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BRADDOCK et al.(10) **Pub. No.: US 2018/0371434 A1**(43) **Pub. Date: Dec. 27, 2018**(54) **COMPOSITIONS FOR TREATING ECTOPIC
CALCIFICATION DISORDERS, AND
METHODS USING SAME**(71) Applicant: **YALE UNIVERSITY**, New Haven, CT
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(2013.01)

(57)

ABSTRACTThe present invention includes compositions and methods
for treating disease and disorders associated with pathologi-
cal calcification or pathological ossification.**Specification includes a Sequence Listing.**

FIG. 1A

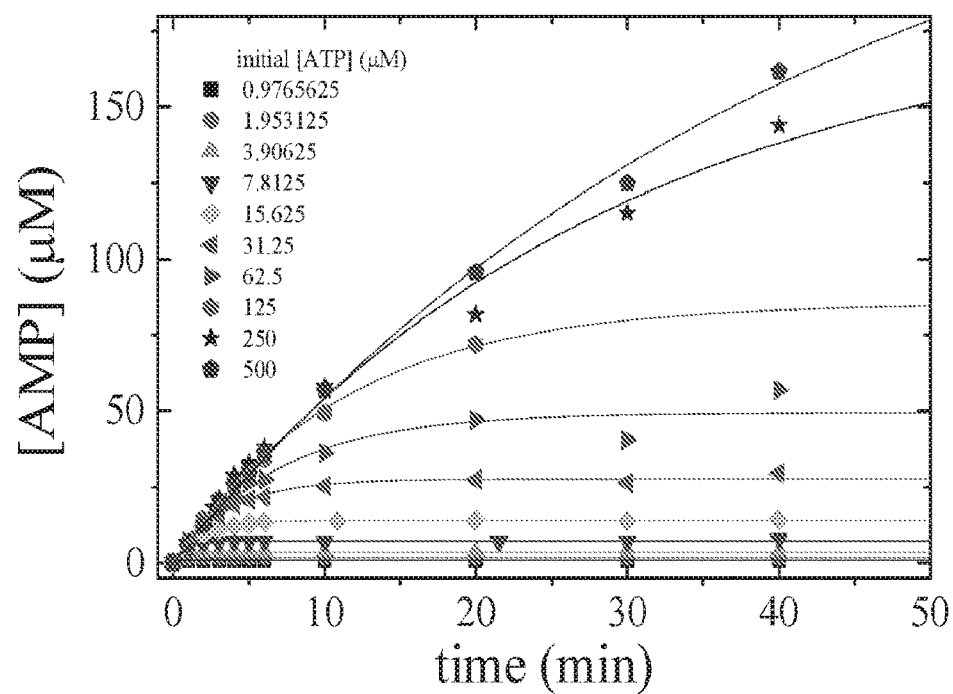


FIG. 1B

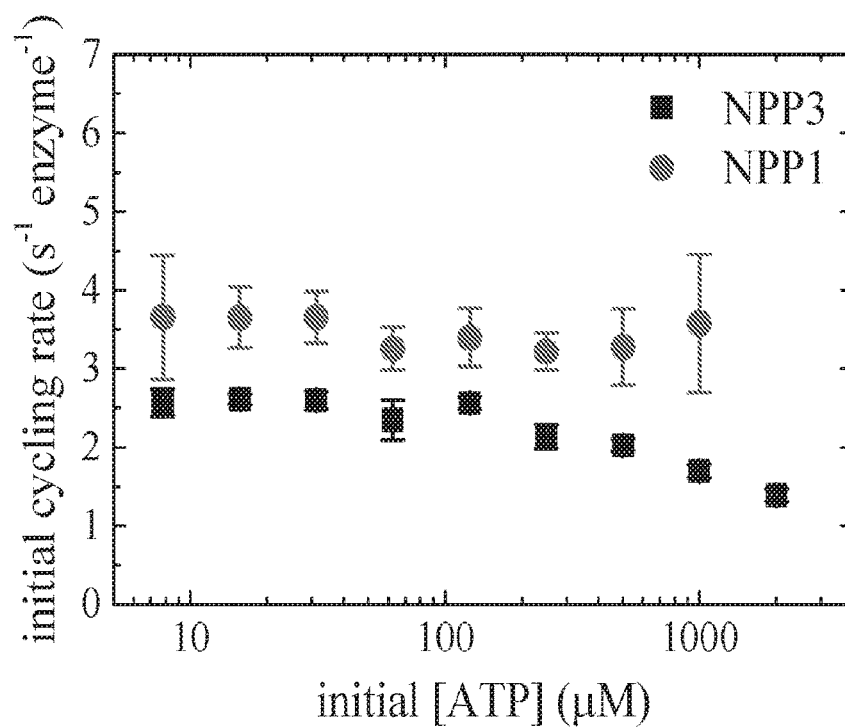


FIG. 1C

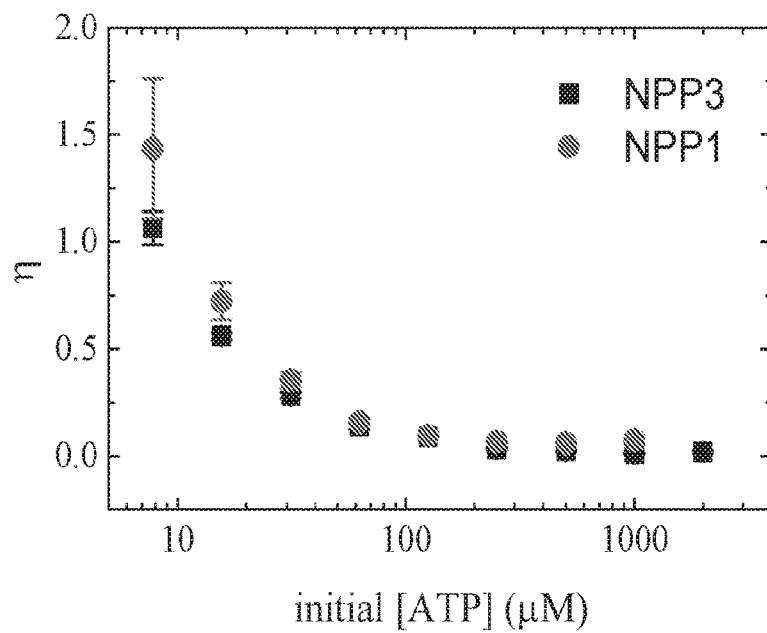
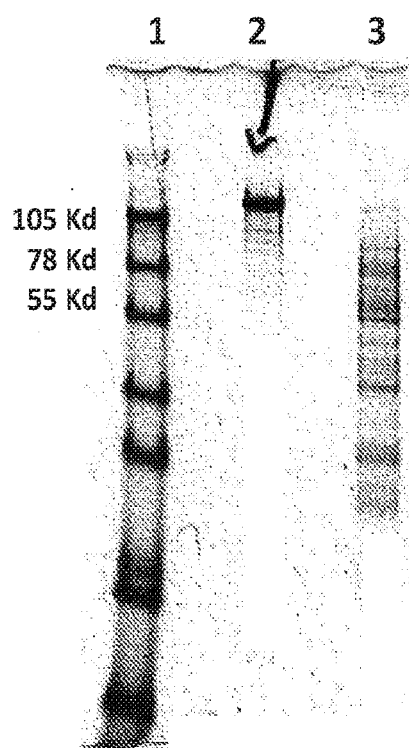


