phe Glu Glu Pro Asp Ser Ser Gly His Ala Gly Gly Pro Val
290 295 300

Ser Ala Arg Val Ile Lys Ala Leu Gln Val Val Asp His Ala Phe Gly
305 310 315 320

Met Leu Met Glu Gly Leu Lys Gln Arg Asn Leu His Asn Cys Val Asn
325 330 335

Ile Ile Leu Leu Ala Asp His Gly Met Asp Gln Thr Tyr Cys Asn Lys

340 345 350

Met Glu Tyr Met Thr Asp Tyr Phe Pro Arg Ile Asn Phe Phe Tyr Met
355 360 365

Tyr Glu Gly Pro Ala Pro Arg Ile Arg Ala His Asn Ile Pro His Asp
370 375 380

Phe Phe Ser Phe Asn Ser Glu Glu Ile Val Arg Asn Leu Ser Cys Arg
385 390 395 400

Lys Pro Asp Gln His Phe Lys Pro Tyr Leu Thr Pro Asp Leu Pro Lys
405 410 415

Arg Leu His Tyr Ala Lys Asn Val Arg Ile Asp Lys Val His Leu Phe
420 425 430

Val Asp Gln Gln Trp Leu Ala Val Arg Ser Lys Ser Asn Thr Asn Cys
435 440 445

Gly Gly Gly Asn His Gly Tyr Asn Asn Glu Phe Arg Ser Met Glu Ala
450 455 460

Ile Phe Leu Ala His Gly Pro Ser Phe Lys Glu Lys Thr Glu Val Glu
465 470 475 480

Pro Phe Glu Asn Ile Glu Val Tyr Asn Leu Met Cys Asp Leu Leu Arg
485 490 495

Ile Gln Pro Ala Pro Asn Asn Gly Thr His Gly Ser Leu Asn His Leu
500 505 510

Leu Lys Val Pro Phe Tyr Glu Pro Ser His Ala Glu Glu Val Ser Lys
515 520 525

Phe Ser Val Cys Gly Phe Ala Asn Pro Leu Pro Thr Glu Ser Leu Asp
530 535 540

Cys Phe Cys Pro His Leu Gln Asn Ser Thr Gln Leu Glu Gln Val Asn
545 550 555 560

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

Asn Leu Pro Phe Gly Arg Pro Arg Val Leu Gln Lys Asn Val Asp His
580 585 590

Cys Leu Leu Tyr His Arg Glu Tyr Val Ser Gly Phe Gly Lys Ala Met
595 600 605

Arg Met Pro Met Trp Ser Ser Tyr Thr Val Pro Gln Leu Gly Asp Thr

610 615 620

Ser Pro Leu Pro Pro Thr Val Pro Asp Cys Leu Arg Ala Asp Val Arg
625 630 635 640

Val Pro Pro Ser Glu Ser Gln Lys Cys Ser Phe Tyr Leu Ala Asp Lys
645 650 655

Asn Ile Thr His Gly Phe Leu Tyr Pro Pro Ala Ser Asn Arg Thr Ser
660 665 670

Asp Ser Gln Tyr Asp Ala Leu Ile Thr Ser Asn Leu Val Pro Met Tyr
675 680 685

Glu Glu Phe Arg Lys Met Trp Asp Tyr Phe His Ser Val Leu Leu Ile
690 695 700

Lys His Ala Thr Glu Arg Asn Gly Val Asn Val Val Ser Gly Pro Ile
705 710 715 720

Phe Asp Tyr Asn Tyr Asp Gly His Phe Asp Ala Pro Asp Glu Ile Thr
725 730 735

Lys His Leu Ala Asn Thr Asp Val Pro Ile Pro Thr His Tyr Phe Val
740 745 750

Val Leu Thr Ser Cys Lys Asn Lys Ser His Thr Pro Glu Asn Cys Pro
755 760 765

Gly Trp Leu Asp Val Leu Pro Phe Ile Ile Pro His Arg Pro Thr Asn
770 775 780

Val Glu Ser Cys Pro Glu Gly Lys Pro Glu Ala Leu Trp Val Glu Glu
785 790 795 800

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

Gly Leu Asp Phe Tyr Gln Asp Lys Val Gln Pro Val Ser Glu Ile Leu
820 825 830

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

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
850 855 860

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
865 870 875 880

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro

885 890 895

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
900 905 910

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
915 920 925

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
930 935 940

Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
945 950 955 960

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

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

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
995 1000 1005

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
1010 1015 1020

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
1025 1030 1035

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
1040 1045 1050

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
1055 1060 1065

Ser Pro Gly Lys
1070

<210> 22
<211> 1074
<212> PRT
<213> Artificial Sequence

<220>
<223> ENPP5-NPP3-Fc sequence

<400> 22

Met Thr Ser Lys Phe Leu Leu Val Ser Phe Ile Leu Ala Ala Leu Ser
1 5 10 15

Leu Ser Thr Thr Phe Ser Lys Gln Gly Ser Cys Arg Lys Lys Cys Phe
20 25 30

```
Asp Ala Ser Phe Arg Gly Leu Glu Asn Cys Arg Cys Asp Val Ala Cys
        35                  40                  45

Lys Asp Arg Gly Asp Cys Cys Trp Asp Phe Glu Asp Thr Cys Val Glu
    50                  55                  60

Ser Thr Arg Ile Trp Met Cys Asn Lys Phe Arg Cys Gly Glu Arg Leu
65                  70                  75                  80

Glu Ala Ser Leu Cys Ser Cys Ser Asp Asp Cys Leu Gln Arg Lys Asp
                85                  90                  95

Cys Cys Ala Asp Tyr Lys Ser Val Cys Gln Gly Glu Thr Ser Trp Leu
            100                 105                 110

Glu Glu Asn Cys Asp Thr Ala Gln Gln Ser Gln Cys Pro Glu Gly Phe
        115                 120                 125

Asp Leu Pro Pro Val Ile Leu Phe Ser Met Asp Gly Phe Arg Ala Glu
    130                 135                 140

Tyr Leu Tyr Thr Trp Asp Thr Leu Met Pro Asn Ile Asn Lys Leu Lys
145                 150                 155                 160

Thr Cys Gly Ile His Ser Lys Tyr Met Arg Ala Met Tyr Pro Thr Lys
                165                 170                 175

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

His Gly Ile Ile Asp Asn Asn Met Tyr Asp Val Asn Leu Asn Lys Asn
        195                 200                 205

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

Gln Pro Met Trp Leu Thr Ala Met Tyr Gln Gly Leu Lys Ala Ala Thr
225                 230                 235                 240

Tyr Phe Trp Pro Gly Ser Glu Val Ala Ile Asn Gly Ser Phe Pro Ser
                245                 250                 255

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

Thr Leu Leu Lys Trp Leu Asp Leu Pro Lys Ala Glu Arg Pro Arg Phe
        275                 280                 285

Tyr Thr Met Tyr Phe Glu Glu Pro Asp Ser Ser Gly His Ala Gly Gly
    290                 295                 300
