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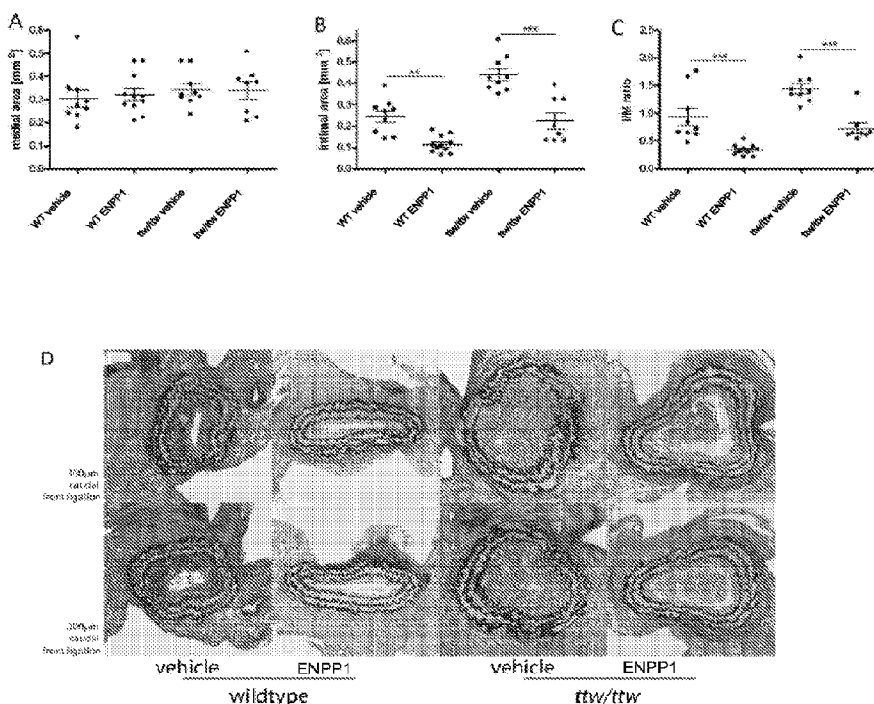
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(54) **Titre : COMPOSITIONS ET METHODES D'INHIBITION DE LA PROLIFERATION DE CELLULES MUSCULAIRES LISSES VASCULAIRES**

(54) **Title: COMPOSITIONS AND METHODS FOR INHIBITING VASCULAR SMOOTH MUSCLE CELL PROLIFERATION**

FIG. 5

Therapeutic Treatment



(57) **Abrégé/Abstract:**

The present disclosure provides compositions and methods for treating vascular smooth muscle cell proliferation in a subject that does not have a deficiency of ectonucleotide pyrophosphatase phosphodiesterase-1 (ENPP1) resulting in a pathological disease of calcification or ossification by administering an ENPP1 agent or an ENPP3 agent.

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Abstract:

The present disclosure provides compositions and methods for treating vascular smooth muscle cell proliferation in a subject that does not have a deficiency of ectonucleotide pyrophosphatase phosphodiesterase- 1 (ENPP1) resulting in a pathological disease of calcification or ossification by administering an ENPP1 agent or an ENPP3 agent.

**COMPOSITIONS AND METHODS FOR INHIBITING VASCULAR SMOOTH
MUSCLE CELL PROLIFERATION**

CROSS REFERENCE

This application claims priority to U.S. Application No. 63/030,870 filed on May 27, 2020, the content of which is herein incorporated by reference in its entirety.

SEQUENCE LISTING

This application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on May 27th, 2021, is named 4427-10102_sequence_ST25.txt and is 340 kilo bytes in size.

BACKGROUND

Myointimal proliferation or myointimal hyperplasia is a complex pathological process of the vascular system characterized by an abnormal proliferation of smooth muscle cells of the vascular wall. Proliferating smooth muscle cells migrate to the subendothelial area and form the hyperplastic lesion, which can cause stenosis and obstruction of the vascular lumen.

Atherosclerosis and neointimal hyperplasia both contribute to cardiovascular disease (CVD), with atherosclerosis resulting in initial native vessel stenosis and neointimal hyperplasia leading to recurrent stenosis after operative intervention. Although stents mitigate the risk of restenosis in selected coronary artery lesions, in-stent restenosis is still a frequent and often intractable clinical problem. Stent placement can directly damage the vessel wall and trigger neointimal hyperplasia that often leads to vessel restenosis, narrowing the lumen despite the stent preventing immediate vessel recoil after angioplasty and later constrictive remodeling. Mechanisms underlying the occurrence and recurrence of neointimal hyperplasia in patients with coronary stents is still not understood.

Neointimal hyperplasia is also the major cause of restenosis after percutaneous coronary interventions such as angioplasty. Neointimal hyperplasia in bypass conduits such as veins and prosthetic grafts greatly limits the long-term success of vascular interventions. Neointimal hyperplasia can affect all forms of vascular grafts, including

both venous and prosthetic conduits used in coronary and peripheral arterial bypass, and arteriovenous fistulae (AVF) created for hemodialysis access.

More than 1 million vascular grafts are implanted annually around the world. Up to 50% of these grafts fail within the 1st 18 months following surgery due to the development of neointimal hyperplasia at the anastomosis site. The lack of treatment to prevent this pathology is a major problem and is yet to be addressed effectively. Therefore, there is a need for efficient treatment to prevent and or reduce neointimal hyperplasia in various clinical interventions.

SUMMARY

The disclosure is based, at least in part, on the unexpected discovery that administration of soluble ENPP1 or ENPP3 can inhibit the undesirable proliferation of vascular smooth muscle cells in subjects who are not deficient in one or both of ENPP1 protein activity or expression. As set forth in the working examples below, the administration of soluble ENPP1 or ENPP3 inhibited proliferation of vascular smooth muscle cells following a tissue injury in wild type mice not deficient in ENPP1 expression or activity.

Accordingly, in one aspect, the disclosure provides a method for reducing and/or preventing the progression of vascular smooth muscle cell proliferation in a subject having a tissue injury. The method includes administering to the subject a therapeutically effective amount of an ENPP1 or an ENPP3 agent to thereby reduce and/or prevent progression of vascular smooth muscle cell proliferation at the site of injury in the subject.

Accordingly, in one aspect, the disclosure provides a method for reducing and/or preventing progression of vascular smooth muscle cell proliferation in a subject having a tissue injury. The method includes administering to the subject a therapeutically effective amount of an ENPP1 or ENPP3 agent to thereby reduce and/or prevent progression of vascular smooth muscle cell proliferation at the site of injury in the subject.

In some embodiments, the subject is not ENPP1 deficient.

In some embodiments of any of the methods described herein, the tissue injury is an injury to any artery or vein. The artery can be, e.g., a coronary artery or carotid artery.

In some embodiments of any of the methods described herein, the tissue injury is a result of stent placement in an artery. In some embodiments of any of the methods described herein, the subject is at risk of developing restenosis. In some embodiments of any of the

methods described herein, the subject suffers from restenosis. In some embodiments of any of the methods described herein, the subject suffers from restenosis in an artery.

In yet another aspect, the disclosure features a method for reducing and/or preventing the progression of vascular smooth muscle cell proliferation in a subject who requires surgery. The method comprises: administering to the subject a therapeutically effective amount of an ENPP1 or ENPP3 agent to thereby reduce and/or prevent progression of vascular smooth muscle cell proliferation at a surgical site in the subject.

In yet another aspect, the disclosure features a method for reducing and/or preventing progression of vascular smooth muscle cell proliferation in a subject who requires surgery. The method comprises: administering to the subject a therapeutically effective amount of an ENPP1 or ENPP3 agent to thereby reduce and/or prevent progression of vascular smooth muscle cell proliferation at a surgical site in the subject.

In some embodiments, any of the methods described herein can also include detecting the presence of and/or measuring the amount of vascular smooth muscle cell proliferation in the subject, e.g., at the site of an injury or at the site of surgery. In some embodiments, such detecting and/or measuring can occur prior to, during, or following administration of an ENPP1 agent or an ENPP3 agent.

In some embodiments of any of the methods described herein, the ENPP1 agent comprises ENPP1 variants that retain enzymatic activity.

In some embodiments of any of the methods described herein, the ENPP3 agent comprises ENPP3 variants that retain enzymatic activity.

In some embodiments of any of the methods described herein, the agent (e.g., the ENPP1 agent or the ENPP3 agent) is administered prior to the surgery.

In some embodiments of any of the methods described herein, the agent (e.g., the ENPP1 agent or the ENPP3 agent) is administered during surgery.