FIG. 2



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

FIG. 3

Construct Map in pcDNA3

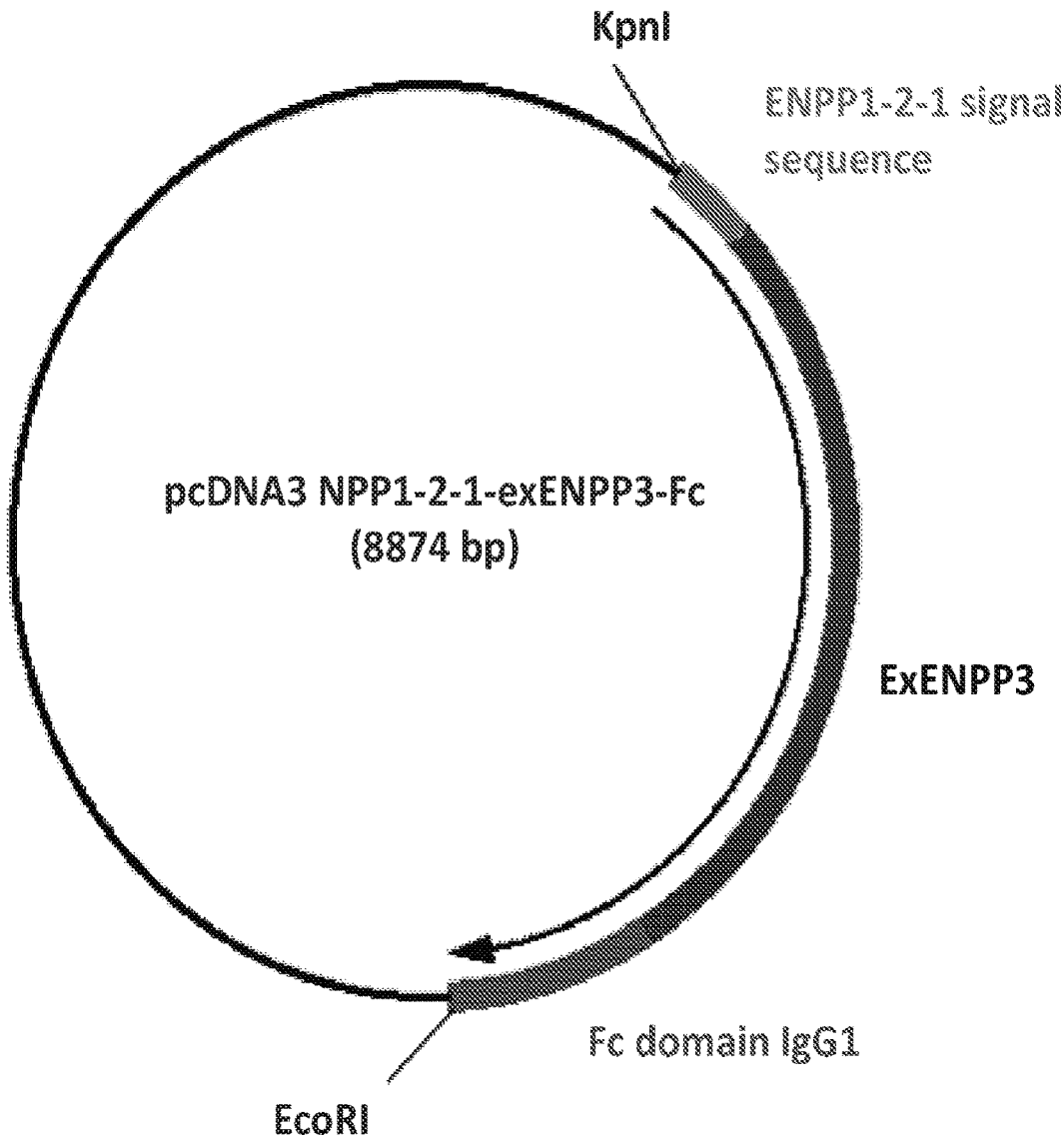


FIG. 4

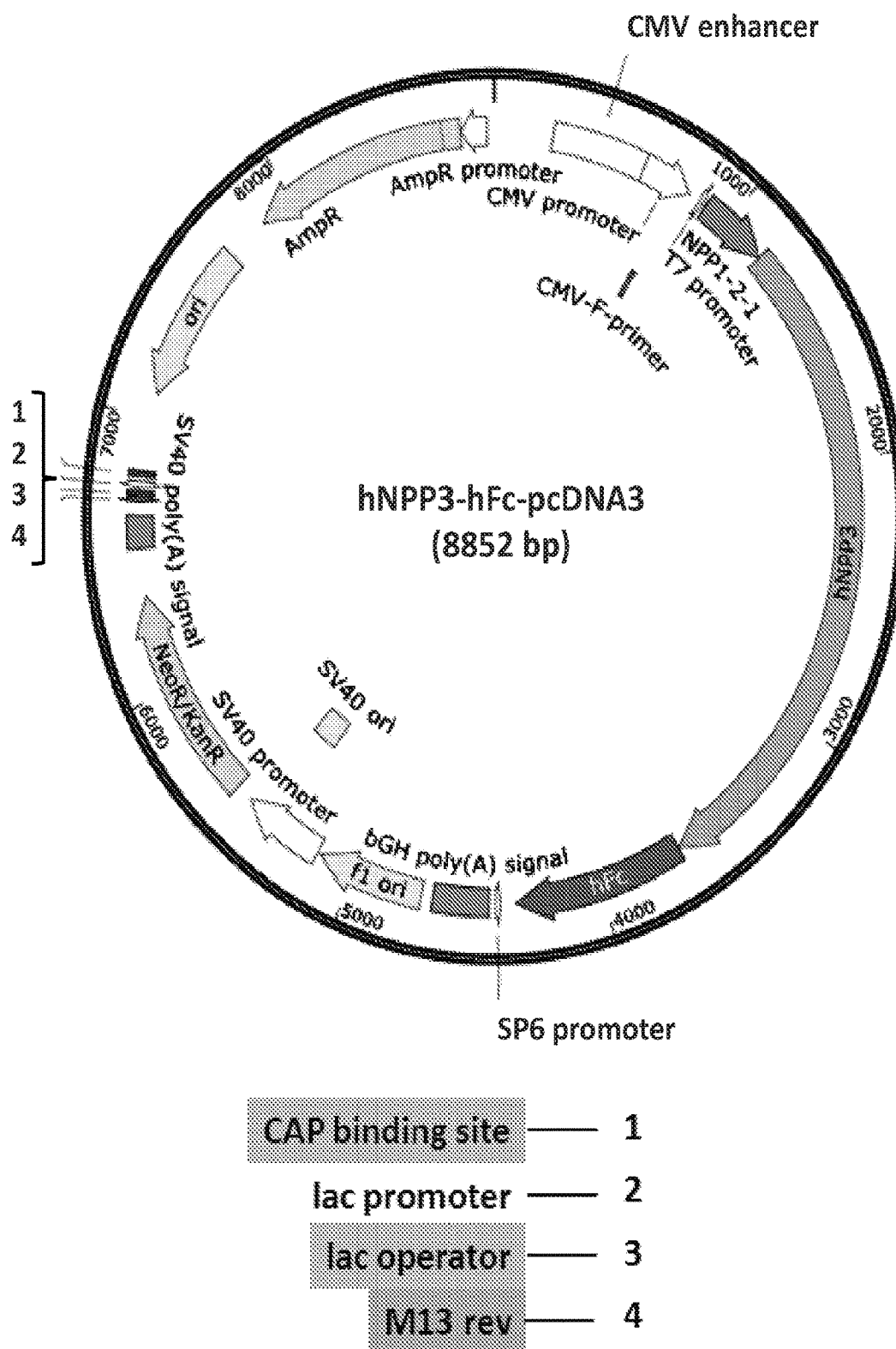
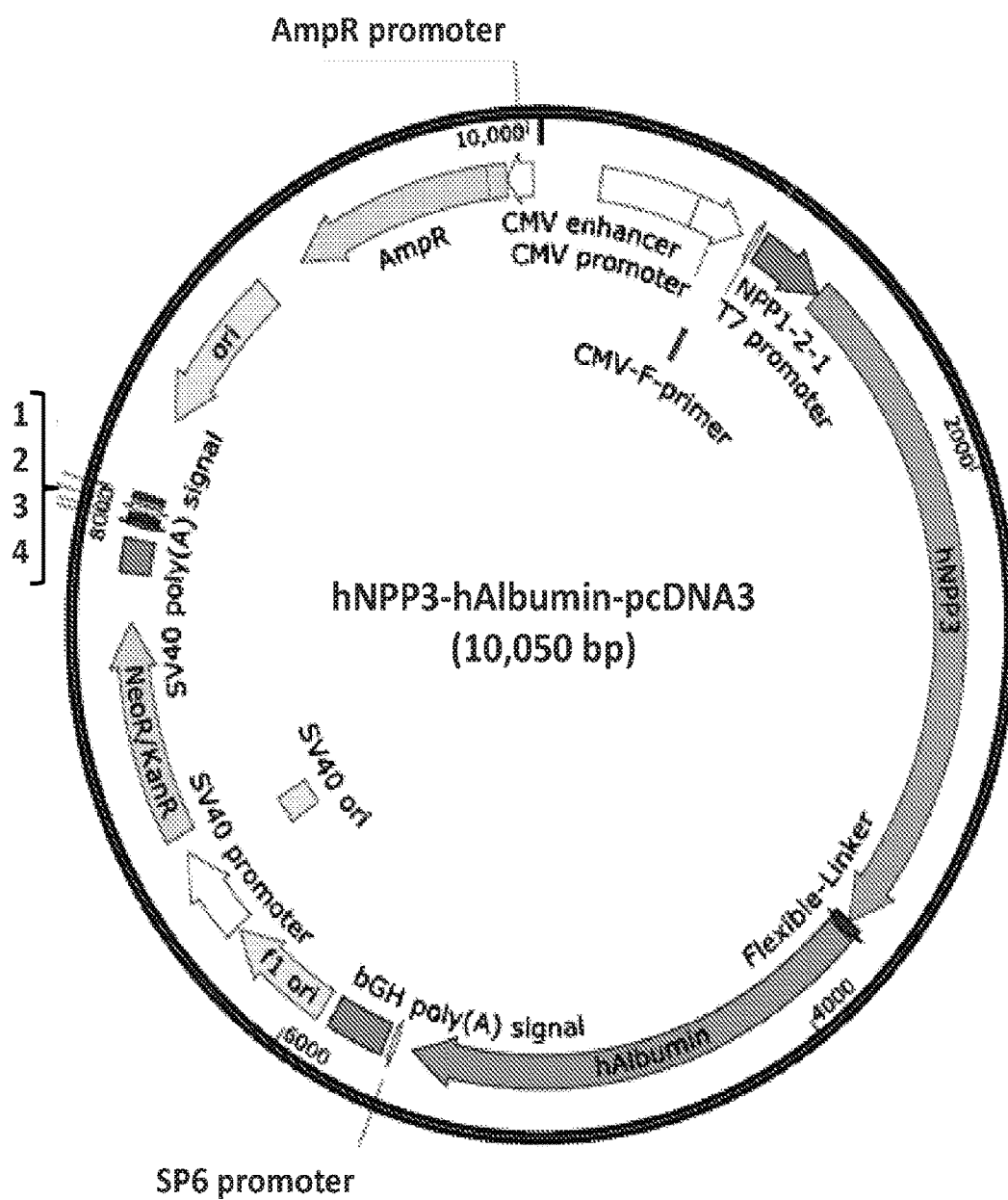


FIG. 5



CAP binding site — 1

lac promoter — 2

lac operator — 3

M13 rev — 4

COMPOSITIONS FOR TREATING ECTOPIC CALCIFICATION DISORDERS, AND METHODS USING SAME

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application No. 62/257,883, filed Nov. 20, 2015, which application is hereby incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] 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.