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Pro Val Ser Ala Arg Val Ile Lys Ala Leu Gln Val Val Asp His Ala
305                 310                 315                 320

Phe Gly Met Leu Met Glu Gly Leu Lys Gln Arg Asn Leu His Asn Cys
                325                 330                 335

Val Asn Ile Ile Leu Leu Ala Asp His Gly Met Asp Gln Thr Tyr Cys
            340                 345                 350

Asn Lys Met Glu Tyr Met Thr Asp Tyr Phe Pro Arg Ile Asn Phe Phe
        355                 360                 365

Tyr Met Tyr Glu Gly Pro Ala Pro Arg Ile Arg Ala His Asn Ile Pro
    370                 375                 380

His Asp Phe Phe Ser Phe Asn Ser Glu Glu Ile Val Arg Asn Leu Ser
385                 390                 395                 400

Cys Arg Lys Pro Asp Gln His Phe Lys Pro Tyr Leu Thr Pro Asp Leu
                405                 410                 415

Pro Lys Arg Leu His Tyr Ala Lys Asn Val Arg Ile Asp Lys Val His
            420                 425                 430

Leu Phe Val Asp Gln Gln Trp Leu Ala Val Arg Ser Lys Ser Asn Thr
        435                 440                 445

Asn Cys Gly Gly Gly Asn His Gly Tyr Asn Asn Glu Phe Arg Ser Met
    450                 455                 460

Glu Ala Ile Phe Leu Ala His Gly Pro Ser Phe Lys Glu Lys Thr Glu
465                 470                 475                 480

Val Glu Pro Phe Glu Asn Ile Glu Val Tyr Asn Leu Met Cys Asp Leu
                485                 490                 495

Leu Arg Ile Gln Pro Ala Pro Asn Asn Gly Thr His Gly Ser Leu Asn
            500                 505                 510

His Leu Leu Lys Val Pro Phe Tyr Glu Pro Ser His Ala Glu Glu Val
        515                 520                 525

Ser Lys Phe Ser Val Cys Gly Phe Ala Asn Pro Leu Pro Thr Glu Ser
    530                 535                 540

Leu Asp Cys Phe Cys Pro His Leu Gln Asn Ser Thr Gln Leu Glu Gln
545                 550                 555                 560

Val Asn Gln Met Leu Asn Leu Thr Gln Glu Glu Ile Thr Ala Thr Val
                565                 570                 575
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Lys Val Asn Leu Pro Phe Gly Arg Pro Arg Val Leu Gln Lys Asn Val
            580                 585                 590

Asp His Cys Leu Leu Tyr His Arg Glu Tyr Val Ser Gly Phe Gly Lys
        595                 600                 605

Ala Met Arg Met Pro Met Trp Ser Ser Tyr Thr Val Pro Gln Leu Gly
    610                 615                 620

Asp Thr Ser Pro Leu Pro Pro Thr Val Pro Asp Cys Leu Arg Ala Asp
625                 630                 635                 640

Val Arg Val Pro Pro Ser Glu Ser Gln Lys Cys Ser Phe Tyr Leu Ala
                645                 650                 655

Asp Lys Asn Ile Thr His Gly Phe Leu Tyr Pro Pro Ala Ser Asn Arg
            660                 665                 670

Thr Ser Asp Ser Gln Tyr Asp Ala Leu Ile Thr Ser Asn Leu Val Pro
        675                 680                 685

Met Tyr Glu Glu Phe Arg Lys Met Trp Asp Tyr Phe His Ser Val Leu
    690                 695                 700

Leu Ile Lys His Ala Thr Glu Arg Asn Gly Val Asn Val Val Ser Gly
705                 710                 715                 720

Pro Ile Phe Asp Tyr Asn Tyr Asp Gly His Phe Asp Ala Pro Asp Glu
                725                 730                 735

Ile Thr Lys His Leu Ala Asn Thr Asp Val Pro Ile Pro Thr His Tyr
            740                 745                 750

Phe Val Val Leu Thr Ser Cys Lys Asn Lys Ser His Thr Pro Glu Asn
        755                 760                 765

Cys Pro Gly Trp Leu Asp Val Leu Pro Phe Ile Ile Pro His Arg Pro
    770                 775                 780

Thr Asn Val Glu Ser Cys Pro Glu Gly Lys Pro Glu Ala Leu Trp Val
785                 790                 795                 800

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

Leu Thr Gly Leu Asp Phe Tyr Gln Asp Lys Val Gln Pro Val Ser Glu
            820                 825                 830

Ile Leu Gln Leu Lys Thr Tyr Leu Pro Thr Phe Glu Thr Thr Ile Asp
        835                 840                 845
```

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Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
    850                 855                 860

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
865                 870                 875                 880

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
                885                 890                 895

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

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
        915                 920                 925

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
    930                 935                 940

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
945                 950                 955                 960

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

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

Thr Cys Leu Val Lys Gly Phe Tyr  Pro Ser Asp Ile Ala  Val Glu Trp
        995                 1000                1005

Glu Ser  Asn Gly Gln Pro Glu  Asn Asn Tyr Lys Thr  Thr Pro Pro
    1010                1015                1020

Val Leu  Asp Ser Asp Gly Ser  Phe Phe Leu Tyr Ser  Lys Leu Thr
    1025                1030                1035

Val Asp  Lys Ser Arg Trp Gln  Gln Gly Asn Val Phe  Ser Cys Ser
    1040                1045                1050

Val Met  His Glu Ala Leu His  Asn His Tyr Thr Gln  Lys Ser Leu
    1055                1060                1065

Ser Leu  Ser Pro Gly Lys
    1070
```

<210> 23
<211> 623
<212> PRT
<213> Artificial Sequence

<220>
<223> Albumin sequence

<400> 23