In some embodiments of any of the methods described herein, the agent (e.g., the ENPP1 agent or the ENPP3 agent) is administered after surgery.

In some embodiments of any of the methods described herein, the agent (e.g., the ENPP1 agent or the ENPP3 agent) is administered prior to, during and/or after surgery.

In some embodiments, any of the methods described herein further comprise performing the surgery.

In some embodiments of any of the methods described herein, the surgery comprises artery bypass grafting.

In some embodiments of any of the methods described herein, the surgery comprises placement of an arterial stent.

In some embodiments of any of the methods described herein, the surgery comprises angioplasty.

In another aspect, the disclosure provides a method of prophylaxis against vascular smooth muscle cell proliferation in a subject who is at risk for non-surgical tissue injury. The method includes administering to the subject a therapeutically effective amount of an ENPP1 or ENPP3 agent to thereby prevent the progression of vascular smooth muscle cell proliferation or reduce the extent of vascular smooth muscle cell proliferation at a site of non-surgical tissue injury in the subject. In some embodiments, the non-surgical tissue injury comprises blunt force trauma. In some embodiments, the subject is at risk of any one of the following: a cardiovascular disorder that is associated with undesirable smooth muscle cell proliferation, atherosclerotic cardiovascular disorder, a myocardial infarction, a stroke, developing coronary artery disease.

In another aspect, the disclosure provides a method of prophylaxis against vascular smooth muscle cell proliferation in a subject who is at risk for non-surgical tissue injury. The method includes administering to the subject a therapeutically effective amount of an ENPP1 or ENPP3 agent to thereby prevent the progression of vascular smooth muscle cell proliferation or reduce the extent of vascular smooth muscle cell proliferation at a site of non-surgical tissue injury in the subject. In some embodiments, the non-surgical tissue injury comprises blunt force trauma. In some embodiments, the subject is at risk of any one of the following: a cardiovascular disorder that is associated with undesirable smooth muscle cell proliferation, atherosclerotic cardiovascular disorder, a myocardial infarction, a stroke, developing coronary artery disease.

In some embodiments of any of the methods described herein, the subject is not ENPP1 Deficient.

In another aspect, the disclosure features a method for treating a subject suffering a myocardial infarction or a stroke. The method comprises administering to the subject a therapeutically effective amount of an ENPP1 or ENPP3 agent to thereby treat the myocardial infarction or stroke.

In another aspect, the disclosure features a method for treating a subject suffering a myocardial infarction or a stroke. The method comprises administering to the subject a therapeutically effective amount of an ENP1 or ENPP3 agent to thereby treat the myocardial infarction or stroke.

In yet another aspect, the disclosure features a method for reducing and/or preventing the progression of vascular smooth muscle cell proliferation in a subject suffering a myocardial infarction or a stroke. The method includes: administering to the subject a therapeutically effective amount of an ENPP1 or ENPP3 agent to thereby reduce and/or prevent the progression of vascular smooth muscle cell proliferation in vasculature associated with the subject's myocardial infarction or stroke.

In yet another aspect, the disclosure features a method for reducing and/or preventing progression of vascular smooth muscle cell proliferation in a subject suffering a myocardial infarction or a stroke. The method includes: administering to the subject a therapeutically effective amount of an ENPP1 or ENPP3 agent to thereby reduce and/or prevent progression of vascular smooth muscle cell proliferation in vasculature associated with the subject's myocardial infarction or stroke.

In some embodiments of any of the methods described herein, the subject is not ENPP1 Deficient.

In some embodiments of any of the methods described herein, the subject is not afflicted with Generalized Arterial Calcification of Infancy (GACI) or Autosomal Recessive Hypophosphatemic Rickets Type 2 (ARHR2).

In some embodiments of any of the methods described herein, the vascular smooth muscle cell proliferation is at the tunica intima of an arterial wall of the subject.

In some embodiments of any of the methods described herein, the tissue injury comprises vascular trauma.

In some embodiments of any of the methods described herein, the surgery comprises coronary intervention, such as scalpel incision or ablation.

In some embodiments of any of the methods described herein, the method includes performing the surgery while simultaneously administering the ENPP1 agent or the ENPP3 agent.

In some embodiments of any of the methods described herein, the method includes administering the ENPP1 agent or the ENPP3 agent prior to surgery or vascular intervention.

In some embodiments of any of the methods described herein, the method includes administering the agent, performing surgery while simultaneously administering the ENPP1 agent or ENPP3 agent, and optionally administering the agent after surgery.

In some embodiments of any of the methods described herein, the method includes administering the ENPP1 agent or ENPP3 agent, performing surgery, and optionally administering the agent after surgery.

In some embodiments of any of the methods described herein, the subject suffers from myocardial ischemia.

In some an embodiments of any of the methods described herein, the ENPP1 agent or ENPP3 agent is administered after treatment for said myocardial infarction and/or said stroke.

In some embodiments of any of the methods described herein, the ENPP1 agent comprises or is an ENPP1 polypeptide.

In some embodiments of any of the methods described herein, the ENPP1 agent comprises or is a nucleic acid encoding an ENPP1 polypeptide.

In some embodiments of any of the methods described herein, the ENPP1 agent comprises or is a viral vector comprising a nucleic acid encoding an ENPP1 polypeptide.

In some embodiments of any of the methods described herein, the ENPP3 agent comprises or is an ENPP3 polypeptide.

In some embodiments of any of the methods described herein, the ENPP3 agent comprises or is a nucleic acid encoding an ENPP3 polypeptide.

In some embodiments of any of the methods described herein, the ENPP3 agent comprises or is a viral vector comprising a nucleic acid encoding an ENPP3 polypeptide.

In some embodiments of any of the methods described herein, the ENPP1 polypeptide comprises the extracellular domain of ENPP1.

In some embodiments of any of the methods described herein, the ENPP1 polypeptide comprises the catalytic domain of ENPP1.

In some embodiments of any of the methods described herein, the ENPP1 polypeptide comprises amino acids 99 to 925 of SEQ ID NO:1.

In some embodiments of any of the methods described herein, the ENPP3 polypeptide comprises amino acids 49 to 875 of SEQ ID NO:7.

In some embodiments of any of the methods described herein, the ENPP1 agent or the ENPP3 agent comprises a heterologous moiety. In some embodiments, the heterologous moiety is a heterologous protein.

In some embodiments of any of the methods described herein, the heterologous moiety increases the half-life of the ENPP1 agent or the ENPP3 agent in a mammal, relative to the half-life of the ENPP1 agent or ENPP3 agent without the heterologous moiety.

In some embodiments of any of the methods described herein, the heterologous moiety is an Fc region of an immunoglobulin molecule, such as an IgG1. In some embodiments, the immunoglobulin is a human immunoglobulin.

In some embodiments of any of the methods described herein, the heterologous moiety is an albumin molecule.

In some embodiments of any of the methods described herein, the heterologous moiety is carboxy-terminal to the ENPP1 polypeptide or ENPP3 polypeptide.

In some embodiments of any of the methods described herein, the ENPP1 agent or the ENPP3 agent comprises a linker.

In some embodiments of any of the methods described herein, the linker separates the ENPP1 polypeptide or ENPP3 polypeptide and the heterologous protein.

In some embodiments of any of the methods described herein, the linker comprises the following amino acid sequence: (GGGGS)_n, wherein n is an integer from 1 to 10.

In some embodiments of any of the methods described herein, the heterologous moiety ENPP1 agent or ENPP3 agent is subcutaneously administered to the subject.

In some embodiments of any of the methods described herein, the ENPP1 agent or the ENPP3 agent is intravenously administered to the subject.

In yet another aspect, the disclosure features a coated stent comprising a vascular stent; and a coating on the stent, the coating comprising an ENPP1 agent; and a carrier for said ENPP1 agent, wherein said coating is configured to release said ENPP1 agent from the stent at a rate of 1-10 µg/ml per day.

In some embodiments of any of the stents described herein, the ENPP1 agent in an amount between 1 wt % and 50 wt %, based on a total weight of the coating.

In some embodiments of any of the stents described herein, the ENPP1 agent is selected from a group consisting of: ENPP1, ENPP1-Fc, ENPP1-Albumin, and ENPP1 mRNA.

In some embodiments of any of the stents described herein, the ENPP1 agent comprises ENPP1 variants that retain enzymatic activity.

In some embodiments of any of the stents described herein, the ENPP3 agent comprises ENPP3 variants that retain enzymatic activity.

In some embodiments of any of the stents described herein, the carrier is non-reactive with said ENPP1 agent.