[0003] 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.

[0004] 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.

[0005] 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.

[0006] 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.

[0007] 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.

[0008] 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.

[0009] 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.

[0010] 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 hyper-

tension. Fibrous thickening of the endocardium and atrio-ventricular 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.

[0011] Conditions in which serum phosphate levels are reduced or elevated are referred to as hypophosphatemia and hyperphosphatemia, respectively. Hypophosphatemia, which often results from renal phosphate wasting, is caused by a number of genetic disorders including X-linked hypophosphatemic rickets (XLH), hereditary hypophosphatemic rickets with hypercalciuria (HHRH), hypophosphatemic bone disease (HBD), and autosomal dominant hypophosphatemic rickets (ADHR). The exact molecular mechanisms by which proper serum phosphate concentrations are maintained are poorly understood.

[0012] 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

[0013] 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.

[0014] 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 μ M 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 μ M 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 $2.59(\pm 0.04) \text{ s}^{-1} \text{ enzyme}^{-1}$, from the weighted average of the measured rates with 7.8, 15.6, 31.3, 62.5, 125 μ M substrate concentration, seeming slower than that for hNPP1 $3.46(\pm 0.44) \text{ s}^{-1} \text{ enzyme}^{-1}$. The K_M can be estimated $<8 \mu\text{M}$. At substrate $[\text{ATP}] > 125 \mu\text{M}$, hNPP3 ATPase cycling rate gradually decreased. FIG. 1C illustrates substrate concentration dependent η . The decreas-

ing 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.

[0015] FIG. 2 illustrates a non-limiting purification profile of NPP3 fusion protein through a Coomassie stained sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel, wherein the purified NPP3 protein is shown in relation to certain size markers.

[0016] FIG. 3 illustrates a non-limiting plasmid construct map of human NPP121-NPP3-Fc in the plasmid, cloned using the indicated restriction endonuclease sites.

[0017] FIG. 4 illustrates a non-limiting plasmid construct map of human NPP121-NPP3-Fc in the plasmid pcDNA3, cloned using IN-FUSION® technology.

[0018] FIG. 5 illustrates a non-limiting plasmid construct map of human NPP121-NPP3-Albumin in the plasmid pcDNA3.

BRIEF SUMMARY OF IRE INVENTION

[0019] 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 NPP I 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.

[0020] In certain embodiments, the polypeptide of the invention has formula (I): EXPORT-PROTEIN-Z-DO-MAIN-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), DSSSEK-FLRRIGRFG (SEQ ID NO:12), EEEEEEEPRGDT (SEQ ID NO:13), APWHLSSQYSRT (SEQ ID NO:14), STLPIPHFSRE (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.

[0021] 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.

[0022] In certain embodiments, DOMAIN is a human IgG Fe 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 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.

[0023] 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.

[0024] 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.

[0025] 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.

[0026] 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.

[0027] 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).

[0028] 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.

[0029] 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.

[0030] 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 μ M. In yet other embodiments, the administered amount raises the level of plasma PPi in the subject to at least about 1.5 μ M.

[0031] 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

[0032] 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 PP_i, as demonstrated herein.

[0033] In certain aspects, the present invention provides compositions, such as but not limited to fusion proteins, that elevate plasma PP_i in physiologic states where plasma PP_i 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 PP_i 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.

[0034] In certain embodiments, low plasma PP_i is defined as plasma PP_i 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 Bruch's 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 PP_i physiologic states via administration of the fusion proteins described herein.

[0035] 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 polymorphism in the ENPP1 coding region in which a Q residue is substituted for a K residue 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).

[0036] 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 PP_i 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 PP_i 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.

[0037] 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.

[0038] 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

[0039] 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.

[0040] As used herein, each of the following terms has the meaning associated with it in this section.

[0041] 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.

[0042] 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.

[0043] “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.

[0044] As used herein, the term “AMR” refers to autosomal dominant hypophosphatemic rickets.

[0045] 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 albumin and porcine serum albumin are also contemplated within the invention.

[0046] 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.

[0047] 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).

[0048] 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.

[0049] As used herein, the term “Ap3P” refers to adenosine-(5’)-triphospho-(5’)-adenosine or a salt thereof.

[0050] As used herein, the term “ARHR2” refers to autosomal recessive hypophosphatemic rickets type-2.

[0051] As used herein, the term “CKD” refers to chronic kidney disease.

[0052] As used herein, the term “CKD-MBD” refers to chronic kidney disease-bone/mineral disorder.

[0053] 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.

[0054] 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.

[0055] 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.

[0056] 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.

[0057] As used herein, the term “CUA” refers to calcific uremic arteriolopathy.

[0058] 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.

[0059] 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.

[0060] 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.

[0061] 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.

[0062] “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.

[0063] 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.

[0064] 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).

[0065] As used herein, the term “HBD” refers to hypophosphatemic bone disease.

[0066] As used herein, the term “HHRH” refers to hereditary hypophosphatemic rickets with hypercalciuria.

[0067] “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 TATGCC share 50% homology. Generally, a comparison is made when two sequences are aligned to give maximum homology.

[0068] As used herein, the term “IIAC” refers to idiopathic infantile arterial calcification.

[0069] 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.

[0070] 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 pro-

duced in the primary immune response in most subjects. It is the most efficient immunoglobulin in agglutination, complement fixation, and other antibody responses, and is important in defense against bacteria and viruses. IgD is the immunoglobulin that has no known antibody function, but may serve as an antigen receptor. IgE is the immunoglobulin that mediates immediate hypersensitivity by causing release of mediators from mast cells and basophils upon exposure to allergen.

[0071] “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.

[0072] “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.

[0073] 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.

[0074] As used herein, the term “MWVC” refers to medial wall vascular calcification.

[0075] As used herein, the term “NPP” refers to ectonucleotide pyrophosphatase/phosphodiesterase.

[0076] 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.

[0077] 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. Pat. 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.”

[0078] As used herein, the term “OPLL” refers to ossification of posterior longitudinal ligament.

[0079] 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.

[0080] 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.

[0081] 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 com-

pound 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.

[0082] 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.

[0083] 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 .

[0084] 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.

[0085] 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.

[0086] 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, (Hu, B; 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, W.

[0087] 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.

[0088] As used herein, the term “PXE” refers to pseudoxanthoma elasticum.

[0089] “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.

[0090] 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.

[0091] 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.

[0092] 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.

[0093] 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.

[0094] 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

[0095] In certain embodiments, the polypeptide of the invention has formula (I): EXPORT-PROTEIN-Z-DO-MAIN-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), DSSSEKFLRRIRGRFG (SEQ ID NO:12), EEEEEPRGDT (SEQ ID NO:13), APWHLSSQYSRT (SEQ ID NO:14), STLPIPHFSRE (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.

[0096] In certain embodiments, the polypeptide comprises the extracellular domain of ENPP3 (SEQ ID NO:1) or a biologically active fragment (or region) thereof.

[0097] 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.

[0098] 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.

[0099] In certain embodiments, DOMAIN is a human IgG Fc domain selected from the group consisting of IgG 1, 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.

[0100] 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.

[0101] 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.

[0102] 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.

[0103] 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.

[0104] 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

[0105] 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.

[0106] 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.

[0107] 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).

[0108] 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.

[0109] 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.

[0110] 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.

[0111] 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 μ M. In yet other embodiments, the administered amount raises the level of plasma PPi in the subject to at least about 1.2 μ M. In yet other embodiments, the administered amount raises the level of plasma PPi in the subject to at least about 1.4 μ M. In yet other embodiments, the administered amount raises the level of plasma PPi in the subject to at least about 1.5 μ M. In certain embodiments, the administered amount raises the level of plasma PPi in the subject to at least about 2 μ M. In certain embodiments, the administered amount raises the level of plasma PPi in the subject to at least about 4 μ M.

[0112] 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.

[0113] 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.

[0114] 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.

[0115] 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

[0116] The invention envisions the use of a pharmaceutical composition comprising a polypeptide of the invention within the methods of the invention.

[0117] 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.

[0118] 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.

[0119] 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.

[0120] 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 routes 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.

[0121] 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.

[0122] 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.

[0123] 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.

[0124] 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.

[0125] 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.

[0126] 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.

[0127] 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.