```
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Met
1               5                   10                  15

Lys Trp Val Thr Phe Leu Leu Leu Leu Phe Val Ser Gly Ser Ala Phe
            20                  25                  30

Ser Arg Gly Val Phe Arg Arg Glu Ala His Lys Ser Glu Ile Ala His
        35                  40                  45

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

Ala Phe Ser Gln Tyr Leu Gln Lys Cys Ser Tyr Asp Glu His Ala Lys
65                  70                  75                  80

Leu Val Gln Glu Val Thr Asp Phe Ala Lys Thr Cys Val Ala Asp Glu
                85                  90                  95

Ser Ala Ala Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp Lys
            100                 105                 110

Leu Cys Ala Ile Pro Asn Leu Arg Glu Asn Tyr Gly Glu Leu Ala Asp
        115                 120                 125

Cys Cys Thr Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln His
    130                 135                 140

Lys Asp Asp Asn Pro Ser Leu Pro Pro Phe Glu Arg Pro Glu Ala Glu
145                 150                 155                 160

Ala Met Cys Thr Ser Phe Lys Glu Asn Pro Thr Thr Phe Met Gly His
                165                 170                 175

Tyr Leu His Glu Val Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu
            180                 185                 190

Leu Leu Tyr Tyr Ala Glu Gln Tyr Asn Glu Ile Leu Thr Gln Cys Cys
        195                 200                 205

Ala Glu Ala Asp Lys Glu Ser Cys Leu Thr Pro Lys Leu Asp Gly Val
    210                 215                 220

Lys Glu Lys Ala Leu Val Ser Ser Val Arg Gln Arg Met Lys Cys Ser
225                 230                 235                 240

Ser Met Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala
                245                 250                 255

Arg Leu Ser Gln Thr Phe Pro Asn Ala Asp Phe Ala Glu Ile Thr Lys
```

260 265 270

Leu Ala Thr Asp Leu Thr Lys Val Asn Lys Glu Cys Cys His Gly Asp
275 280 285

Leu Leu Glu Cys Ala Asp Asp Arg Ala Glu Leu Ala Lys Tyr Met Cys
290 295 300

Glu Asn Gln Ala Thr Ile Ser Ser Lys Leu Gln Thr Cys Cys Asp Lys
305 310 315 320

Pro Leu Leu Lys Lys Ala His Cys Leu Ser Glu Val Glu His Asp Thr
325 330 335

Met Pro Ala Asp Leu Pro Ala Ile Ala Ala Asp Phe Val Glu Asp Gln
340 345 350

Glu Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly Thr
355 360 365

Phe Leu Tyr Glu Tyr Ser Arg Arg His Pro Asp Tyr Ser Val Ser Leu
370 375 380

Leu Leu Arg Leu Ala Lys Lys Tyr Glu Ala Thr Leu Glu Lys Cys Cys
385 390 395 400

Ala Glu Ala Asn Pro Pro Ala Cys Tyr Gly Thr Val Leu Ala Glu Phe
405 410 415

Gln Pro Leu Val Glu Glu Pro Lys Asn Leu Val Lys Thr Asn Cys Asp
420 425 430

Leu Tyr Glu Lys Leu Gly Glu Tyr Gly Phe Gln Asn Ala Ile Leu Val
435 440 445

Arg Tyr Thr Gln Lys Ala Pro Gln Val Ser Thr Pro Thr Leu Val Glu
450 455 460

Ala Ala Arg Asn Leu Gly Arg Val Gly Thr Lys Cys Cys Thr Leu Pro
465 470 475 480

Glu Asp Gln Arg Leu Pro Cys Val Glu Asp Tyr Leu Ser Ala Ile Leu
485 490 495

Asn Arg Val Cys Leu Leu His Glu Lys Thr Pro Val Ser Glu His Val
500 505 510

Thr Lys Cys Cys Ser Gly Ser Leu Val Glu Arg Arg Pro Cys Phe Ser
515 520 525

Ala Leu Thr Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Lys Ala Glu

530 535 540

Thr Phe Thr Phe His Ser Asp Ile Cys Thr Leu Pro Glu Lys Glu Lys
545 550 555 560

Gln Ile Lys Lys Gln Thr Ala Leu Ala Glu Leu Val Lys His Lys Pro
565 570 575

Lys Ala Thr Ala Glu Gln Leu Lys Thr Val Met Asp Asp Phe Ala Gln
580 585 590

Phe Leu Asp Thr Cys Cys Lys Ala Ala Asp Lys Asp Thr Cys Phe Ser
595 600 605

Thr Glu Gly Pro Asn Leu Val Thr Arg Cys Lys Asp Ala Leu Ala
610 615 620

<210> 24
<211> 1542
<212> PRT
<213> Artificial Sequence

<220>
<223> ENPP121-NPP3-Albumin sequence

<400> 24

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 Lys
85 90 95

Gln Gly Ser Cys Arg Lys Lys Cys Phe Asp Ala Ser Phe Arg Gly Leu
100 105 110

Glu Asn Cys Arg Cys Asp Val Ala Cys Lys Asp Arg Gly Asp Cys Cys
115 120 125

Trp Asp Phe Glu Asp Thr Cys Val Glu Ser Thr Arg Ile Trp Met Cys
130 135 140

```
Asn Lys Phe Arg Cys Gly Glu Arg Leu Glu Ala Ser Leu Cys Ser Cys
145                 150                 155                 160

Ser Asp Asp Cys Leu Gln Arg Lys Asp Cys Cys Ala Asp Tyr Lys Ser
                165                 170                 175

Val Cys Gln Gly Glu Thr Ser Trp Leu Glu Glu Asn Cys Asp Thr Ala
            180                 185                 190

Gln Gln Ser Gln Cys Pro Glu Gly Phe Asp Leu Pro Pro Val Ile Leu
        195                 200                 205

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

Leu Met Pro Asn Ile Asn Lys Leu Lys Thr Cys Gly Ile His Ser Lys
225                 230                 235                 240

Tyr Met Arg Ala Met Tyr Pro Thr Lys Thr Phe Pro Asn His Tyr Thr
                245                 250                 255

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

Met Tyr Asp Val Asn Leu Asn Lys Asn Phe Ser Leu Ser Ser Lys Glu
        275                 280                 285

Gln Asn Asn Pro Ala Trp Trp His Gly Gln Pro Met Trp Leu Thr Ala
    290                 295                 300