In some embodiments of any of the stents described herein, the carrier comprises a polymeric carrier that is physically bound to said ENPP1 agent.

In some embodiments of any of the stents described herein, the carrier comprises a polymeric carrier that is chemically bound to said ENPP1 agent.

In some embodiments of any of the stents described herein, the carrier comprises a polymeric biodegradable carrier.

In some embodiments of any of the stents described herein, the carrier comprises a nonpolymeric carrier.

In some embodiments of any of the stents described herein, the nonpolymeric carrier is selected from a group consisting of: Vitamin E, Vitamin E acetate, Vitamin E succinate, oleic acid, peanut oil and cottonseed oil.

In some embodiments of any of the methods described herein, the carrier is liquid at body temperature. In some embodiments of any of the methods described herein the carrier is solid at body temperature.

In yet another aspect, the disclosure features a method for reducing and/or preventing progression of vascular smooth muscle cell proliferation in a subject having a tissue injury, the method comprising: implanting an arterial stent coated with an ENPP1 agent into an artery of the subject proximal to said tissue injury, wherein said implanted stent is configured to release said ENPP1 agent in an amount effective to reduce and/or prevent progression of vascular smooth muscle cell proliferation at a site of injury in the subject, wherein the subject is not ENPP1 deficient, thereby to reduce and/or prevent progression of vascular smooth muscle cell proliferation at said site of injury in said subject.

In some embodiments of any of the methods described herein, the tissue injury comprises stent placement in an artery.

In some embodiments of any of the methods described herein, the tissue injury is due to a prior placement of a non-eluting arterial stent in said artery or due to a prior placement of an eluting arterial stent in said artery which elutes therapeutic agents other than said ENPP1 agent.

In some embodiments of any of the methods described herein, the subject is at risk of developing restenosis.

In yet another aspect, the disclosure features a method for reducing and/or preventing progression of vascular smooth muscle cell proliferation in a subject who has a condition requiring surgery at a surgical site, the method comprising: implanting an arterial stent coated with an ENPP1 agent into an artery proximal to said surgical site in the subject, wherein said implanted stent is configured to release said ENPP1 agent in an amount effective to reduce and/or prevent progression of vascular smooth muscle cell proliferation, wherein the subject is not ENPP1 deficient, thereby to reduce and/or prevent progression of vascular smooth muscle cell proliferation at said surgical site.

In some embodiments of any of the methods described herein, the agent is administered to the subject prior to, during and/or after surgery.

In some embodiments of any of the methods described herein, further comprises performing the surgery.

In some embodiments of any of the methods described herein, the surgery comprises artery bypass grafting.

In some embodiments of any of the methods described herein, the condition requiring surgery is due to a prior placement of a non-eluting arterial stent in said artery.

In some embodiments of any of the methods described herein, the condition requiring surgery is due to a prior placement of an eluting arterial stent in said artery which elutes therapeutic agents other than said ENPP1 agent.

In some embodiments of any of the methods described herein, the surgery comprises angioplasty.

In yet another aspect, the disclosure features a method for ameliorating a myocardial infarction or a stroke in a subject suffering therefrom, the method comprising: implanting an arterial stent coated with an ENPP1 agent into an artery of said subject, wherein said implanted stent is configured to release said ENPP1 agent in an amount effective to ameliorate a myocardial infarction or stroke, thereby to ameliorating said myocardial infarction or stroke.

In yet another aspect, the disclosure features a method for reducing and/or preventing progression of vascular smooth muscle cell proliferation in a subject suffering a myocardial infarction or a stroke, the method comprising: implanting an arterial stent coated with an ENPP1 agent into an artery of a subject, wherein said implanted stent is configured to release said ENPP1 agent in an amount effective to reduce and/or prevent progression of vascular smooth muscle cell proliferation in vasculature associated with a myocardial infarction or stroke, thereby to reduce and/or prevent progression of vascular smooth muscle cell proliferation in vasculature of said subject associated with myocardial infarction or stroke.

In some embodiments of any of the methods described herein, the subject is not ENPP1 deficient.

In some embodiments of any of the methods described herein, the ENPP1 agent comprises an ENPP1 polypeptide.

In some embodiments of any of the methods described herein, the ENPP1 agent is a nucleic acid encoding an ENPP1 polypeptide.

In some embodiments of any of the methods described herein, the ENPP1 agent comprises a viral vector comprising a nucleic acid encoding an ENPP1 polypeptide.

In some embodiments of any of the methods described herein, the ENPP1 polypeptide comprises the extracellular domain of ENPP1.

In some embodiments of any of the methods described herein, the ENPP1 polypeptide comprises the catalytic domain of ENPP1.

In some embodiments of any of the methods described herein, the ENPP1 polypeptide comprises amino acids 99 to 925 of SEQ ID NO:1.

In some embodiments of any of the methods described herein, the ENPP1 polypeptide comprises a heterologous protein.

In some embodiments of any of the methods described herein, the heterologous protein increases the circulating half-life of the ENPP1 polypeptide in mammal.

In some embodiments of any of the methods described herein, the heterologous protein is an Fc region of an immunoglobulin molecule.

In some embodiments of any of the methods described herein, the immunoglobulin molecule is an IgG1 molecule.

In some embodiments of any of the methods described herein, the heterologous protein is an albumin molecule.

In some embodiments of any of the methods described herein, the heterologous protein is carboxy-terminal to the ENPP1 polypeptide.

In some embodiments of any of the methods described herein, the ENPP1 agent comprises a linker.

In some embodiments of any of the methods described herein, the linker separates the ENPP1 polypeptide and the heterologous protein.

In some embodiments of any of the methods described herein, the linker comprises the following amino acid sequence: (GGGGS)_n, wherein n is an integer from 1 to 10.

In yet another aspect, the disclosure features a method for reducing and/or preventing progression of vascular smooth muscle cell proliferation in a subject having a tissue injury, the method comprising: implanting an arterial stent coated with an ENPP3 agent into an artery of a subject proximal to said tissue injury, wherein said implanted stent is configured to release said ENPP3 agent in an amount effective to reduce and/or prevent progression of vascular smooth muscle cell proliferation at a site of injury in the subject, thereby to reduce and/or prevent progression of vascular smooth muscle cell proliferation at said site of injury in said subject.

In some embodiments of any of the methods described herein, the tissue injury comprises injury to an artery.

In some embodiments of any of the methods described herein, the tissue injury comprises stent placement in an artery.

In some embodiments of any of the methods described herein, the subject is at risk of developing restenosis.

In yet another aspect, the disclosure features a method for reducing and/or preventing progression of vascular smooth muscle cell proliferation in a subject who has a condition requiring surgery at a surgical site, the method comprising: implanting an arterial stent coated with an ENPP3 agent into an artery proximal to said surgical site in the subject, wherein said implanted stent is configured to release said ENPP3 agent in an amount effective to reduce and/or prevent progression of vascular smooth muscle cell proliferation, thereby to reduce and/or prevent progression of vascular smooth muscle cell proliferation at said surgical site. In some embodiments of any of the methods described herein, the agent is administered to the subject prior to, during and/or after surgery.

In some embodiments of any of the methods described herein, further comprises performing the surgery.

In some embodiments of any of the methods described herein, the surgery comprises artery bypass grafting.

In some embodiments of any of the methods described herein, the condition requiring surgery is due to a prior placement of a non-eluting arterial stent in said artery.

In some embodiments of any of the methods described herein, the condition requiring surgery is due to a prior placement of an eluting arterial stent in said artery which elutes therapeutic agents other than said ENPP3 agent.

In some embodiments of any of the methods described herein, the surgery comprises angioplasty.

In yet another aspect, the disclosure features a method for ameliorating a myocardial infarction or a stroke in a subject suffering therefrom, the method comprising: implanting an arterial stent coated with an ENPP3 agent into an artery of said subject, wherein said implanted stent is configured to release said ENPP3 agent in an amount effective to ameliorate a myocardial infarction or stroke, thereby to ameliorating said myocardial infarction or stroke.

In yet another aspect, the disclosure features a method for reducing and/or preventing progression of vascular smooth muscle cell proliferation in a subject suffering a myocardial

infarction or a stroke, the method comprising: implanting an arterial stent coated with an ENPP3 agent into an artery of a subject, wherein said implanted stent is configured to release said ENPP3 agent in an amount effective to reduce and/or prevent progression of vascular smooth muscle cell proliferation in vasculature associated with a myocardial infarction or stroke, thereby to reduce and/or prevent progression of vascular smooth muscle cell proliferation in vasculature of said subject associated with myocardial infarction or stroke.