[0128] 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 0.2% 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.

[0129] 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.

[0130] 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.

[0131] 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.

[0132] 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.

[0133] 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 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

[0134] 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.

[0135] 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.

[0136] 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.

[0137] 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.

[0138] A medical doctor, 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.

[0139] 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.

[0140] 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 physician taking all other factors about the patient into account.

[0141] Compounds of the invention for administration may be in the range of from about 1 μ g to about 7,500 mg, about 20 μ g to about 7,000 mg, about 40 μ g, to about 6,500 mg, about 80 μ g to about 6,000 mg, about 100 μ g to about 5,500 mg, about 200 μ g to about 5,000 mg, about 400 μ g to about 4,000 mg, about 800 μ g 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.

[0142] In some embodiments, the dose of a compound of the invention is from about 0.5 μ g 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, one embodiment, 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.

[0143] 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.

[0144] 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

[0145] 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.

[0146] 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

[0147] For oral application, particularly suitable are tablets, dragees, liquids, drops, suppositories, or capsules, caplets and gels. 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.

[0148] 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. Pat. 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.

[0149] 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.

[0150] 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.

[0151] 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).

[0152] 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.

[0153] A tablet comprising the active ingredient may, for example, be made by compressing or molding the active ingredient, optionally with one or more additional ingredients. Compressed tablets may be prepared by compressing,

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

[0154] 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.

[0155] 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 inciting 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.

[0156] U.S. Pat. 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.

[0157] 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

[0158] 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.

[0159] 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 sterile pyrogen-free water) prior to parenteral administration of the reconstituted composition.

[0160] 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

[0161] Additional dosage forms of this invention include dosage forms as described in U.S. Pat. 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

[0162] 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, multi-layer 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.

[0163] 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.

[0164] 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.

[0165] 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.

[0166] 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.

[0167] The term sustained release is used in its conventional sense to refer to a drug formulation that provides for gradual release of a drug over an extended period of time, and that may, although not necessarily, result in substantially constant blood levels of a drug over an extended time period. The period of time may be as long as a month or more and should be a release which is longer than the same amount of agent administered in bolus form. For sustained release, the compounds may be formulated with a suitable polymer or hydrophobic material which provides sustained, release properties to the compounds. As such, the compounds for use the method of the invention may be administered in the form of microparticles, for example, by injection or in the form of wafers or discs by implantation. In 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.

[0168] The term delayed release is used herein in its conventional sense to refer to a drug formulation that provides for an initial release of the drug after some delay following drug administration and that may, although not necessarily, includes a delay of from about 10 minutes up to about 12 hours. The term pulsatile release is used herein in its conventional sense to refer to a drug formulation that provides release of the drug in such a way as to produce pulsed plasma profiles of the drug after drug administration. The term immediate release is used in its conventional sense to refer to a drug formulation that provides for release of the drug immediately after drug administration.

[0169] 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.

[0170] 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.

[0171] 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.

[0172] 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.

[0173] 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

[0174] 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:

[0175]

Extracellular domain of ENPP3 (SEQ ID NO: 1)

EKQGSCKKCC FDASFRG

LENCRCDVAC KDRGDCCWDF EDTCVESTRI WMCNKFRGCE TRLEASLCSC SDDCLQRKDC

CADYKSVQCG ETSWLENCND TAQQSQCPGEG FDLPPVILFS MDGFRAEYLY TWDTLMPNIN

KLKTCGIHSK YMRAMYPTKT FPNHYTIVTG LYPESHGIID NNMYDVNLNK NFSLSKEQN

NPAWWHGQPM NLTAMYQGLK AATYFWPGSE VAINGSFPSI YMPYNGSVPF EERISTLLKW

LDLPKAERPR FYTMYFEEDP SSGHAGGPVS ARVIKALQVV DHAFGMLMEG LKQRNLHNCV

NIILLADHGM DQTYCNKMEY MTDYFPRINF FYMYEGPAPR IRAHNIPHDF FSNSEIEIVR

NLSCRKPDQH FKPYLTPDLP KRLHYAKNVR IDKVHLFVDQ QWLAVRSKSN TNCGGGNHGY

NNEFRSMEAI FLAHGPSFKE KTEVEPFENI EVYNLMCDLL RIQPAPNNGT HGSLNHLKLV

PFYEPShAE VSKFSVCGFA NPLPTESLDC FCPHLQNSTQ LEQVNLMLNL TQEEITATVK

VNLFPGRPRV LQKNVDHCLL YHREYVSGFG KAMRMPMWSS YTVPLGDTN PLPPTVPDCL

RADVRVPSE SQKCSFYLD KNITHGFLYP PASNRTSDSQ YDALITSNLV PMYEEFRKMW

DYFHSVLLIK HATERNGVNV VSGPIFDYNY DGHFDAPDEI TKHLANTDVP IPHYFVVLT

SCKNKSHTPE NCPGWLDVLP FIIPHRPTNV ESCPEGKPEA LWVEERFTHA 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)

MTSKFLVSVF ILAALSLSST 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

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

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

(RQQ)_n, wherein n is an integer ranging between 1 and 20. SEQ ID NO: 8

-continued

(KR)_n, wherein n is an integer ranging between 1 and 20. SEQ ID NO: 9

R_n, wherein n is an integer ranging between 1 and 20. SEQ ID NO: 10

(KR)_n, wherein n is an integer ranging between 1 and 20. SEQ ID NO: 11

DSSSEEEKFLRRIGRFG SEQ ID NO: 12

EEEEEEPRGDT SEQ ID NO: 13

APWHLSSQYSRT SEQ ID NO: 14

STLPIPHEFSRE SEQ ID NO: 15

VTKHLNQISQSY SEQ ID NO: 16

E_n, wherein n is an integer ranging between 1 and 20. SEQ ID NO: 17

D_n, wherein n is an integer ranging between 1 and 20. SEQ ID NO: 18

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

MERDGCAGGG SRGGEGGRAP REGPAGNGRD RGRSHAAEAP GDPQAAASLL APMDVGEEPL

EKAARARTAK DPNTYKIISL FTFAGVNIC LGFTAKQSGC RKKCFDASFR GLENCRCDDVA

CKDRGDCCWD FEDTCVESTIR IWMCNKFRCG ERLEASLCSC SDDCLQRKDC CADYKSVQCQ

ETSWLEENDC TAQQSQCPG FDLPPVILFS MDGFRAEYLY TWDTLMPNIN KLKTCGIHKS

YMRAMYPTKT FPNHYTIVTG LYPESHGIID NNMYDVNLNK NFSLSKEQN NPAWWHQPM

WLTAMYQGLK AATYFWPGE VAINGSFPI YMPYNGSVPF EERISTLLKW LDLPKAERPR

FYTMEEEPD SSGHAGGPVS ARVIKALQVV DHAFGLMEG LKQRNLHNCV NIILLADHGM

DQTYCNKMEY MTDYFPRINF FYMEGPAPR IRAHNIPHDF FSNSEIEVR NLSCRKPDQH

FKPYLTPDLP KRLHYAKNVR IDKVHLFVDQ QWLAVRSKSN TNCGGGNHGY NNEFRSMEAI

FLAHGPSFKE KTEVEPFENI EVYNLMCDLL RIQAPNNGT HGSLNHLKV PFYEPShAE

VSKFSVCGFA NPLPTESLDC FCPHLQNSTQ LEQVNQMLNL TQEEITATVK VNLPPGRPRV

LQKNVDHCLL YHREYVSGFG KAMRMPWSS YTVPLGDT S PLPPTVEDCL RADVRVPPSE

SQKCSFYLD KNITHGFLYP PASNRTSDSQ YDALITSNLV PMYEEFRKMW DYFHSVLLIK

HATERNGVNV VSGPIFDYNY DGHFDAPDEI TKHLANTDVP IPHYFVVLV SCKNKSHTPE

NCPGWLVDLP FIIPHRPTNV ESCPEGKPEA LWVEERFTH IARVRDVELL TGLDFYQDKV

QPVSEILQLK TYLPTFETTI DKTHTCPPCP APELLGGPSV FLFTPKTKDT LMISRTPEVT

CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYE

CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE

WESNGQPENN YKTTTPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS

LSLSPGK

[0176] 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.

[0177] 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).

[0178] 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.