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

Val Ala Ile Asn Gly Ser Phe Pro Ser Ile Tyr Met Pro Tyr Asn Gly
                325                 330                 335

Ser Val Pro Phe Glu Glu Arg Ile Ser Thr Leu Leu Lys Trp Leu Asp
            340                 345                 350

Leu Pro Lys Ala Glu Arg Pro Arg Phe Tyr Thr Met Tyr Phe Glu Glu
        355                 360                 365

Pro Asp Ser Ser Gly His Ala Gly Gly Pro Val Ser Ala Arg Val Ile
    370                 375                 380

Lys Ala Leu Gln Val Val Asp His Ala Phe Gly Met Leu Met Glu Gly
385                 390                 395                 400

Leu Lys Gln Arg Asn Leu His Asn Cys Val Asn Ile Ile Leu Leu Ala
                405                 410                 415
```

```
Asp His Gly Met Asp Gln Thr Tyr Cys Asn Lys Met Glu Tyr Met Thr
            420                 425                 430

Asp Tyr Phe Pro Arg Ile Asn Phe Phe Tyr Met Tyr Glu Gly Pro Ala
        435                 440                 445

Pro Arg Ile Arg Ala His Asn Ile Pro His Asp Phe Phe Ser Phe Asn
    450                 455                 460

Ser Glu Glu Ile Val Arg Asn Leu Ser Cys Arg Lys Pro Asp Gln His
465                 470                 475                 480

Phe Lys Pro Tyr Leu Thr Pro Asp Leu Pro Lys Arg Leu His Tyr Ala
                485                 490                 495

Lys Asn Val Arg Ile Asp Lys Val His Leu Phe Val Asp Gln Gln Trp
            500                 505                 510

Leu Ala Val Arg Ser Lys Ser Asn Thr Asn Cys Gly Gly Gly Asn His
        515                 520                 525

Gly Tyr Asn Asn Glu Phe Arg Ser Met Glu Ala Ile Phe Leu Ala His
    530                 535                 540

Gly Pro Ser Phe Lys Glu Lys Thr Glu Val Glu Pro Phe Glu Asn Ile
545                 550                 555                 560

Glu Val Tyr Asn Leu Met Cys Asp Leu Leu Arg Ile Gln Pro Ala Pro
                565                 570                 575

Asn Asn Gly Thr His Gly Ser Leu Asn His Leu Leu Lys Val Pro Phe
            580                 585                 590

Tyr Glu Pro Ser His Ala Glu Glu Val Ser Lys Phe Ser Val Cys Gly
        595                 600                 605

Phe Ala Asn Pro Leu Pro Thr Glu Ser Leu Asp Cys Phe Cys Pro His
    610                 615                 620

Leu Gln Asn Ser Thr Gln Leu Glu Gln Val Asn Gln Met Leu Asn Leu
625                 630                 635                 640

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

Arg Pro Arg Val Leu Gln Lys Asn Val Asp His Cys Leu Leu Tyr His
            660                 665                 670