In some embodiments of any of the methods described herein, the subject is not ENPP1 deficient.

In some embodiments of any of the methods described herein, the ENPP3 agent comprises an ENPP3 polypeptide.

In some embodiments of any of the methods described herein, the ENPP3 agent is a nucleic acid encoding an ENPP3 polypeptide.

In some embodiments of any of the methods described herein, the ENPP3 agent comprises a viral vector comprising a nucleic acid encoding an ENPP3 polypeptide.

In some embodiments of any of the methods described herein, the ENPP3 polypeptide comprises a heterologous protein.

In some embodiments of any of the methods described herein, the ENPP3 polypeptide comprises the extracellular domain of ENPP3.

In some embodiments of any of the methods described herein, the ENPP3 polypeptide comprises the catalytic domain of ENPP3.

In some embodiments of any of the methods described herein, the ENPP3 polypeptide comprises amino acids 49-875 of SEQ ID NO: 7.

In some embodiments of any of the methods described herein, the ENPP3 polypeptide comprises a heterologous protein.

In some embodiments of any of the methods described herein, the heterologous protein increases the circulating half-life of the ENPP3 polypeptide in mammal.

In some embodiments of any of the methods described herein, the heterologous protein is an Fc region of an immunoglobulin molecule.

In some embodiments of any of the methods described herein, the immunoglobulin molecule is an IgG1 molecule.

In some embodiments of any of the methods described herein, the heterologous protein is an albumin molecule.

In some embodiments of any of the methods described herein, the heterologous protein is carboxy-terminal to the ENPP3 polypeptide.

In some embodiments of any of the methods described herein, the ENPP3 agent comprises a linker

In some embodiments of any of the methods described herein, the linker separates the ENPP3 polypeptide and the heterologous protein.

In some embodiments of any of the methods described herein, the linker comprises the following amino acid sequence: (GGGGS)_n, wherein n is an integer from 1 to 10.

In yet another aspect, the disclosure features a coated stent comprising a vascular stent; and a coating on the stent, the coating comprising an ENPP3 agent; and a carrier for said ENPP3 agent, wherein said coating is configured to release said ENPP3 agent from the stent at a rate of 1-10 µg/ml per day.

In some embodiments of any of the methods described herein, the ENPP3 agent is in an amount between 1 wt % and 50 wt %, based on a total weight of the coating.

In some embodiments of any of the methods described herein, the ENPP3 agent is selected from a group consisting of: ENPP3, ENPP3-Fc, ENPP3-Albumin, and ENPP3 mRNA

In some embodiments of any of the methods described herein, the carrier is non-reactive with said ENPP3 agent.

In some embodiments of any of the methods described herein, the carrier comprises a polymeric carrier that is physically bound to said ENPP3 agent.

In some embodiments of any of the methods described herein, the carrier comprises a polymeric carrier that is chemically bound to said ENPP3 agent.

In some embodiments of any of the methods described herein, the carrier comprises a polymeric biodegradable carrier.

In some embodiments of any of the methods described herein, the carrier comprises a nonpolymeric carrier.

In some embodiments of any of the methods described herein, the nonpolymeric carrier is selected from a group consisting of: Vitamin E, Vitamin E acetate, Vitamin E succinate, oleic acid, peanut oil and cottonseed oil.

In some embodiments of any of the methods described herein, the carrier is liquid at body temperature.

In some embodiments of any of the methods described herein, the carrier is solid at body temperature.

Other features and advantages of the disclosure will be apparent from the following detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the schematic diagram of prophylactic treatment regimen of WT and *ttw/ttw* mice prior to carotid ligation. WT and *ttw/ttw* mice were treated 7 days prior to carotid ligation with ENPP1-Fc at an exemplary dosage of 10mg/kg weight by subcutaneous injection every other day. The control cohorts, WT and *ttw/ttw* mice, were injected with vehicle containing tris buffered saline, at pH 7.4. All mice were then dissected at 14 days after carotid ligation and the mice were approximately 9 weeks of age.

Fig. 2A shows a schematic diagram of the carotid artery ligation and sectioning for histological analysis. For morphometrical measurements of the ligated carotid arteries, 5µm sections immediately proximal of the ligation site were taken. A total of 12 sections per animal (every 25µm) were analyzed proximal from the ligation site, spanning a distance of approximately 250 µm. The medial area, the intimal area and the intima/media ratio (I/M ratio) were calculated for each section and a representative stained section is shown in Figure 2B.

Fig. 3 shows the histological analysis of the vasculature. Representative stained sections from either 100 µm (top) or 200 µm (bottom) from the ligation in WT mice/vehicle treated, WT mice/ENPP1-Fc treated, *ttw/ttw* mice/vehicle treated and *ttw/ttw* mice/ENPP1-Fc treated are shown from left to right, respectively. Von Gieson's solution stains elastic collagen fibers and distinguishes the internal (IEL) and external elastic lamina (EEL) from the lumen of the vessel (L). In the WT mice, the carotid ligation caused intimal hyperplasia resulting in narrowing of the lumen, with more severe narrowing closer to the ligature (100 µm) and less severe occlusion further away (200 µm). In contrast, in the *ttw/ttw* mice the degree of intimal hyperplasia appeared to be increased, as the lumen at 200 µm is almost completely occluded. Both WT and *ttw/ttw* mice show a decrease in proliferation of vascular smooth muscle cells (VSMC) upon ENPP1-Fc administration. The effect of a decrease in VSMC proliferation upon treatment with ENPP1-Fc is more pronounced in *ttw/ttw* mice but it is surprising to see the reduction in VSMC proliferation also in WT mice. It appears that even in WT mice, which do not have ENPP1 deficiency, the administration of ENPP1-Fc greatly reduces VSMC proliferation. The *ttw/ttw* mice and WT mice treated with ENPP1-Fc showed much less intimal hyperplasia than those treated with vehicle. This suggests that the administration of ENPP1-Fc prior to and after the carotid ligation protected against and reversed intimal hyperplasia.

Fig 4A-C and D-F show the morphometric quantitation of the results. Fig 4G shows the histological analysis of the vasculature. The sections were stained in the same manner as

describe above. Measurement of the circumference of the external and internal elastic lamina and the luminal border allows quantitation of the medial (M) and intimal (I) areas.

Administration of ENPP1-Fc prevents intimal proliferation after carotid ligation in WT- and *ttw/ttw*- mice. ENPP1-Fc treatment was started 7 days prior to carotid ligation, and serial sections of the left carotid arteries were taken 14 days (A-C) or 21 days (D-F) after carotid ligation. Morphometric quantitation was performed on medial (A & D) and intimal (B & E) areas, and the I/M ratio was calculated (C & F). Values are presented as the mean $\pm$ SEM, $n \geq 9$ each group, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (one-way ANOVA multiple group comparison followed by the Bonferroni's post hoc test).

The medial area, between the external and internal lamina, remained constant (Figure 4A). The intimal area around the lumen showed a statistically-significant increase in vehicle-treated WT mice relative to ENPP1-Fc treated WT mice (Figure 4B). Likewise, the intimal area around the lumen showed a statistically-significant increase in vehicle-treated *ttw/ttw* mice relative to ENPP1-Fc treated *ttw/ttw* mice (Figure 4B). The ENPP1-Fc-treated *ttw/ttw* mice were similar to ENPP1-Fc treated WT mice in both the intimal area and the I/M ratio, with the results again being statistically significant (Figure 4C).

Fig. 5 (A-C) shows that therapeutic administration of ENPP1-Fc inhibits intimal proliferation after carotid ligation in WT- and *ttw/ttw*- mice. Fig 5D shows the histological analysis of the vasculature. The sections were stained in the same manner as describe above. ENPP1-Fc treatment was started 7 days after carotid ligation, and serial sections of the left carotid arteries were taken 14 days after carotid ligation. Morphometric quantitation was performed on medial (A) and intimal (B) areas, and the I/M ratio was calculated (C). Values are presented as the mean $\pm$ SEM, $n=7$ for WT, $n=10$ for vehicle-treated *ttw/ttw* or rhENPP1-treated *ttw/ttw*- mice, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (one-way ANOVA multiple group comparison followed by the Bonferroni's post hoc test).