[0179] 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
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGK

ENPP7-NPP3-Fc sequence

(SEQ ID NO: 21)

MRGPAVLLTV ALATLLAPGA KQGSC RKKCFDASFR GLENCRCDDVA
CKDRGDCCWD FEDTCVESTRIWMCNKPRCG ERLEASLCSC SDDCLQRKDC CADYKSVCQG
ETSWLEENCD TAQQSQCPG FDLPPVILFS MDGFRAEYLY TWDTLMPNIN KLKTCGIHKS
YMRAMYPTKT FPNHYTIVTG LYPESHGIID NNMYDVNLNK NFSLSSEQN NPAWHGQPM
WLTAMYQLK AATYFWPGSE VAINGSFPSI YMPYNGSVPF EERISTLLKW LDLPKAERPR
FYTMFEEDP SSGHAGGPVS ARVIKALQVV DHAFGLMEG LKQRNLHNCV NIILLADHGM
DQTYCNKMEY MTDYFPRINF FYMEGPAPR IRAHNIPHDF FSNSEIEVR NLSCRKPDQH
FKPYLTPDLP KRLHYAKNVR IDKVHLFVDQ QWLAVRSKSN TNCGGNGHY NNEFRSMEAI
FLAHGPSFKE KTEVEPFENI EVYNLMCDLL RIQPAPNNGT HGSLNHLKLV PFYEPHAE
VSKFSVCGFA NPLPTESLDC FCPHLQNSTQ LEQVNQMLNL TQEEITATVK VNLPPGRPRV
LQKNVDHCLL YHREYVSGFG KAMRMPMWSS YTVPLGDTSLPPTVPDCL RADVRVPPSE
SQKCSFYLD KNITHGLYP PASNRTSDSQ YDALITSNLV PMYEEFRKMW DYFHSVLLIK
HATEPNGVNV VSGPIFDYNY DGHFDAPDEI TKHLANTDVP IPTHYFVVLTSCKNKSHTPE
NCPGWLVDLP FIIPHRPTNV ESCPEGKPEA LWVEERFTAH IARVRDVELL TGLDFYQDKV
QPVSEILQLK TYLPTFETTI DKTHTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT
CVVVDVSHED PEVKFNWYVD GVEVHSNKT PREEQYNSTY RVVSVLTVLH QDWLNGKEYK
CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE
WESNGQPENN YKTTTPVLD DGSFFLYSKL TVDKSRWQQG NVFS CSVMHE ALHNHYTQKS
LSLSPGK

ENPP5-NPP3-Fc sequence

(SEQ ID NO: 22)

MTSKFLLVSF ILAALSLSLT FSKQSGC RKKCFDASFR GLENCRCDDVA
 CKDRGDCCWD FEDTCVESTIR IWMCNKFRCG ERLEASLCSC SDDCLQRKDC CADYKSVQCQ
 ETSWLEENCD TAQSQSQPEG FDLPPVILFS MDGFRAEYLY TWDTLMPNIN KLKTCGIHSK
 YMRAMYPTKT FPNHYTIVTG LYPESHGIID NNMYDVNLNK NTSLSKSKEQN NPAWWHGQPM
 WLTAMYQGLK AATYFWPGSE VAINGSFPSI YMPYNGSVPF EERISTLLKW LDLPKAERPR
 FYTMYFEEPD SSGHAGGPVS ARVIKALQVV DHAFGMLMEG LKQRNLHNCV NIILLADHGM
 DQTYCNKMEY MTDYFPRINF FYMYEGPAPR IRAHNIPHDF FSNSEIEIVR NLSCRKPDQH
 FKPYLTPDLP KRLHYAKNVR IDKVHLFVDQ QWLAVRSKSN TNCGGGNHGY NNEFRSMEAI
 FLAHGPSFKE KTEVEPFENI EVYNLMCDLL RIQPAPNNGT HGSLNHLKLV PFYEPHAE
 VSKFSVCGFA NPLPTESLDC FCPHLQNSTQ LEQVNQMLNL TQEEITATVK VNLPPGRPRV
 LQKNVDHCLL YHREYVSGFG KAMRMPMWSS YTVPLQGDTS PLPPTVPDCL RADVRVPPSE
 SQKCSFYLDAD KNITHGFLYP PASNRTSDSQ YDALITSNLV PMYEEFRKMW DYFHSVLLIK
 HATERNGVNV VSGPIFDYNY DGHFDAPDEI TKHLANTDVP IPTHYFVVLV SCKNKSHTPE
 NCPGWLDVLP FIIPHRPTNV ESCPEGKPEA LWVEERFTHA IARVRDVELL TGLDFYQDKV
 QPVSEILQLK TYLPTFETTI DKTHTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT
CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK
CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE
WESNGQPENN YKTTTPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVME ALHNHYTQKS
LSLSPGK

[0180] 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 Fe domain, albumin domain is used.

[0181] In certain embodiments, the NPP3 C-terminus and the Fe/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)

GGGGSGGGSGGGGSMKWTFLLLLFVSGSAFSGVFRFAHAKSEIAHRYNDLGEQHFGLVLIASQ
 YLQKCSYDEHAKLVQEVTDFAKTCVADESAANCDKSLHTLFQDKLCAIPNLRENYGELADCCCTKQEP
 RNECFLOHKDDNPSLPPFERPEAEAMCTSFKENPTTFMGHYLHEVARRHPYFYAPELLYYAEQYNEIL
 TQCCAEADKESCLTPKLDGVKEKALVSSVRQRMKCSSMQKGERAFKAWAVARLSQTFPNADFAEITK
 LATDLTKVNEKCCGDLLEACDDRAELAKYMCENQATISSKLQTCCKPLKKAHCLSEVEHDTMPAD
 LPAIAADFVEDQEVCKNYAEAKDVLGTFLYEYSRRHPDYSVSLLLRLAKKYEATLEKCCAEANPPAC
 YGTVLAEPQPLVEEPKNLVKTNCDLYEKLGEYGFQNAILVRYTQKAPQVSTPTLVEAARNLGRVGTCK
 CTLPEDQRLPCVEDYLSAILNRVCLLHEKTPVSEHVTKCCSGSLVERRPCFSALTVDETYVPKEFKAE
 TFTFHSICTLPEKEKQIKKQALAEVLKHKPKATAEQKLTVMDDFAQFLDTCCKAADKDTCFSTEGP
 NLVTRCKDALA

- continued

ENPP121-NPP3-Albumin sequence

(SEQ ID NO: 24)

MERDGCAGGG SRGGEGGRAP REGPAGNGRD RGRSHAAEAP GDPQAAASLL APMDVGEEPL**EKAARARTAK DPNTYKIISL** FTFAVGVNIC LGFTAKQGSC RKKCFDASFR GLENCRCDDVA

CKDRGDCCWD FEDTCVESTR IWMCNKFRCG ERLEASLCSC SDDCLQRKDC CADYKSVCCG

ETSWLEEND TAQSQCPEG FDLPPVILFS MDGFRAEYLY TWDTLMPNIN KLKTCGIHSK

YMRAMYPTKT FPNHYTIVTG LYPESHGIID NNMYDVNLNK NFSLSSEQN NPAWWHGQPM

WLTAMYQGLK AATYFWPGE VAINGSFPSI YMPYNGSVPF EERISTLLKW LDLPKAERPR

FYTMFYEEPD SSGHAGGPVS ARVIKALQVV DHAFGMLMEG LKQRNLHNCV NIILLADHGM

DQTYCNKMEY MTDYFPRINF FYMYEGPAPR IRAHNIPHDF FSNSEIEVR NLSCKRPDQH

FKPYLTPDLR KRLHYAKNVR IDKVHLFVDQ QWLAVRSKSN TNCGGGNHGY NNEFRSMEAI

FLAHGPSFKE KTEVEPFENI EVYNLMCDLL RIQPAPNNGT HGSLNHLKLV PFYEPHAE

VSKFSVCGFA NPLPTESLDC FCPHLQNSTQ LEQVQMLNL TQEEITATVK VNLPPGRPRV

LQKNVDHCLL YHREYVSGFG KAMRMPMWS YTPQLGDTL PLPPTVPDCL RADVRVPPSE

SQKCSFYLD KNITHGFLYP PASNRTSDSQ YDALITSNLV PMYEEFRKMW DYFHSVLLIK

HATERNGVNV VSGPIFDYNY DGHFDAPDEI TKHLANTDVP IPTHYFVVLV SCKNKSHTPE

NCPGWLDVLP FIIPHRPTNV ESCPEGKPEA LWVEERFTHA IARVRDVELL TGLDFYQDKV

QPVSEILQLK TYLPTFETTI

GGSGGGGSG GGSMSKMWTF LLLLFVSGSA FSRGVFRREA HKSEIAHRYN DLGEQHFHGLVLIAFSQYLQ KCSYDEHAKL VQEVTDFAKT CVADESAANC DKSLLHFLGD KLCAIPNLRENYGELADCCCT KQEPERNECF LQHKDDNPSL PPFERPEAEA MCTSFKENPT TFMGHYLHEVARRHPYFYAP ELLYYAQYN EILTQCCAEA DKESCLTPKL DGVKEKALVS SVRQRMKCSSMOKFGERAFK AWAVARLSQT FPNADFAEIT KLATDLTKVN KECCHGDLLE CADDRALAKYMCENQATIS SKLOTCCDKP LLKKAHCLSE VEHDTPADL PAIAADFVED QEVCKNYAEAKDVFLGTFLY EYSRRHPDYS VSLLRLAKK YEATLEKCCA EANPPACYGT VLAEPQPLVEEPKNLVKTNC DLYEKLGEYG FQNAILVRYT QKAPQVSTPT LVEAARNLGR VGTKCCTLPEDQRLPCVEDY LSAILNRVCL LHEKTPVSEH VTKCCSGSLV ERRPCFSALT VDETYVPKEFKAETFTHESD ICTLPEKEQ IKKQATALAEL VKHKPKATAE QLKTVMDDFA QFLDTCCAADKDTCFSTEG PNLVTRCKDA LA

[0182] 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.