Arg Glu Tyr Val Ser Gly Phe Gly Lys Ala Met Arg Met Pro Met Trp
        675                 680                 685
```

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Ser Ser Tyr Thr Val Pro Gln Leu Gly Asp Thr Ser Pro Leu Pro Pro
    690                 695                 700

Thr Val Pro Asp Cys Leu Arg Ala Asp Val Arg Val Pro Pro Ser Glu
705                 710                 715                 720

Ser Gln Lys Cys Ser Phe Tyr Leu Ala Asp Lys Asn Ile Thr His Gly
                725                 730                 735

Phe Leu Tyr Pro Pro Ala Ser Asn Arg Thr Ser Asp Ser Gln Tyr Asp
            740                 745                 750

Ala Leu Ile Thr Ser Asn Leu Val Pro Met Tyr Glu Glu Phe Arg Lys
        755                 760                 765

Met Trp Asp Tyr Phe His Ser Val Leu Leu Ile Lys His Ala Thr Glu
    770                 775                 780

Arg Asn Gly Val Asn Val Val Ser Gly Pro Ile Phe Asp Tyr Asn Tyr
785                 790                 795                 800

Asp Gly His Phe Asp Ala Pro Asp Glu Ile Thr Lys His Leu Ala Asn
                805                 810                 815

Thr Asp Val Pro Ile Pro Thr His Tyr Phe Val Val Leu Thr Ser Cys
            820                 825                 830

Lys Asn Lys Ser His Thr Pro Glu Asn Cys Pro Gly Trp Leu Asp Val
        835                 840                 845

Leu Pro Phe Ile Ile Pro His Arg Pro Thr Asn Val Glu Ser Cys Pro
    850                 855                 860

Glu Gly Lys Pro Glu Ala Leu Trp Val Glu Glu Arg Phe Thr Ala His
865                 870                 875                 880

Ile Ala Arg Val Arg Asp Val Glu Leu Leu Thr Gly Leu Asp Phe Tyr
                885                 890                 895

Gln Asp Lys Val Gln Pro Val Ser Glu Ile Leu Gln Leu Lys Thr Tyr
            900                 905                 910

Leu Pro Thr Phe Glu Thr Thr Ile Gly Gly Gly Ser Gly Gly Gly Gly
        915                 920                 925

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

Phe Val Ser Gly Ser Ala Phe Ser Arg Gly Val Phe Arg Arg Glu Ala
945                 950                 955                 960
```

```
His Lys Ser Glu Ile Ala His Arg Tyr Asn Asp Leu Gly Glu Gln His
               965                 970                 975

Phe Lys Gly Leu Val Leu Ile Ala Phe Ser Gln Tyr Leu Gln Lys Cys
           980                 985                 990

Ser Tyr Asp Glu His Ala Lys Leu  Val Gln Glu Val Thr  Asp Phe Ala
       995                 1000                1005

Lys Thr  Cys Val Ala Asp Glu  Ser Ala Ala Asn Cys  Asp Lys Ser
    1010                1015                1020

Leu His  Thr Leu Phe Gly Asp  Lys Leu Cys Ala Ile  Pro Asn Leu
    1025                1030                1035

Arg Glu  Asn Tyr Gly Glu Leu  Ala Asp Cys Cys Thr  Lys Gln Glu
    1040                1045                1050

Pro Glu  Arg Asn Glu Cys Phe  Leu Gln His Lys Asp  Asp Asn Pro
    1055                1060                1065

Ser Leu  Pro Pro Phe Glu Arg  Pro Glu Ala Glu Ala  Met Cys Thr
    1070                1075                1080

Ser Phe  Lys Glu Asn Pro Thr  Thr Phe Met Gly His  Tyr Leu His
    1085                1090                1095

Glu Val  Ala Arg Arg His Pro  Tyr Phe Tyr Ala Pro  Glu Leu Leu
    1100                1105                1110

Tyr Tyr  Ala Glu Gln Tyr Asn  Glu Ile Leu Thr Gln  Cys Cys Ala
    1115                1120                1125

Glu Ala  Asp Lys Glu Ser Cys  Leu Thr Pro Lys Leu  Asp Gly Val
    1130                1135                1140

Lys Glu  Lys Ala Leu Val Ser  Ser Val Arg Gln Arg  Met Lys Cys
    1145                1150                1155

Ser Ser  Met Gln Lys Phe Gly  Glu Arg Ala Phe Lys  Ala Trp Ala
    1160                1165                1170

Val Ala  Arg Leu Ser Gln Thr  Phe Pro Asn Ala Asp  Phe Ala Glu
    1175                1180                1185

Ile Thr  Lys Leu Ala Thr Asp  Leu Thr Lys Val Asn  Lys Glu Cys
    1190                1195                1200

Cys His  Gly Asp Leu Leu Glu  Cys Ala Asp Asp Arg  Ala Glu Leu
    1205                1210                1215
```

```
Ala Lys  Tyr Met Cys Glu Asn  Gln Ala Thr Ile Ser  Ser Lys Leu
    1220                1225                1230

Gln Thr  Cys Cys Asp Lys Pro  Leu Leu Lys Lys Ala  His Cys Leu
    1235                1240                1245

Ser Glu  Val Glu His Asp Thr  Met Pro Ala Asp Leu  Pro Ala Ile
    1250                1255                1260

Ala Ala  Asp Phe Val Glu Asp  Gln Glu Val Cys Lys  Asn Tyr Ala
    1265                1270                1275

Glu Ala  Lys Asp Val Phe Leu  Gly Thr Phe Leu Tyr  Glu Tyr Ser
    1280                1285                1290

Arg Arg  His Pro Asp Tyr Ser  Val Ser Leu Leu Leu  Arg Leu Ala
    1295                1300                1305

Lys Lys  Tyr Glu Ala Thr Leu  Glu Lys Cys Cys Ala  Glu Ala Asn
    1310                1315                1320

Pro Pro  Ala Cys Tyr Gly Thr  Val Leu Ala Glu Phe  Gln Pro Leu
    1325                1330                1335

Val Glu  Glu Pro Lys Asn Leu  Val Lys Thr Asn Cys  Asp Leu Tyr
    1340                1345                1350

Glu Lys  Leu Gly Glu Tyr Gly  Phe Gln Asn Ala Ile  Leu Val Arg
    1355                1360                1365

Tyr Thr  Gln Lys Ala Pro Gln  Val Ser Thr Pro Thr  Leu Val Glu
    1370                1375                1380

Ala Ala  Arg Asn Leu Gly Arg  Val Gly Thr Lys Cys  Cys Thr Leu
    1385                1390                1395

Pro Glu  Asp Gln Arg Leu Pro  Cys Val Glu Asp Tyr  Leu Ser Ala
    1400                1405                1410

Ile Leu  Asn Arg Val Cys Leu  Leu His Glu Lys Thr  Pro Val Ser
    1415                1420                1425

Glu His  Val Thr Lys Cys Cys  Ser Gly Ser Leu Val  Glu Arg Arg
    1430                1435                1440

Pro Cys  Phe Ser Ala Leu Thr  Val Asp Glu Thr Tyr  Val Pro Lys
    1445                1450                1455

Glu Phe  Lys Ala Glu Thr Phe  Thr Phe His Ser Asp  Ile Cys Thr
    1460                1465                1470
```

```
Leu Pro  Glu Lys Glu Lys Gln  Ile Lys Lys Gln Thr  Ala Leu Ala
    1475                1480                1485


Glu Leu  Val Lys His Lys Pro  Lys Ala Thr Ala Glu  Gln Leu Lys
    1490                1495                1500


Thr Val  Met Asp Asp Phe Ala  Gln Phe Leu Asp Thr  Cys Cys Lys
    1505                1510                1515


Ala Ala  Asp Lys Asp Thr Cys  Phe Ser Thr Glu Gly  Pro Asn Leu
    1520                1525                1530


Val Thr  Arg Cys Lys Asp Ala  Leu Ala
    1535                1540
```

<210> 25
<211> 1467
<212> PRT
<213> Artificial Sequence

<220>
<223> ENPP7-NPP3-Albumin

<400> 25

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


Ala Pro Gly Ala Lys Gln Gly Ser Cys Arg Lys Lys Cys Phe Asp Ala
            20                  25                  30


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


Arg Gly Asp Cys Cys Trp Asp Phe Glu Asp Thr Cys Val Glu Ser Thr
    50                  55                  60


Arg Ile Trp Met Cys Asn Lys Phe Arg Cys Gly Glu Arg Leu Glu Ala
65                  70                  75                  80


Ser Leu Cys Ser Cys Ser Asp Asp Cys Leu Gln Arg Lys Asp Cys Cys
                85                  90                  95


Ala Asp Tyr Lys Ser Val Cys Gln Gly Glu Thr Ser Trp Leu Glu Glu
            100                 105                 110


Asn Cys Asp Thr Ala Gln Gln Ser Gln Cys Pro Glu Gly Phe Asp Leu
        115                 120                 125


Pro Pro Val Ile Leu Phe Ser Met Asp Gly Phe Arg Ala Glu Tyr Leu
    130                 135                 140