Evaluation of the therapeutic effects of ENPP1-Fc was initiated at 7 days post ligation, when neointimal hyperplasia was definitely present. The medial area, between the external and internal lamina, remained constant in all groups of mice (figure 5 A). Therapeutic treatment with ENPP1-Fc beginning at 7 days post ligation led to a significant reduction of the intimal area in ENPP1-Fc treated *ttw/ttw*- mice compared to vehicle treated *ttw/ttw* -mice (figure 5 B, $p < 0.05$), whereas a trend towards reduction was observed between ENPP1-Fc treated and vehicle treated WT-mice. The I/M ratio of both ENPP1-Fc treated

WT- and *ttw/ttw*- mice was significantly decreased compared to the levels of vehicle treated WT- and *ttw/ttw*- mice (figure 5 C, $p < 0.05$, both).

Fig.6A-C shows medial area, intimal area and I/M ratio graphs for determination of the best starting point and design of therapeutic treatment of *ttw/ttw*- and WT- mice. For determination of the best starting point, medial (A) and intimal (B) area and I/M ratio (C) of *ttw/ttw*- mice ligated for 7, 10 and 14 days were evaluated. Based on these data, carotid ligation in *ttw/ttw*- and WT- mice was performed in mice at 7 weeks of age and administration of ENPP1-Fc (10mg/kg weight, subcutaneously, every other day) or vehicle (TBS, pH7.4) started 7 days after carotid ligation (at 8 weeks of age), when intimal hyperplasia in carotid ligated *ttw/ttw*- mice is definitely present in vessels, and also significantly different compared to 14 days ligated *ttw/ttw*- mice ($p < 0.001$ for intimal area and I/M ratio, B and C). Values are presented as the mean $\pm$ SEM, * $p < 0.05$, *** $p < 0.001$ (one-way ANOVA multiple group comparison followed by the Bonferroni's post hoc test).

Fig.7 shows histological sections indicating degradation of intimal carotid tissue after carotid ligation for 21 days in *ttw/ttw*- mice. Histological analysis of the carotid artery of *ttw/ttw*- mice, which were ligated for 21 days (Elastica von Gieson's stain). Sections were made 200, 150, 100 and 50 μ m from point of ligation from *ttw/ttw*- mice showing degradation of intimal area and elastic fibers (Fig 7A). Positive TUNEL staining of carotids from *ttw/ttw*- mice ligated for 21 days compared to negative staining in carotids from WT- mice, approximately 300 μ m caudal from ligation (Fig. 7B). Negative control: staining was performed without TUNEL enzyme; positive control: degradation of DNA using DNase I grade I.

Fig 8 shows comparison of preventive and therapeutic administration of ENPP1-Fc on intimal proliferation after carotid ligation in WT- and *ttw/ttw*- mice. Preventive ENPP1-Fc treatment was started 7 days prior to carotid ligation, whereas therapeutic ENPP1-Fc treatment was started 7 days after carotid ligation. Serial sections of the left carotid arteries of all animals were taken 14 days after carotid ligation. Morphometric quantitation was performed on medial (A) and intimal (B) areas, and the I/M ratio was calculated (C). Values are presented as the mean $\pm$ SEM, $n \geq 8$ for each group, * $p < 0.05$, *** $p < 0.001$ (one-way ANOVA multiple group comparison followed by the Bonferroni's post hoc test).

Fig. 9A is a cross-section of an artery experiencing restenosis in the presence of an uncoated stent. The endothelium normally serves as a solid barrier between the layer of

smooth muscle cells **14** and the arterial lumen **20**. Small tears **16** in the endothelium **12** can expose smooth muscle cells **14**, which can then migrate into the arterial lumen **20** and hyperproliferate into a mass **18** which can partially or completely occlude the lumen **20** even though an uncoated stent **21** is placed, during a procedure **60** such as angioplasty, in the artery **10** to keep the arterial lumen **20** open. Fig. 9B is a cross-section of an artery **10** containing a coated stent **22**. The stent has a coating **24** containing a carrier and a bioactive compound such as ENPP1 agent **65** that inhibits and or prevents restenosis. By using a stent having this coating **24**, the tears **16** shown in FIG. 9A in the endothelium **12** may be reduced or eliminated. Additionally, the mass **18** created by a proliferation of smooth muscle cells **14**, as shown in FIG. 9A, is eliminated or substantially reduced.

DETAILED DESCRIPTION

Definitions

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

For clarity, "*NPPI*" and "*ENPPI*" refer to the same protein and are used interchangeably herein. As used herein, the term "*ENPPI protein*" or "*ENPPI polypeptide*" refers to ectonucleotide pyrophosphatase/phosphodiesterase-1 protein encoded by the *ENPPI* gene that is capable of cleaving ATP to generate PP_i and also reduces ectopic calcification in soft tissue.

ENPP1 protein is a type II transmembrane glycoprotein and cleaves a variety of substrates, including phosphodiester bonds of nucleotides and nucleotide sugars and pyrophosphate bonds of nucleotides and nucleotide sugars. ENPP1 protein has a transmembrane domain and soluble extracellular domain. The extracellular domain is further subdivided into somatomedin B domain, catalytic domain and the nuclease domain. The sequence and structure of wild-type ENPP1 is described in detail in PCT Application Publication No. WO 2014/126965 to Braddock, et al., which is incorporated herein in its entirety by reference.

ENPP1 polypeptides as used herein encompass polypeptides that exhibit ENPP1 enzymatic activity, mutants of ENPP1 that retain ENPP1 enzymatic activity, fragments of

ENPP1 or variants of ENPP1 including deletion variants that exhibit ENPP1 enzymatic activity, as noted below.

ENPP3 polypeptides as used herein encompass polypeptides that exhibit enzymatic activity, mutants of ENPP3 that retain enzymatic activity, fragments of ENPP3 or variants of ENPP3 including deletion variants that exhibit enzymatic activity as noted below.

Some examples of ENPP1 and ENPP3 polypeptides, mutants, or mutant fragments thereof, have been previously disclosed in International PCT Application Publications No. WO/2014/126965- Braddock et al., WO/2016/187408-Braddock et al., WO/2017/087936- Braddock et al., and WO2018/027024-Braddock et al., all of which are incorporated by reference in their entireties herein.

“*Enzymatically active*” with respect to an ENPP1 polypeptide or an ENPP3 polypeptide, or, as used herein, “enzymatic activity” with respect to an ENPP1 polypeptide or an ENPP3 polypeptide, is defined as possessing ATP hydrolytic activity into AMP and PP_i and/or AP3a hydrolysis to ADP and AMP. NPP1 and NPP3 readily hydrolyze ATP into AMP and PP_i. The steady-state Michaelis-Menten enzymatic constants of NPP1 are determined using ATP as a substrate. NPP1 can be demonstrated to cleave ATP by HPLC analysis of the enzymatic reaction, and the identity of the substrates and products of the reaction are confirmed by using ATP, AMP, and ADP standards. The ATP substrate degrades over time in the presence of NPP1, with the accumulation of the enzymatic product AMP. Using varying concentrations of ATP substrate, the initial rate velocities for NPP1 are derived in the presence of ATP, and the data is fit to a curve to derive the enzymatic rate constants. At physiologic pH, the kinetic rate constants of NPP1 are $K_m=144 \mu\text{M}$ and $k_{cat}=7.8 \text{ s}^{-1}$.

As used herein the term “*plasma pyrophosphate (PP_i) levels*” refers to the amount of pyrophosphate present in plasma of animals. In certain embodiments, animals include rat, mouse, cat, dog, human, cow and horse. It is necessary to measure PP_i in the plasma rather than serum because of release from platelets. There are several ways to measure PP_i, one of which is by enzymatic assay using uridine-diphosphoglucose (UDPG) pyrophosphorylase (Iust & Seegmiller, 1976, *Clin. Chim. Acta* 66:241-249; Cheung & Suhadolnik, 1977, *Anal. Biochem.* 83:61-63) with modifications.

Typically, plasma PP_i levels in healthy human subjects range from about 1 μM to about 3 μM , in some cases between 1-2 μM . A normal level of ENPP1 in plasma refers to the amount of ENPP1 protein required to maintain a normal level of plasma pyrophosphate (PP_i) in a healthy subject. A normal level of PP_i in healthy humans corresponds to 2-3 μM .

Subjects who have a deficiency of ENPP1 exhibit low PP_i levels which range from at least 10% below normal levels, at least 20% below normal levels, at least 30% below normal levels, at least 40% below normal levels, at least 50% below normal levels, at least 60% below normal levels, at least 70% below normal levels, at least 80% below normal levels and combinations thereof. In patients afflicted with GACI, the PP_i levels are found to be less than 1 μ m and in some cases are below a detectable level. In patients afflicted with PXE, the PP_i levels are below 0.5 μ m. (*Arterioscler Thromb Vasc Biol.* 2014 Sep;34(9):1985-9; Braddock *et al.*, *Nat Commun.* 2015; 6: 10006.)