[0183] 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 GLENCRCDDVA

CKDRGDCCWD FEDTCVESTR IWMCNKFRCG ERLEASLCSC SDDCLQRKDC CADYKSVCCG

ETSWLEEND TAQSQCPEG FDLPPVILFS MDGFRAEYLY TWDTLMPNIN KLKTCGIHSK

-continued

YMRAMYPTKT FPNHYTIVTG LYPESHGIID NNMYDVNLNK NFSLSKEQN NPAWWHGQPM
 WLTAMYQGLK AATYFWPGSE VAINGSFPSI YMPYNGSVPF EERISTLLKW LDLPKAERPR
 FYTMYFEEPD SSGHAGGPVS ARVIKALQVV DHAFGMLMEG LKQRNLHNCV NIILLADHGM
 DQTYCNKMEY MTDYFPRINF FYMYEGPAPR IRAHNIPHDF FSNSEIEIVR NLSCRKPDQH
 FKPYLTPDLP KRLHYAKNVR IDKVHLFVDQ QWLAVRSKSN TNCGGGNHGY NNEFRSMEAI
 FLAHGPSFKE KTEVEPFENI EVYNLMCDLL RIQPAPNNGT HGSLNHLKLV PFYEPShAEE
 VSKFSVCGFA NPLPTESLDC FCPHLQNSTQ LEQVNQMLNL TQEEITATVK VNLPPGPRPV
 LQKNVDHCLL YHREYVSGFG KAMRMPMWSS YTVPLGSDTS PLPPTVPDCL RADVRVPPSE
 SQKCSFYLDK KNITHGLFLY PASNRTSTQ YDALITSNLV PMYEEFRKMW DYFHSVLLIK
 HATERNGVNV VSGPIFDYNY DGHFDAPDEI TKHLANTDVP IPHXYFVVLV SKNKSHTPE
 NCPGWLDVLP FIIPHRPTNV ESCPEGKPEA LWVEERFTHA LARVRDVELL TGLDFYQDKV
 QPVSEILQLK TYLPTFETTI

GGSGGGGSG GGGSMKWTF LLLLFVSGSA FSRGVFRREA HKSEIAHRYN DLGEQHFKGL
VLIAFSQYLQ KCSYDEHAKL VQEVTDFAKT CVADESAANC DKSLLTLFGD KLCAIPNLRE
NYGELADCC TQEPERNECF LQHKDDNPSL PFERPEAEA MCTSFKENPT TFMGHYLHEV
ARRHPYFYAP ELLYYAEQYN EILTQCCAEA DKESCLTPKL DGVKEKALVS SVRQRMKCSS
MQKFGERAFL AWAVARLSQT FPNADFAEIT KLATDLTKVN KECCHGDLLE CADDRALAK
YMCENQATIS SKLQTCDDKP LLKKAHCLSE VEHDTMPADL PAIAADFVED QEVCKNYAEA
KDVLGTLFLY EYSRRHPDYS VSLLLRLAKK YEATLEKCCA EANPPACYGT VLAEPQPLVE
EPKNLVKTNC DLYEKLGEYG FQNAILVRYT QKAPQVSTPT LVEAARNLGR VGTKCCTLPE
DQRLPCVEDY LSAILNRVCL LHEKTPVSEH VTKGCSGLV ERRPCFSALT VDETYVPKEF
KAETFTFHSD ICTLPEKEKQ IKKQATALAEL VKHKPKATAE QLKTVMDDFA QFLDTCCCAA
DKDTCFSTEG PNLVTRCKDA LA

[0184] Single underlined residues=signal peptide sequence from NPP7; Double underlined residues=amino acid sequence of spacer sequence and albumin domain.

[0185] 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)

MTSKFLLSVF ILAALSSTT FSKQGSC RKKCFDASFR GLENCRCDA
 CKDRGDCCWD FEDTCVESTI IWMCNKFRCG ERLEASLCSC SDDCLQRKDC CADYKSVCCG
 ETSWLEENDC TAQQSQCPGE FDLPPVILFS MDGFRAEYLY TWDTLMPNIN KLKTCGIHSK
 YMRAMYPTKT FPNHYTIVTG LYPESHGIID NNMYDVNLNK NFSLSKEQN NPAWWHGQPM
 WLTAMYQGLK AATYFWPGSE VAINGSFPSI YMPYNGSVPF EERISTLLKW LDLPKAERPR
 FYTMYFEEPD SSGHAGGPVS ARVIKALQVV DHAFGMLMEG LKQRNLHNCV NIILLADHGM
 DQTYCNKMEY MTDYFPRINF FYMYEGPAPR IRAHNIPHDF FSNSEIEIVR NLSCRKPDQH
 FKPYLTPDLP KRLHYAKNVR IDKVHLFVDQ QWLAVRSKSN TNCGGGNHGY NNEFRSMEAI
 FLAHGPSFKE KTEVEPFENI EVYNLMCDLL RIQPAPNNGT HGSLNHLKLV PFYEPShAEE

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VSKFSVCGFA NPLPTESLDC FCPHLQNSTQ LEQVNQMLNL TQEEITATVK VNLPPGRPRV
 LQKNVDHCLL YHREYVSGFG KAMRMPMWSS YTVPLGDSY PLPPTVPDCL RADVRVPPSE
 SQKCSFYLDL KNITHGFLYP PASNRTSDSQ YDALITSNLV PMYEEFRKMW DYFHSVLLIK
 HATERNGVMV VSGPIFDYNY DGHFDAPDEI TKHLANTDVP IPHYFVVLV SCKNKSHTPE
 NCPGWLDVLP FIIPHRPTNV ESCPEGKPEA LWVEERFTHA IARVRDVELL TGLDFYQDKV
 QPVSEILQLK TYLPTFETTI
GGSGGGGSG GGSMMKWTF LLLLFVSGSA FSRGVFRREA HKSEIAHRYN DLGEQHFKGL
VLIASFQYLQ KCSYDEHAKL VQEVTDFAKT CVADESAANC DKSLLTLFGD KLCAIPNLRE
NYGELADCTT KQEPERNECF LQHKDDNPSL PPFERPEAEA MCTSFKENPT TFMGHYLHEV
ARRHPYFYAP ELLYYAEQYN EILTQCCAEA DKESCLTPKL DGVKEKALVS SVRQRMKCSS
MQKFGERAFL AWAVARLSQT FPNADFAEIT KLATDLTKVN KECCHGDLLE CADDRAEALAK
YMCENQATIS SKLQTCDDKP LLKKAHCLSE VEHDTMPADL PAIAADFVED QEVCKNYAEA
KDVLGLTFLY EYSRRHPDYS VSLLLRLAKK YEATLEKCCA EANPPACYGT VLAEPQPLVE
EPKNLVKTNC DLYEKLGEYG FQNAILVRYT QKAPQVSTPT LVEAARNLGR VGTKCCTLPE
DQRLPCVEDY LSAILNRVCL LHEKTPVSEH VTKGCSGLV ERRPCFSALT VDETYVPKEF
KAETFTFHSD ICTLPEKEKQ IKKQATALAEL VKHKPKATAE QPKTVMDDFA QPLDTCCCAA
DKDTCFSTEG PNLVTRCKDA LA

[0186] 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)

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 CAACACCTACAAGATCATCTCCCTCTTCACTTTCGCCGTCGGAGTCAACATCTGCCTGGG
 ATTACACGCCGAAAAGCAAGGACGCTGCAGGAAGAAGTGCTTTGATGCATCATTTAGAGG
 ACTGGAGAAGTCCCGGTGTGATGTGGCATGTAAAGACCGAGGTGATGCTGCTGGGATTT
 TGAAGACACCTGTGTGGAATCAACTCGAATATGGATGTGCAATAAATTTCTGTTGGGAGA
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 CACAGCCAGCAGTCTCAGTGCCAGAGGGTTTGACCTGCCACCAAGTTATCTTGTGTTTC
 TATGGATGGATTTAGAGCTGAATATTTATACATGGGATACTTTAATGCCAAATATCAA
 TAAACTGAAAACATGTGGAATTCATTCAAAATACATGAGAGCTATGTATCTTACCAAAAC
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Nucleotide sequence of NPP121-NPP3-Fc (SEQ ID NO: 28)
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CATCCTGAAGCAAAAAGAAATGCCCTGTGCAGAAGACTATCTATCCGTGGTCTGAACCAG
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AAAGAGTTTAATGCTGAAACATTACCTTCCATGCAGATATATGCACACTTCTGAGAAG
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Nucleotide sequence of hNPP3-hFc-pcDNA3

(SEQ ID NO: 29)

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Example 1

[0187] FIGS. 1A-1C comprise graphs illustrating studies of hNPP3 steady state ATP hydrolysis activity.

[0188] 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 μ M 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.

[0189] 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 50 nM hNPP3 and hNPP1 depleted ATP substrate within 1 minute at 0.98, 1.95 and 3.9 μ M 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 $2.59(\pm 0.04) \text{ s}^{-1} \text{ enzyme}^{-1}$, from the weighted average of the measured rates at 7.8, 15.6, 31.3, 62.5, 125 μ M substrate. The turnover rate of hNPP1 was $3.46(\pm 0.44) \text{ s}^{-1} \text{ enzyme}^{-1}$. The K_M for ATP substrate was estimated to be $<8 \mu\text{M}$.