Tyr Thr Trp Asp Thr Leu Met Pro Asn Ile Asn Lys Leu Lys Thr Cys
```

145 150 155 160

Gly Ile His Ser Lys Tyr Met Arg Ala Met Tyr Pro Thr Lys Thr Phe
165 170 175

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

Ile Ile Asp Asn Asn Met Tyr Asp Val Asn Leu Asn Lys Asn Phe Ser
195 200 205

Leu Ser Ser Lys Glu Gln Asn Asn Pro Ala Trp Trp His Gly Gln Pro
210 215 220

Met Trp Leu Thr Ala Met Tyr Gln Gly Leu Lys Ala Ala Thr Tyr Phe
225 230 235 240

Trp Pro Gly Ser Glu Val Ala Ile Asn Gly Ser Phe Pro Ser Ile Tyr
245 250 255

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

Leu Lys Trp Leu Asp Leu Pro Lys Ala Glu Arg Pro Arg Phe Tyr Thr
275 280 285

Met Tyr Phe Glu Glu Pro Asp Ser Ser Gly His Ala Gly Gly Pro Val
290 295 300

Ser Ala Arg Val Ile Lys Ala Leu Gln Val Val Asp His Ala Phe Gly
305 310 315 320

Met Leu Met Glu Gly Leu Lys Gln Arg Asn Leu His Asn Cys Val Asn
325 330 335

Ile Ile Leu Leu Ala Asp His Gly Met Asp Gln Thr Tyr Cys Asn Lys
340 345 350

Met Glu Tyr Met Thr Asp Tyr Phe Pro Arg Ile Asn Phe Phe Tyr Met
355 360 365

Tyr Glu Gly Pro Ala Pro Arg Ile Arg Ala His Asn Ile Pro His Asp
370 375 380

Phe Phe Ser Phe Asn Ser Glu Glu Ile Val Arg Asn Leu Ser Cys Arg
385 390 395 400

Lys Pro Asp Gln His Phe Lys Pro Tyr Leu Thr Pro Asp Leu Pro Lys
405 410 415

Arg Leu His Tyr Ala Lys Asn Val Arg Ile Asp Lys Val His Leu Phe

420 425 430

Val Asp Gln Gln Trp Leu Ala Val Arg Ser Lys Ser Asn Thr Asn Cys
435 440 445

Gly Gly Gly Asn His Gly Tyr Asn Asn Glu Phe Arg Ser Met Glu Ala
450 455 460

Ile Phe Leu Ala His Gly Pro Ser Phe Lys Glu Lys Thr Glu Val Glu
465 470 475 480

Pro Phe Glu Asn Ile Glu Val Tyr Asn Leu Met Cys Asp Leu Leu Arg
485 490 495

Ile Gln Pro Ala Pro Asn Asn Gly Thr His Gly Ser Leu Asn His Leu
500 505 510

Leu Lys Val Pro Phe Tyr Glu Pro Ser His Ala Glu Glu Val Ser Lys
515 520 525

Phe Ser Val Cys Gly Phe Ala Asn Pro Leu Pro Thr Glu Ser Leu Asp
530 535 540

Cys Phe Cys Pro His Leu Gln Asn Ser Thr Gln Leu Glu Gln Val Asn
545 550 555 560

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

Asn Leu Pro Phe Gly Arg Pro Arg Val Leu Gln Lys Asn Val Asp His
580 585 590

Cys Leu Leu Tyr His Arg Glu Tyr Val Ser Gly Phe Gly Lys Ala Met
595 600 605

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

Ser Pro Leu Pro Pro Thr Val Pro Asp Cys Leu Arg Ala Asp Val Arg
625 630 635 640

Val Pro Pro Ser Glu Ser Gln Lys Cys Ser Phe Tyr Leu Ala Asp Lys
645 650 655

Asn Ile Thr His Gly Phe Leu Tyr Pro Pro Ala Ser Asn Arg Thr Ser
660 665 670

Asp Ser Gln Tyr Asp Ala Leu Ile Thr Ser Asn Leu Val Pro Met Tyr
675 680 685

Glu Glu Phe Arg Lys Met Trp Asp Tyr Phe His Ser Val Leu Leu Ile

690 695 700

Lys His Ala Thr Glu Arg Asn Gly Val Asn Val Val Ser Gly Pro Ile
705 710 715 720

Phe Asp Tyr Asn Tyr Asp Gly His Phe Asp Ala Pro Asp Glu Ile Thr
725 730 735

Lys His Leu Ala Asn Thr Asp Val Pro Ile Pro Thr His Tyr Phe Val
740 745 750

Val Leu Thr Ser Cys Lys Asn Lys Ser His Thr Pro Glu Asn Cys Pro
755 760 765

Gly Trp Leu Asp Val Leu Pro Phe Ile Ile Pro His Arg Pro Thr Asn
770 775 780

Val Glu Ser Cys Pro Glu Gly Lys Pro Glu Ala Leu Trp Val Glu Glu
785 790 795 800

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

Gly Leu Asp Phe Tyr Gln Asp Lys Val Gln Pro Val Ser Glu Ile Leu
820 825 830

Gln Leu Lys Thr Tyr Leu Pro Thr Phe Glu Thr Thr Ile Gly Gly Gly
835 840 845

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Met Lys Trp Val Thr
850 855 860

Phe Leu Leu Leu Leu Phe Val Ser Gly Ser Ala Phe Ser Arg Gly Val
865 870 875 880

Phe Arg Arg Glu Ala His Lys Ser Glu Ile Ala His Arg Tyr Asn Asp
885 890 895

Leu Gly Glu Gln His Phe Lys Gly Leu Val Leu Ile Ala Phe Ser Gln
900 905 910

Tyr Leu Gln Lys Cys Ser Tyr Asp Glu His Ala Lys Leu Val Gln Glu
915 920 925

Val Thr Asp Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Ala Asn
930 935 940

Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Ala Ile
945 950 955 960

Pro Asn Leu Arg Glu Asn Tyr Gly Glu Leu Ala Asp Cys Cys Thr Lys

965 970 975

Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn
980 985 990

Pro Ser Leu Pro Pro Phe Glu Arg Pro Glu Ala Glu Ala Met Cys Thr
995 1000 1005

Ser Phe Lys Glu Asn Pro Thr Thr Phe Met Gly His Tyr Leu His
1010 1015 1020

Glu Val Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu
1025 1030 1035

Tyr Tyr Ala Glu Gln Tyr Asn Glu Ile Leu Thr Gln Cys Cys Ala
1040 1045 1050

Glu Ala Asp Lys Glu Ser Cys Leu Thr Pro Lys Leu Asp Gly Val
1055 1060 1065

Lys Glu Lys Ala Leu Val Ser Ser Val Arg Gln Arg Met Lys Cys
1070 1075 1080

Ser Ser Met Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala
1085 1090 1095

Val Ala Arg Leu Ser Gln Thr Phe Pro Asn Ala Asp Phe Ala Glu
1100 1105 1110

Ile Thr Lys Leu Ala Thr Asp Leu Thr Lys Val Asn Lys Glu Cys
1115 1120 1125

Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Glu Leu
1130 1135 1140

Ala Lys Tyr Met Cys Glu Asn Gln Ala Thr Ile Ser Ser Lys Leu
1145 1150 1155

Gln Thr Cys Cys Asp Lys Pro Leu Leu Lys Lys Ala His Cys Leu
1160 1165 1170

Ser Glu Val Glu His Asp Thr Met Pro Ala Asp Leu Pro Ala Ile
1175 1180 1185

Ala Ala Asp Phe Val Glu Asp Gln Glu Val Cys Lys Asn Tyr Ala