As used herein, the term “ PP_i ” refers to pyrophosphate.

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, including missense and nonsense mutations, insertions, deletions, frameshifts and premature terminations.

As used herein, the term “*ENPP1 precursor protein*” refers to ENPP1 polypeptide with its signal peptide sequence at the ENPP1 N-terminus. Upon proteolysis, the signal sequence is cleaved from ENPP1 to provide the ENPP1 protein. Signal peptide sequences useful within the disclosure include, but are not limited to, Albumin signal sequence, Azurocidin signal sequence, ENPP1 signal peptide sequence, ENPP2 signal peptide sequence, ENPP7 signal peptide sequence, and/or ENPP5 signal peptide sequence.

As used herein, the term “*ENPP3 precursor protein*” refers to ENPP3 polypeptide with its signal peptide sequence at the ENPP3 N-terminus. Upon proteolysis, the signal sequence is cleaved from ENPP3 to provide the ENPP3 protein. Signal peptide sequences useful within the disclosure include, but are not limited to, Albumin signal peptide sequence, Azurocidin signal peptide sequence, ENPP1 signal peptide sequence, ENPP2 signal peptide sequence, ENPP7 signal peptide sequence, and/or ENPP5 signal peptide sequence.

As used herein, the term “*Azurocidin signal peptide sequence*” refers to the signal peptide derived from human Azurocidin. Azurocidin, also known as cationic antimicrobial protein CAP37 or heparin-binding protein (HBP), is a protein that in humans is encoded by the AZU1 gene. The nucleotide sequence encoding Azurocidin signal peptide (MTRLTVLALLAGLLASSRA (SE ID NO: 42) is fused with the nucleotide sequence of NPP1 or NPP3 gene which when encoded generates ENPP1 precursor protein or ENPP3 precursor protein. (*Optimized signal peptides for the development of high expressing CHO cell lines*, Kober *et al.*, *Biotechnol Bioeng.* 2013 Apr;110(4):1164-73)

The term “*ENPP1-Fc construct*” refers to ENPP1 recombinantly fused and/or chemically conjugated (including both covalent and non-covalent conjugations) to an FcR binding domain of an IgG molecule (preferably, a human IgG). In certain embodiments, the C-terminus of ENPP1 is fused or conjugated to the N-terminus of the FcR binding domain.

As used herein, the term “*ENPP3-Fc construct*” refers to ENPP3 recombinantly fused and/or chemically conjugated (including both covalent and non-covalent conjugations) to an FcR binding domain of an IgG molecule (preferably, a human IgG). In certain embodiments, the C-terminus of ENPP1 is fused or conjugated to the N-terminus of the FcR binding domain.

As used herein, the term “*Fc*” refers to a human IgG (immunoglobulin) Fc domain. Subtypes of IgG such as IgG1, IgG2, IgG3, and IgG4 are contemplated for use as Fc domains. The “*Fc region or Fc polypeptide*” is the portion of an IgG molecule that correlates to a crystallizable fragment obtained by papain digestion of an IgG molecule. The Fc region comprises the C-terminal half of the two heavy chains of an IgG molecule that are linked by disulfide bonds. It has no antigen binding activity but contains the carbohydrate moiety and the binding sites for complement and Fc receptors, including the FcRn receptor. The Fc fragment contains the entire second constant domain CH2 (residues 231-340 of human IgG1, according to the Kabat numbering system) and the third constant domain CH3 (residues 341-447). The term “IgG hinge-Fc region” or “hinge-Fc fragment” refers to a region of an IgG molecule consisting of the Fc region (residues 231 -447) and a hinge region (residues 216-230) extending from the N-terminus of the Fc region. The term “constant domain” refers to the portion of an immunoglobulin molecule having a more conserved amino acid sequence relative to the other portion of the immunoglobulin, the variable domain, which contains the antigen binding site. The constant domain contains the CH1, CH2 and CH3 domains of the heavy chain and the CHL domain of the light chain.

As used herein the term “*functional equivalent variant*”, as used herein, relates to a polypeptide substantially homologous to the sequences of ENPP1 or ENPP3 (defined above) and that preserves the enzymatic and biological activities of ENPP1 or ENPP3, respectively. Methods for determining whether a variant preserves the biological activity of the native ENPP1 or ENPP3 are widely known to the skilled person and include any of the assays used in the experimental part of said application. Particularly, functionally equivalent variants of ENPP1 or ENPP3 delivered by viral vectors is encompassed by the present disclosure. The functionally equivalent variants of ENPP1 or ENPP3 are polypeptides substantially homologous to the native ENPP1 or ENPP3 respectively. The expression “substantially

homologous”, relates to a protein sequence when said protein sequence has a degree of identity with respect to the ENPP1 or ENPP3 sequences described above of at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% respectively and still retaining at least 50%, 55%, 60%, 70%, 80% or 90% activity of wild type ENPP1 or ENPP3 protein with respect to enzymatic activity

The degree of identity between two polypeptides is determined using computer algorithms and methods that are widely known for the persons skilled in the art. The identity between two amino acid sequences is preferably determined by using the BLASTP algorithm (*BLAST Manual*, Altschul, S., et al., NCBI NLM NIH Bethesda, Md. 20894, Altschul, S., et al., *J. Mol. Biol.* 215: 403-410 (1990)), though other similar algorithms can also be used. BLAST and BLAST 2.0 are used, with the parameters described herein, to determine percent sequence identity. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information.

“*Functionally equivalent variants*” of ENPP1 or ENPP3 may be obtained by replacing nucleotides within the polynucleotide accounting for codon preference in the host cell that is to be used to produce the ENPP1 or ENPP3 respectively. Such “codon optimization” can be determined via computer algorithms which incorporate codon frequency tables such as “Human high.cod” for codon preference as provided by the University of Wisconsin Package Version 9.0, Genetics Computer Group, Madison, Wis. The variants of ENPP1 or ENPP3 polypeptides are expected to retain at least 50%, 55%, 60%, 70%, 80% or 90% activity of wild type ENPP1 or ENPP3 protein with respect to enzymatic activity.

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 most frequently observed in a population and is thus arbitrarily designed the “normal” or “wild-type” form of the human NPP1 or NPP3 genes. In contrast, the term “functionally equivalent” refers to an NPP1 or NPP3 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.

“About” as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of + 20% or + 10%, more preferably + 5%, even more preferably + 1%, and still more preferably + 0.1% from the specified value, as such variations are appropriate to perform the disclosed methods.

As defined herein, the term “*subject*”, “*individual*” or “*patient*” refers to mammal preferably a human.

As defined herein, the term “*moiety*” refers to a chemical component or biological molecule that can be covalently or non-covalently linked to ENPP1 or ENPP3 protein and has the ability to confer a desired property to the protein to which it is attached. For example, the term moiety can refer to a bone targeting peptide such as polyaspartic acid or polyglutamic acid (of 4-20 consecutive asp or glu residues) or a molecule that extends the half-life of ENPP1 or ENPP3 polypeptide. Some other examples of half-life extending moieties include Fc, albumin, transferrin, polyethylene glycol (PEG), homo-amino acid polymer (HAP), proline-alanine-serine polymer (PAS), elastin-like peptide (ELP), and gelatin-like protein (GLK).

As defined herein, the phrase “*medial area*” is the area between lamina elastica externa and lamina elastica interna of an artery.

As defined herein, the phrase “*intimal area*” and said intimal area is the area between said lamina elastica interna and lumen of an artery.

As defined herein, the phrase “*lamina elastica externa*” refers to a layer of elastic connective tissue lying immediately outside the smooth muscle of the tunica media of an artery.

As defined herein, the phrase “*lamina elastica interna*” refers to a layer of elastic tissue that forms the outermost part of the tunica intima of blood vessels.

As defined herein, the phrase “*lumen*” refers to the interior of a vessel, such as the central space in an artery, vein or capillary through which blood flow occurs.

As defined herein, the phrase “*surgery*” refers to an invasive medical procedure that involves coronary interventions which result in tissue injury by scalpel incision or radiofrequency ablation or cryoablation or laser ablation.

As defined herein, the phrase “*tissue injury*” refers to proliferation or onset of proliferation and migration of vascular smooth muscle eventually resulting in the thickening

of arterial walls and decreased arterial lumen space resulting restenosis after percutaneous coronary interventions such as stenting or angioplasty.