[0190] 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

[0191] 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 (Phi):

[0192] 1. enpp1asj/asj model of Generalized Arterial Calcification of Infancy (GACI); Li, et al., 2013, *Disease Models & Mech.* 6(5):1227-35.

[0193] 2. enpp12asj/2asj model of Generalized Arterial Calcification of Infancy (GACI); Li, et al., 2014, *PloS one* 9(12):e113542.

[0194] 3. ABCC6^{-/-} mouse model of Pseudoxanthoma Elasticum (PXE); Jiang, et al., 2007, *J. Invest. Derm.* 127(6):1392-402.

[0195] 4. HYP mouse model of X-linked hypophosphatasia (XLH); Liang, et al., 2009, *Calcif. Tissue Int.* 85(3):235-46.

[0196] 5. LmnaG609G/+ mouse model of Hutchison-Gilford Progeria Syndrome; Villa-Bellosta, et al., 2013, *Circulation* 127(24):2442-51.

[0197] 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).

[0198] 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.

[0199] 8. Mouse model of chronic kidney disease (CKD) on the adenine diet; Jia, et al., 2013, *BMC Nephrol.* 14:116.

[0200] 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.

[0201] 10. ENPP1 knockout mouse model of GACI and osteopenia; Mackenzie, et al., 2012, *PloS one* 7(2):e32177.

[0202] 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 Hypophosphatemic Rickets type 2 (ARHR2) (Levy-Litan, et al., 2010, *Am. J. Human Gen.* 86(2):273-8.

[0203] 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

[0204] 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.

[0205] 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.

[0206] 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 shak-

ing flasks or bio-reactors previously described for ENPP1 (Albright, et al., 2015, Nat Commun. 6:10006).

[0207] 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 5200 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.

[0208] 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 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 Coomassie 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

[0209] 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](http://www.dot.mayomedicallaboratories.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/](http://www.dot.specialtylabs dot com/about_us/)).

[0210] 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.

[0211] 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 USA 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 reliably measure PPi concentrations, and can provide clinical utility as predictive diagnostic identifying patients at risk for the above mentioned disorders.

[0212] 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.

SEQUENCE LISTING

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between 1 and 20

<400> SEQUENCE: 8

Arg Gln Gln
1

<210> SEQ ID NO 9
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically synthesized
<220> FEATURE:
<221> NAME/KEY: REPEAT
<222> LOCATION: (1)..(2)
<223> OTHER INFORMATION: (KR)n, wherein n is an integer ranging
between 1 and 20

<400> SEQUENCE: 9

Lys Arg
1

<210> SEQ ID NO 10

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<211> LENGTH: 1
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically synthesized
<220> FEATURE:
<221> NAME/KEY: REPEAT
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: (R)n, wherein n is an integer ranging
between 1 and 20

<400> SEQUENCE: 10

Arg
1

<210> SEQ ID NO 11
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically synthesized
<220> FEATURE:
<221> NAME/KEY: REPEAT
<222> LOCATION: (1)..(2)
<223> OTHER INFORMATION: (KR)n, wherein n is an integer ranging
between 1 and 20

<400> SEQUENCE: 11

Lys Arg
1

<210> SEQ ID NO 12
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically synthesized

<400> SEQUENCE: 12

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

<210> SEQ ID NO 13
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically synthesized

<400> SEQUENCE: 13

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

<210> SEQ ID NO 14
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically synthesized

<400> SEQUENCE: 14

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

<210> SEQ ID NO 15
<211> LENGTH: 12

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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically synthesized

<400> SEQUENCE: 15

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

<210> SEQ ID NO 16
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically synthesized

<400> SEQUENCE: 16

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

<210> SEQ ID NO 17
<211> LENGTH: 1
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically synthesized
<220> FEATURE:
<221> NAME/KEY: REPEAT
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: (E)n, wherein n is an integer ranging
between 1 and 20

<400> SEQUENCE: 17

Glu
1

<210> SEQ ID NO 18
<211> LENGTH: 1
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically synthesized
<220> FEATURE:
<221> NAME/KEY: REPEAT
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: (D)n, wherein n is an integer ranging
between 1 and 20

<400> SEQUENCE: 18

Asp
1

<210> SEQ ID NO 19
<211> LENGTH: 1147
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ENPP121-NPP3-Fc sequence

<400> SEQUENCE: 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

-continued

35					40					45					
Leu 50	Leu	Ala	Pro	Met	Asp	Val 55	Gly	Glu	Glu	Pro	Leu 60	Glu	Lys	Ala	Ala
Arg 65	Ala	Arg	Thr	Ala	Lys 70	Asp	Pro	Asn	Thr	Tyr 75	Lys	Ile	Ile	Ser	Leu 80
Phe	Thr	Phe	Ala	Val 85	Gly	Val	Asn	Ile	Cys 90	Leu	Gly	Phe	Thr	Ala 95	Lys
Gln	Gly	Ser	Cys 100	Arg	Lys	Lys	Cys	Phe 105	Asp	Ala	Ser	Phe	Arg 110	Gly	Leu
Glu	Asn	Cys 115	Arg	Cys	Asp	Val	Ala 120	Cys	Lys	Asp	Arg	Gly 125	Asp	Cys	Cys
Trp	Asp 130	Phe	Glu	Asp	Thr	Cys 135	Val	Glu	Ser	Thr	Arg 140	Ile	Trp	Met	Cys
Asn 145	Lys	Phe	Arg	Cys	Gly 150	Glu	Arg	Leu	Glu	Ala 155	Ser	Leu	Cys	Ser	Cys 160
Ser	Asp	Asp	Cys 165	Leu	Gln	Arg	Lys	Asp	Cys 170	Cys	Ala	Asp	Tyr	Lys 175	Ser
Val	Cys	Gln	Gly 180	Glu	Thr	Ser	Trp	Leu 185	Glu	Glu	Asn	Cys	Asp 190	Thr	Ala
Gln	Gln	Ser 195	Gln	Cys	Pro	Glu	Gly 200	Phe	Asp	Leu	Pro	Pro 205	Val	Ile	Leu
Phe	Ser 210	Met	Asp	Gly	Phe	Arg 215	Ala	Glu	Tyr	Leu	Tyr 220	Thr	Trp	Asp	Thr
Leu 225	Met	Pro	Asn	Ile	Asn 230	Lys	Leu	Lys	Thr	Cys 235	Gly	Ile	His	Ser	Lys 240
Tyr	Met	Arg	Ala	Met 245	Tyr	Pro	Thr	Lys	Thr 250	Phe	Pro	Asn	His	Tyr 255	Thr
Ile	Val	Thr	Gly 260	Leu	Tyr	Pro	Glu	Ser 265	His	Gly	Ile	Ile	Asp 270	Asn	Asn
Met	Tyr 275	Asp	Val	Asn	Leu	Asn	Lys 280	Asn	Phe	Ser	Leu	Ser 285	Ser	Lys	Glu
Gln	Asn 290	Asn	Pro	Ala	Trp	Trp 295	His	Gly	Gln	Pro	Met 300	Trp	Leu	Thr	Ala
Met 305	Tyr	Gln	Gly	Leu	Lys 310	Ala	Ala	Thr	Tyr	Phe 315	Trp	Pro	Gly	Ser	Glu 320
Val	Ala	Ile	Asn	Gly 325	Ser	Phe	Pro	Ser	Ile 330	Tyr	Met	Pro	Tyr	Asn 335	Gly
Ser	Val	Pro	Phe 340	Glu	Glu	Arg	Ile	Ser 345	Thr	Leu	Leu	Lys 350	Trp	Leu	Asp
Leu	Pro	Lys 355	Ala	Glu	Arg	Pro	Arg 360	Phe	Tyr	Thr	Met	Tyr 365	Phe	Glu	Glu
Pro	Asp 370	Ser	Ser	Gly	His	Ala 375	Gly	Gly	Pro	Val	Ser 380	Ala	Arg	Val	Ile
Lys 385	Ala	Leu	Gln	Val	Val 390	Asp	His	Ala	Phe	Gly 395	Met	Leu	Met	Glu	Gly 400
Leu	Lys	Gln	Arg	Asn 405	Leu	His	Asn	Cys	Val 410	Asn	Ile	Ile	Leu	Leu	Ala
Asp	His	Gly 420	Met	Asp	Gln	Thr	Tyr	Cys 425	Asn	Lys	Met	Glu 430	Tyr	Met	Thr
Asp	Tyr	Phe 435	Pro	Arg	Ile	Asn	Phe 440	Phe	Tyr	Met	Tyr	Glu 445	Gly	Pro	Ala

-continued

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			

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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

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<210> SEQ ID NO 20

<211> LENGTH: 227

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: IgG Fc sequence

<400> SEQUENCE: 20

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

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

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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> SEQ ID NO 21
 <211> LENGTH: 1072
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: ENPP7-NPP3-Fc sequence