1190 1195 1200

Glu Ala Lys Asp Val Phe Leu Gly Thr Phe Leu Tyr Glu Tyr Ser
1205 1210 1215

Arg Arg His Pro Asp Tyr Ser Val Ser Leu Leu Leu Arg Leu Ala

1220 1225 1230

Lys Lys Tyr Glu Ala Thr Leu Glu Lys Cys Cys Ala Glu Ala Asn
1235 1240 1245

Pro Pro Ala Cys Tyr Gly Thr Val Leu Ala Glu Phe Gln Pro Leu
1250 1255 1260

Val Glu Glu Pro Lys Asn Leu Val Lys Thr Asn Cys Asp Leu Tyr
1265 1270 1275

Glu Lys Leu Gly Glu Tyr Gly Phe Gln Asn Ala Ile Leu Val Arg
1280 1285 1290

Tyr Thr Gln Lys Ala Pro Gln Val Ser Thr Pro Thr Leu Val Glu
1295 1300 1305

Ala Ala Arg Asn Leu Gly Arg Val Gly Thr Lys Cys Cys Thr Leu
1310 1315 1320

Pro Glu Asp Gln Arg Leu Pro Cys Val Glu Asp Tyr Leu Ser Ala
1325 1330 1335

Ile Leu Asn Arg Val Cys Leu Leu His Glu Lys Thr Pro Val Ser
1340 1345 1350

Glu His Val Thr Lys Cys Cys Ser Gly Ser Leu Val Glu Arg Arg
1355 1360 1365

Pro Cys Phe Ser Ala Leu Thr Val Asp Glu Thr Tyr Val Pro Lys
1370 1375 1380

Glu Phe Lys Ala Glu Thr Phe Thr Phe His Ser Asp Ile Cys Thr
1385 1390 1395

Leu Pro Glu Lys Glu Lys Gln Ile Lys Lys Gln Thr Ala Leu Ala
1400 1405 1410

Glu Leu Val Lys His Lys Pro Lys Ala Thr Ala Glu Gln Leu Lys
1415 1420 1425

Thr Val Met Asp Asp Phe Ala Gln Phe Leu Asp Thr Cys Cys Lys
1430 1435 1440

Ala Ala Asp Lys Asp Thr Cys Phe Ser Thr Glu Gly Pro Asn Leu
1445 1450 1455

Val Thr Arg Cys Lys Asp Ala Leu Ala
1460 1465

<210> 26

<211> 1469
<212> PRT
<213> Artificial Sequence

<220>
<223> ENPP5-NPP3-albumin sequence

<400> 26

Met Thr Ser Lys Phe Leu Leu Val Ser Phe Ile Leu Ala Ala Leu Ser
1 5 10 15

Leu Ser Thr Thr Phe Ser Lys Gln Gly Ser Cys Arg Lys Lys Cys Phe
20 25 30

Asp Ala Ser Phe Arg Gly Leu Glu Asn Cys Arg Cys Asp Val Ala Cys
35 40 45

Lys Asp Arg Gly Asp Cys Cys Trp Asp Phe Glu Asp Thr Cys Val Glu
50 55 60

Ser Thr Arg Ile Trp Met Cys Asn Lys Phe Arg Cys Gly Glu Arg Leu
65 70 75 80

Glu Ala Ser Leu Cys Ser Cys Ser Asp Asp Cys Leu Gln Arg Lys Asp
85 90 95

Cys Cys Ala Asp Tyr Lys Ser Val Cys Gln Gly Glu Thr Ser Trp Leu
100 105 110

Glu Glu Asn Cys Asp Thr Ala Gln Gln Ser Gln Cys Pro Glu Gly Phe
115 120 125

Asp Leu Pro Pro Val Ile Leu Phe Ser Met Asp Gly Phe Arg Ala Glu
130 135 140

Tyr Leu Tyr Thr Trp Asp Thr Leu Met Pro Asn Ile Asn Lys Leu Lys
145 150 155 160

Thr Cys Gly Ile His Ser Lys Tyr Met Arg Ala Met Tyr Pro Thr Lys
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Thr Phe Pro Asn His Tyr Thr Ile Val Thr Gly Leu Tyr Pro Glu Ser
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His Gly Ile Ile Asp Asn Asn Met Tyr Asp Val Asn Leu Asn Lys Asn
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Phe Ser Leu Ser Ser Lys Glu Gln Asn Asn Pro Ala Trp Trp His Gly
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Gln Pro Met Trp Leu Thr Ala Met Tyr Gln Gly Leu Lys Ala Ala Thr
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Tyr Phe Trp Pro Gly Ser Glu Val Ala Ile Asn Gly Ser Phe Pro Ser
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Tyr Thr Met Tyr Phe Glu Glu Pro Asp Ser Ser Gly His Ala Gly Gly
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Pro Val Ser Ala Arg Val Ile Lys Ala Leu Gln Val Val Asp His Ala
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Phe Gly Met Leu Met Glu Gly Leu Lys Gln Arg Asn Leu His Asn Cys
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Val Asn Ile Ile Leu Leu Ala Asp His Gly Met Asp Gln Thr Tyr Cys
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Asn Lys Met Glu Tyr Met Thr Asp Tyr Phe Pro Arg Ile Asn Phe Phe
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Tyr Met Tyr Glu Gly Pro Ala Pro Arg Ile Arg Ala His Asn Ile Pro
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His Asp Phe Phe Ser Phe Asn Ser Glu Glu Ile Val Arg Asn Leu Ser
385                 390                 395                 400

Cys Arg Lys Pro Asp Gln His Phe Lys Pro Tyr Leu Thr Pro Asp Leu
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Pro Lys Arg Leu His Tyr Ala Lys Asn Val Arg Ile Asp Lys Val His
            420                 425                 430

Leu Phe Val Asp Gln Gln Trp Leu Ala Val Arg Ser Lys Ser Asn Thr
        435                 440                 445

Asn Cys Gly Gly Gly Asn His Gly Tyr Asn Asn Glu Phe Arg Ser Met
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Glu Ala Ile Phe Leu Ala His Gly Pro Ser Phe Lys Glu Lys Thr Glu
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Val Glu Pro Phe Glu Asn Ile Glu Val Tyr Asn Leu Met Cys Asp Leu
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Leu Arg Ile Gln Pro Ala Pro Asn Asn Gly Thr His Gly Ser Leu Asn
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His Leu Leu Lys Val Pro Phe Tyr Glu Pro Ser His Ala Glu Glu Val
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Ser Lys Phe Ser Val Cys Gly Phe Ala Asn Pro Leu Pro Thr Glu Ser
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Asp His Cys Leu Leu Tyr His Arg Glu Tyr Val Ser Gly Phe Gly Lys
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Ala Met Arg Met Pro Met Trp Ser Ser Tyr Thr Val Pro Gln Leu Gly
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Asp Thr Ser Pro Leu Pro Pro Thr Val Pro Asp Cys Leu Arg Ala Asp