As defined herein, the phrase “*deficient for NPP1*” or “*ENPP1 deficiency*” refers to having a loss of function mutation in ENPP1 protein or in a gene encoding the protein that result in a diagnosis of Generalized Arterial Calcification of Infancy. (GACI), or a diagnosis of being at risk of developing or of being afflicted with autosomal recessive hypophosphatemic rickets type 2 (ARHR2).

As defined herein, the phrase “*vascular trauma*” refers to an injury to a blood vessel—an artery, which carries blood to an extremity, or a vein, which returns blood to the heart. Vascular injuries may also be caused by invasive procedures, such as percutaneous transluminal coronary angioplasty, and vascular bypass surgery.

As defined herein the phrase “*accidental trauma*” refers to a blood vessel such as artery by a blunt injury that occurs when a blood vessel is crushed or stretched due to exertion of physical force or penetrating injury which occurs when a blood vessel is punctured, torn or severed. Blunt injury occurs during physical alterations such as boxing and penetrating injury occurs due to sharp objects such as knife or bullet wounds. The trauma or injury can be caused by different factors, such as radiation, viral infections, development of immune complexes, and hyperlipidemia.

As defined herein the phrase “*restenosis*” refers to the recurrence of stenosis. Stenosis refers to the narrowing of a blood vessel, leading to restricted blood flow. Restenosis usually pertains to an artery or other large blood vessel that has become narrowed, received treatment to clear the blockage and subsequently becomes re-narrowed. Restenosis is commonly detected by using one or more of ultrasound, X-ray computed tomography (CT), nuclear imaging, optical imaging or contrast enhanced image or immunohistochemical detection.

As defined herein the phrase “*myointimal proliferation*” refers to the proliferation of vascular smooth muscle cells that occurs at the tunica intima of an arterial wall of an individual.

As used herein, the term “*treatment*” or “*treating*” is defined as the application or administration of soluble NPP1 (alone or in combination with another pharmaceutical agent), to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient (e.g., for diagnosis or ex vivo applications), who has a disease or disorder, a symptom of a disease or disorder or the potential to develop a disease or disorder, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease or disorder, the symptoms of the disease or disorder, or the potential to develop the

disease or disorder. Such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics.

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

As used herein, the phrase “*reduce or prevent myointimal or neointimal proliferation*” refers to the ability of soluble NPP1 upon administration to reduce the level of proliferation vascular smooth muscle cells at the site of tissue injury thereby reducing the thickening of arterial walls and prevent the occurrence of or reduce the level of restenosis of the artery.

As used herein the term “*coronary intervention*” refers to surgical and non-surgical procedures, such as including balloon angioplasty, angioplasty with stent, rotablation or cutting balloon catheterization that are performed to clear blockage and restore blood flow to the blocked blood vessels.

As used herein the term “non-surgical tissue injury” refers to injuries sustained to a tissue or blood vessel during a traumatic event including but not limited to physical altercations involving the use of blunt force or sharp objects such as a knife, mechanical injury such fall from elevation, workplace injury due to heavy machinery or vehicular injury such as car accidents.

As used herein the term “site of non-surgical tissue injury” refers to the site at which the tissue injury has occurred which includes but not limited to the brain, spinal cord, coronary arterial vessels, and peripheral arterial vessels

As used herein, the term “*site of surgery*” refers to the region of the artery upon which a tissue injury has occurred either due to vascular trauma or accidental trauma.

As used herein the term “*ENPP1 fragment*” refers to a fragment or a portion of ENPP1 protein or an active subsequence of the full-length NPP1 having at least an ENPP1 catalytic domain administered in protein form or in the form of a nucleic acid encoding the same.

As used herein, the term “*ENPP1 agent*” refers to ENPP1 polypeptide or fusion protein or ENPP1 fragment comprising at least catalytic domain capable of producing plasma pyrophosphate (Ppi) by cleavage of adenosine triphosphate (ATP) or a polynucleotide such as cDNA or RNA encoding ENPP1 polypeptide or fusion protein or ENPP1 fragment comprising at least catalytic domain capable of producing Ppi by enzymatic cleavage of ATP or a vector such as a viral vector containing a polynucleotide encoding the same.

As used herein, the term “*stent*” refers to a tubular support placed inside a blood vessel, canal, or duct to aid healing or relieve an obstruction or prevent narrowing of the passage. Stents generally comprise an expandable mesh coil which is made of metal (ex: stainless steel, Cobalt alloy, Nickel-titanium alloy, manganese alloy, molybdenum alloy, platinum alloy, tungsten alloy) or polymers (ex: Silicone).

As used herein, the term “*vascular stent*” refers to a tubular support placed inside an artery or vein of a mammal to aid healing or relieve an obstruction or prevent narrowing of the arterial passage.

As used herein, the term “*coated stent*” or “*eluting stent*” refers to a stent that is coated with a therapeutic molecule such as protein, chemical compound or nucleic acid that gradually elutes from the stent surface (interior or exterior) at the site of implantation thereby providing therapeutic relief. Therapeutic molecules such as ENPP1 agent or ENPP3 agent can be bonded directly to a metal stent, and some are bonded to a matrix polymer, which acts as a drug reservoir to ensure drug retention during deployment and a uniform distribution on the stent. The types, compositions, and designs of the polymers coated on the stent dictate the eluting kinetic of the sustain time release of the drug over a period of weeks or months following the implantation in situ. The coating materials can be categorized as organic vs inorganic, bioerodable vs nonbioerodable, and synthetic vs naturally occurring substances.

As used herein, the term “*coating*” refers to composition comprising a polymeric carrier that is used in conjunction with an ENPP1 agent or ENPP3 agent to coat the stents. The coating may be applied in the form a spray or dried film comprising the ENPP1 agent or ENPP3 agent suspended in a polymeric matrix. The polymeric carrier is in an amount sufficient to provide a polymer matrix or support for the ENPP1 agent or ENPP3 agent. The polymer is preferably non-reactive with the ENPP1 agent or ENPP3 agent, i.e., no chemical reaction occurs when the two are mixed.

As used herein, the term “*solvent*” is defined according to its broadest recognized definition and includes any material into which the carrier (polymer) and the ENPP1 agent or ENPP3 agent can dissolve, fully or partially, at room temperature or from 20° C. to 40° C to form the coating composition. Sterile, double distilled water is a preferred solvent.

As used herein, the term “*site of injury*” refers to a region in the vasculature where the flow of blood or spinal fluid is constricted due to accumulation of one or more of lipids, cholesterol, calcium, and various types of cells, such as smooth muscle cells and platelets. The site of injury is commonly identified by using Cardiac catheterization. During a cardiac catheterization, a long, narrow tube called a catheter is inserted through a plastic introducer

sheath (a short, hollow tube that is inserted into a blood vessel in your arm or leg). The catheter is guided through the blood vessel to the coronary arteries with the aid of an x-ray machine. Contrast material is injected through the catheter and x-ray images (Coronary angiogram) are created as the contrast material moves through the heart's chambers, valves and major vessels. The digital photographs of the contrast material are used to identify the site of the narrowing or blockage in the coronary artery. Additional imaging procedures, called intra-vascular ultrasound (IVUS) and fractional flow reserve (FFR), may be performed along with cardiac catheterization in some cases to obtain detailed images of the walls of the blood vessels.

As used herein "*site of implant*" refers to the region at which the ENPP1 or ENPP3 coated stent is implanted in the vasculature. The coated stents of the invention can be placed at the center of the to the site of tissue injury, immediately adjacent the site of tissue injury or within 200 μm on either side from the center of the site of tissue injury.

As used herein, the term "*myocardial infarction*" refers to permanent damage to the heart muscle that occurs due to the formation of plaques in the interior walls of the arteries resulting in reduced blood flow to the heart and injuring heart muscles because of lack of oxygen supply. The symptoms of MI include chest pain, which travels from left arm to neck, shortness of breath, sweating, nausea, vomiting, abnormal heart beating, anxiety, fatigue, weakness, stress, depression, and other factors.

As used herein the term "*myocardial ischemia*" refers to the condition of the heart muscle that is characterized by a decrease in blood supply to the heart tissue which leads to chest pain or angina pectoris, myocardial infarction is the end point of this ischemia that results in the death of heart tissue due to absence of blood supply. Coronary artery disease (CAD) is considered as a common cause of myocardial ischemia.

As used herein the term "*blunt force trauma*" refers to physical trauma to a body part, either by impact, injury or physical attack or high-velocity impact. Blunt trauma can lead to contusions, abrasions, lacerations, and/or bone fractures. As used herein the term "*non-surgical tissue injury*" or "*penetrating trauma*" refers to trauma to a body part which occurs when an object such as a projectile or knife enters a tissue of the body, creating an open wound.