<400> SEQUENCE: 21

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				

-continued

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

-continued

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									615							620
Ser Pro Leu Pro Pro Thr Val Pro Asp Cys Leu Arg Ala Asp Val Arg									630							640
Val Pro Pro Ser Glu Ser Gln Lys Cys Ser Phe Tyr Leu Ala Asp Lys																
									645							655
Asn Ile Thr His Gly Phe Leu Tyr Pro Pro Ala Ser Asn Arg Thr Ser																
									660							670
Asp Ser Gln Tyr Asp Ala Leu Ile Thr Ser Asn Leu Val Pro Met Tyr																
									675							685
Glu Glu Phe Arg Lys Met Trp Asp Tyr Phe His Ser Val Leu Leu Ile																
									690							700
Lys His Ala Thr Glu Arg Asn Gly Val Asn Val Val Ser Gly Pro Ile																
									710							720
Phe Asp Tyr Asn Tyr Asp Gly His Phe Asp Ala Pro Asp Glu Ile Thr																
									725							735
Lys His Leu Ala Asn Thr Asp Val Pro Ile Pro Thr His Tyr Phe Val																
									740							750
Val Leu Thr Ser Cys Lys Asn Lys Ser His Thr Pro Glu Asn Cys Pro																
									755							765
Gly Trp Leu Asp Val Leu Pro Phe Ile Ile Pro His Arg Pro Thr Asn																
									770							780
Val Glu Ser Cys Pro Glu Gly Lys Pro Glu Ala Leu Trp Val Glu Glu																
									785							800
Arg Phe Thr Ala His Ile Ala Arg Val Arg Asp Val Glu Leu Leu Thr																
									805							815
Gly Leu Asp Phe Tyr Gln Asp Lys Val Gln Pro Val Ser Glu Ile Leu																
									820							830
Gln Leu Lys Thr Tyr Leu Pro Thr Phe Glu Thr Thr Ile Asp Lys Thr																
									835							845
His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser																
									850							860
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg																
									865							880
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro																
									885							895
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala																
									900							910
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val																
									915							925
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr																
									930							940
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr																
									945							960

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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

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<210> SEQ ID NO 22
<211> LENGTH: 1074
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ENPP5-NPP3-Fc sequence

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<400> SEQUENCE: 22

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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

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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				
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		
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					

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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	
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					

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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> SEQ ID NO 23
 <211> LENGTH: 623
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Albumin sequence

<400> SEQUENCE: 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 Arg Ala Glu Leu Ala Lys Tyr Met Cys	

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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> SEQ ID NO 24

<211> LENGTH: 1542

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: ENPP121-NPP3-Albumin sequence

<400> SEQUENCE: 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

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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				

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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	

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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
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
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				

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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> SEQ ID NO 25

<211> LENGTH: 1467

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: ENPP7-NPP3-Albumin

<400> SEQUENCE: 25

Met Arg Gly Pro Ala Val Leu Leu Thr Val Ala Leu Ala Thr Leu Leu

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

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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	

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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					

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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> SEQ ID NO 26

<211> LENGTH: 1469

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: ENPP5-NPP3-albumin sequence

<400> SEQUENCE: 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

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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				
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				
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				
Pro Val Ser Ala Arg Val Ile Lys Ala Leu Gln Val Val Asp His Ala	305					310					315				
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				
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				

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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	
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	Gly	835	840	845	
Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Met	Lys	Trp	850	855	860	
Val	Thr	Phe	Leu	Leu	Leu	Leu	Phe	Val	Ser	Gly	Ser	Ala	Phe	Ser	Arg	865	870	875	880

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Gly	Val	Phe	Arg	Arg	Glu	Ala	His	Lys	Ser	Glu	Ile	Ala	His	Arg	Tyr	885	890	895
Asn	Asp	Leu	Gly	Glu	Gln	His	Phe	Lys	Gly	Leu	Val	Leu	Ile	Ala	Phe	900	905	910
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	930	935	940
Ala	Asn	Cys	Asp	Lys	Ser	Leu	His	Thr	Leu	Phe	Gly	Asp	Lys	Leu	Cys	945	950	955
Ala	Ile	Pro	Asn	Leu	Arg	Glu	Asn	Tyr	Gly	Glu	Leu	Ala	Asp	Cys	Cys	965	970	975
Thr	Lys	Gln	Glu	Pro	Glu	Arg	Asn	Glu	Cys	Phe	Leu	Gln	His	Lys	Asp	980	985	990
Asp	Asn	Pro	Ser	Leu	Pro	Pro	Phe	Glu	Arg	Pro	Glu	Ala	Glu	Ala	Met	995	1000	1005
Cys	Thr	Ser	Phe	Lys	Glu	Asn	Pro	Thr	Thr	Phe	Met	Gly	His	Tyr		1010	1015	1020
Leu	His	Glu	Val	Ala	Arg	Arg	His	Pro	Tyr	Phe	Tyr	Ala	Pro	Glu		1025	1030	1035
Leu	Leu	Tyr	Tyr	Ala	Glu	Gln	Tyr	Asn	Glu	Ile	Leu	Thr	Gln	Cys		1040	1045	1050
Cys	Ala	Glu	Ala	Asp	Lys	Glu	Ser	Cys	Leu	Thr	Pro	Lys	Leu	Asp		1055	1060	1065
Gly	Val	Lys	Glu	Lys	Ala	Leu	Val	Ser	Ser	Val	Arg	Gln	Arg	Met		1070	1075	1080
Lys	Cys	Ser	Ser	Met	Gln	Lys	Phe	Gly	Glu	Arg	Ala	Phe	Lys	Ala		1085	1090	1095
Trp	Ala	Val	Ala	Arg	Leu	Ser	Gln	Thr	Phe	Pro	Asn	Ala	Asp	Phe		1100	1105	1110
Ala	Glu	Ile	Thr	Lys	Leu	Ala	Thr	Asp	Leu	Thr	Lys	Val	Asn	Lys		1115	1120	1125
Glu	Cys	Cys	His	Gly	Asp	Leu	Leu	Glu	Cys	Ala	Asp	Asp	Arg	Ala		1130	1135	1140
Glu	Leu	Ala	Lys	Tyr	Met	Cys	Glu	Asn	Gln	Ala	Thr	Ile	Ser	Ser		1145	1150	1155
Lys	Leu	Gln	Thr	Cys	Cys	Asp	Lys	Pro	Leu	Leu	Lys	Lys	Ala	His		1160	1165	1170
Cys	Leu	Ser	Glu	Val	Glu	His	Asp	Thr	Met	Pro	Ala	Asp	Leu	Pro		1175	1180	1185
Ala	Ile	Ala	Ala	Asp	Phe	Val	Glu	Asp	Gln	Glu	Val	Cys	Lys	Asn		1190	1195	1200
Tyr	Ala	Glu	Ala	Lys	Asp	Val	Phe	Leu	Gly	Thr	Phe	Leu	Tyr	Glu		1205	1210	1215
Tyr	Ser	Arg	Arg	His	Pro	Asp	Tyr	Ser	Val	Ser	Leu	Leu	Leu	Arg		1220	1225	1230
Leu	Ala	Lys	Lys	Tyr	Glu	Ala	Thr	Leu	Glu	Lys	Cys	Cys	Ala	Glu		1235	1240	1245
Ala	Asn	Pro	Pro	Ala	Cys	Tyr	Gly	Thr	Val	Leu	Ala	Glu	Phe	Gln		1250	1255	1260
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1295	1300	1305
Val Glu Ala Ala Arg Asn Leu Gly Arg Val Gly Thr Lys Cys Cys		
1310	1315	1320
Thr Leu Pro Glu Asp Gln Arg Leu Pro Cys Val Glu Asp Tyr Leu		
1325	1330	1335
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		
1355	1360	1365
Arg Arg Pro Cys Phe Ser Ala Leu Thr Val Asp Glu Thr Tyr Val		
1370	1375	1380
Pro Lys Glu Phe Lys Ala Glu Thr Phe Thr Phe His Ser Asp Ile		
1385	1390	1395
Cys Thr Leu Pro Glu Lys Glu Lys Gln Ile Lys Lys Gln Thr Ala		
1400	1405	1410
Leu Ala Glu Leu Val Lys His Lys Pro Lys Ala Thr Ala Glu Gln		
1415	1420	1425
Leu Lys Thr Val Met Asp Asp Phe Ala Gln Phe Leu Asp Thr Cys		
1430	1435	1440
Cys Lys Ala Ala Asp Lys Asp Thr Cys Phe Ser Thr Glu Gly Pro		
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<210> SEQ ID NO 27

<211> LENGTH: 3447

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Nucleotide sequence of NPP121-NPP3-Fc

<400> SEQUENCE: 27

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<210> SEQ ID NO 28

<211> LENGTH: 4638

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Nucleotide sequence of NPP121-NPP3-Fc

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<210> SEQ ID NO 29

<211> LENGTH: 8852

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Nucleotide sequence of hNPP3-hFc-pcDNA3

<400> SEQUENCE: 29

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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), (SEQ ID NO:10), (KR)_n (SEQ ID NO:11), DSSSEKFLRRIGRFG (SEQ ID NO:12), EEEEEPRGDT (SEQ ID NO:13), APWHLSSQYSRT (SEQ ID NO:14), STLPIPHF-SRE (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 ENPP3 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 μ M.

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 μ M.

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 ENPP1 deficiency or ENPP1-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.

37-38. (canceled)

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