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Val Arg Val Pro Pro Ser Glu Ser Gln Lys Cys Ser Phe Tyr Leu Ala
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Asp Lys Asn Ile Thr His Gly Phe Leu Tyr Pro Pro Ala Ser Asn Arg
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Thr Ser Asp Ser Gln Tyr Asp Ala Leu Ile Thr Ser Asn Leu Val Pro
675 680 685

Met Tyr Glu Glu Phe Arg Lys Met Trp Asp Tyr Phe His Ser Val Leu
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Leu Ile Lys His Ala Thr Glu Arg Asn Gly Val Asn Val Val Ser Gly
705 710 715 720

Pro Ile Phe Asp Tyr Asn Tyr Asp Gly His Phe Asp Ala Pro Asp Glu
725 730 735

Ile Thr Lys His Leu Ala Asn Thr Asp Val Pro Ile Pro Thr His Tyr
740 745 750

Phe Val Val Leu Thr Ser Cys Lys Asn Lys Ser His Thr Pro Glu Asn
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Cys Pro Gly Trp Leu Asp Val Leu Pro Phe Ile Ile Pro His Arg Pro
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Glu Glu Arg Phe Thr Ala His Ile Ala Arg Val Arg Asp Val Glu Leu
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Leu Thr Gly Leu Asp Phe Tyr Gln Asp Lys Val Gln Pro Val Ser Glu
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Ile Leu Gln Leu Lys Thr Tyr Leu Pro Thr Phe Glu Thr Thr Ile Gly
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Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Met Lys Trp
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Val Thr Phe Leu Leu Leu Leu Phe Val Ser Gly Ser Ala Phe Ser Arg
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Gly Val Phe Arg Arg Glu Ala His Lys Ser Glu Ile Ala His Arg Tyr
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Asn Asp Leu Gly Glu Gln His Phe Lys Gly Leu Val Leu Ile Ala Phe
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Ser Gln Tyr Leu Gln Lys Cys Ser Tyr Asp Glu His Ala Lys Leu Val
        915                 920                 925

Gln Glu Val Thr Asp Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala
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Ala Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys
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Ala Ile Pro Asn Leu Arg Glu Asn Tyr Gly Glu Leu Ala Asp Cys Cys
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Thr Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp
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Asp Asn Pro Ser Leu Pro Pro Phe  Glu Arg Pro Glu Ala  Glu Ala Met
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Cys Thr  Ser Phe Lys Glu Asn  Pro Thr Thr Phe Met  Gly His Tyr
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Leu His  Glu Val Ala Arg Arg  His Pro Tyr Phe Tyr  Ala Pro Glu
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Lys Cys  Ser Ser Met Gln Lys  Phe Gly Glu Arg Ala  Phe Lys Ala
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Trp Ala  Val Ala Arg Leu Ser  Gln Thr Phe Pro Asn  Ala Asp Phe
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Ala Glu  Ile Thr Lys Leu Ala  Thr Asp Leu Thr Lys  Val Asn Lys
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Glu Cys  Cys His Gly Asp Leu  Leu Glu Cys Ala Asp  Asp Arg Ala
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Glu Leu  Ala Lys Tyr Met Cys  Glu Asn Gln Ala Thr  Ile Ser Ser
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Lys Leu  Gln Thr Cys Cys Asp  Lys Pro Leu Leu Lys  Lys Ala His
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Cys Leu  Ser Glu Val Glu His  Asp Thr Met Pro Ala  Asp Leu Pro
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Ala Ile  Ala Ala Asp Phe Val  Glu Asp Gln Glu Val  Cys Lys Asn
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Tyr Ala  Glu Ala Lys Asp Val  Phe Leu Gly Thr Phe  Leu Tyr Glu
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Tyr Ser  Arg Arg His Pro Asp  Tyr Ser Val Ser Leu  Leu Leu Arg
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Leu Ala  Lys Lys Tyr Glu Ala  Thr Leu Glu Lys Cys  Cys Ala Glu
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Ala Asn  Pro Pro Ala Cys Tyr  Gly Thr Val Leu Ala  Glu Phe Gln
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Pro Leu  Val Glu Glu Pro Lys  Asn Leu Val Lys Thr  Asn Cys Asp
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Leu Tyr  Glu Lys Leu Gly Glu  Tyr Gly Phe Gln Asn  Ala Ile Leu
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Val Arg  Tyr Thr Gln Lys Ala  Pro Gln Val Ser Thr  Pro Thr Leu
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Ser Ala Ile Leu Asn Arg Val Cys Leu Leu His Glu Lys Thr Pro
1340 1345 1350

Val Ser Glu His Val Thr Lys Cys Cys Ser Gly Ser Leu Val Glu
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Arg Arg Pro Cys Phe Ser Ala Leu Thr Val Asp Glu Thr Tyr Val
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Pro Lys Glu Phe Lys Ala Glu Thr Phe Thr Phe His Ser Asp Ile
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Cys Thr Leu Pro Glu Lys Glu Lys Gln Ile Lys Lys Gln Thr Ala
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Leu Ala Glu Leu Val Lys His Lys Pro Lys Ala Thr Ala Glu Gln
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