As used herein the term "*scalpel incision*" refers to incision made in a tissue using a sharp object such as a scalpel during surgical procedure. An incision is a cut made into the tissues of the body to expose the underlying tissue, bone, so that a surgical procedure can be performed.

As used herein the term “*ablation*” refers to the removal or destruction of a body part or tissue or its function. Ablation may be performed by surgery, hormones, drugs, radiofrequency, heat.

As used herein, the term “*effective amount*” refers to an amount of an agent (e.g., NPP1 fusion or NPP3 fusion polypeptides) which, as compared to a corresponding subject who has not received such an amount, sufficient to provide improvement of a condition, disorder, disease, or to provide a decrease in progression or advancement of a condition, disorder, or disease. An effective amount also may result in treating, healing, preventing or ameliorating a condition, disease, or disorder. The term also includes within its scope amounts effective to enhance normal physiological function.

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.

As used here the term “*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 a substantially purified form or can exist in a non-native environment such as, for example, a host cell.

As used herein, “*substantially purified*” refers to being essentially free of other components. For example, a substantially purified polypeptide is a polypeptide that has been separated from other components with which it is normally associated in its naturally occurring state. Non-limiting embodiments include 95% purity, 99% purity, 99.5% purity, 99.9% purity and 100% purity.

As used herein the term “*oligonucleotide*” or “*polynucleotide*” is a nucleic acid ranging from at least 2, in certain embodiments at least 8, 15 or 25 nucleotides in length, but may be up to 50, 100, 1000, or 5000 nucleotides long or a compound that specifically hybridizes to a polynucleotide.

As used herein, the term “*pharmaceutical composition*” or “composition” refers to a mixture of at least one compound useful within the disclosure 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, subcutaneous, intravenous, oral, aerosol, inhalational, rectal,

vaginal, transdermal, intranasal, buccal, sublingual, parenteral, intrathecal, intragastrical, ophthalmic, pulmonary, and topical administration.

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

As used herein, the term “*pathological calcification*” refers to the abnormal deposition of calcium salts in soft tissues, secretory and excretory passages of the body causing it to harden. There are two types, dystrophic calcification which occurs in dying and dead tissue and metastatic calcification which elevated extracellular levels of calcium (hypercalcemia), exceeding the homeostatic capacity of cells and tissues. Calcification can involve cells as well as extracellular matrix components such as collagen in basement membranes and elastic fibers in arterial walls. Some examples of tissues prone to calcification include: Gastric mucosa – the inner epithelial lining of the stomach, Kidneys and lungs, Cornea, Systemic arteries and Pulmonary veins.

As used herein, the term “*pathological ossification*” refers to a pathological condition in which bone arises in tissues not in the osseous system and in connective tissues usually not manifesting osteogenic properties. Ossification is classified into three types depending on the nature of the tissue being affected, endochondral ossification is ossification that occurs in and replaces cartilage. Intramembranous ossification is the ossification of bone that occurs in and replaces connective tissue. Metaplastic ossification the development of bony substance in normally soft body structures; called also heterotrophic ossification.

As used herein, “*reduction of calcification*” is observed by using non-invasive methods like X-rays, micro CT and MRI. Reduction of calcification is also inferred by using radio imaging with ^{99m}Tc-pyrophosphate (^{99m}PYP) uptake. The presence of calcifications in mice are evaluated via post-mortem by micro-computed tomography (CT) scans and histologic sections taken from the heart, aorta and kidneys with the use of dyes such as Hematoxylin and Eosin (H&E) and Alizarin red by following protocols established by Braddock et al. (Nature Communications volume 6, Article number: 10006 (2015))

A “*low level of PP_i*” refers to a condition in which the subject has less than or equal to 2%-5% of normal levels of plasma pyrophosphate (PP_i). Normal levels of Plasma PP_i in

healthy human subjects is approximately 1.8 to 2.6 μM . (Arthritis and Rheumatism, Vol. 22, No. 8 (August 1979))

As used herein the term “*Ectopic calcification*” refers to a condition characterized by a pathologic deposition of calcium salts in tissues or bone growth in soft tissues.

As used herein the term “*Ectopic calcification of soft tissue*” refers to inappropriate biomineralization, typically composed of calcium phosphate, hydroxyapatite, calcium oxalates and ocatcalcium phosphates occurring in soft tissues leading to loss of hardening of soft tissues. “Arterial calcification” refers to ectopic calcification that occurs in arteries and heart valves leading to hardening and or narrowing of arteries. Calcification in arteries is correlated with atherosclerotic plaque burden and increased risk of myocardial infarction, increased ischemic episodes in peripheral vascular disease, and increased risk of dissection following angioplasty.

As used herein, the term “*Venous calcification*” refers to ectopic calcification that occurs in veins that reduces the elasticity of the veins and restricts blood flow which can then lead to increase in blood pressure and coronary defects.

As used herein, the term “*Vascular calcification*” refers to the pathological deposition of mineral in the vascular system. It has a variety of forms, including intimal calcification and medial calcification, but can also be found in the valves of the heart. Vascular calcification is associated with atherosclerosis, diabetes, certain heredity conditions, and kidney disease, especially CKD. Patients with vascular calcification are at higher risk for adverse cardiovascular events. Vascular calcification affects a wide variety of patients. Idiopathic infantile arterial calcification is a rare form of vascular calcification where the arteries of neonates calcify.

As used herein, the term “*Brain calcification*” (BC) refers to a nonspecific neuropathology wherein deposition of calcium and other mineral in blood vessel walls and tissue parenchyma occurs leading to neuronal death and gliosis. Brain calcification is” often associated with various chronic and acute brain disorders including Down’s syndrome, Lewy body disease, Alzheimer’s disease, Parkinson’s disease, vascular dementia, brain tumors, and various endocrinologic conditions. Calcification of heart tissue refers to accumulation of deposits of calcium (possibly including other minerals) in tissues of the heart, such as aorta tissue and coronary tissue.

The terms “*adeno-associated viral vector*”, “*AAV vector*”, “*adeno-associated virus*”, “*AAV virus*”, “*AAV virion*”, “*AAV viral particle*” and “*AAV particle*”, as used interchangeably herein, refer to a viral particle composed of at least one AAV capsid protein

(preferably by all of the capsid proteins of a particular AAV serotype) and an encapsidated recombinant viral genome. The particle comprises a recombinant viral genome having a heterologous polynucleotide comprising a sequence encoding human ENPP1 or human ENPP3 or a functionally equivalent variant thereof,) and a transcriptional regulatory region that at least comprises a promoter flanked by the AAV inverted terminal repeats. The particle is typically referred to as an “*AAV vector particle*” or “*AAV vector*”.

As used herein, the term “*vector*” means a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. In some embodiments, the vector is a plasmid, i.e., a circular double stranded DNA loop into which additional DNA segments may be ligated. In some embodiments, the vector is a viral vector, wherein additional nucleotide sequences may be ligated into the viral genome. In some embodiments, the vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). In other embodiments, the vectors (e.g., non-episomal mammalian vectors) is integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors (expression vectors) are capable of directing the expression of genes to which they are operatively linked.

As used herein, the term “*recombinant host cell*” (or simply “host cell”), as used herein, means a cell into which an exogenous nucleic acid and/or recombinant vector has been introduced. It should be understood that “recombinant host cell” and “host cell” mean not only the particular subject cell but also the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term “host cell” as used herein.

The term “*recombinant viral genome*”, as used herein, refers to an AAV genome in which at least one extraneous expression cassette polynucleotide is inserted into the naturally occurring AAV genome. The genome of the AAV according to the disclosure typically comprises the cis-acting 5' and 3' inverted terminal repeat sequences (ITRs) and an expression cassette.

The term “*expression cassette*”, as used herein, refers to a nucleic acid construct, generated recombinantly or synthetically, with a series of specified nucleic acid elements, which permit transcription of a particular nucleic acid in a target cell. The expression cassette of the recombinant viral genome of the AAV vector according to the disclosure comprises a

transcriptional regulatory region operatively linked to a nucleotide sequence encoding ENPP1 or ENPP3 or a functionally equivalent variant thereof.

The term “*transcriptional regulatory region*”, as used herein, refers to a nucleic acid fragment capable of regulating the expression of one or more genes. The transcriptional regulatory region according to the disclosure includes a promoter and, optionally, an enhancer.

The term “*promoter*”, as used herein, refers to a nucleic acid fragment that functions to control the transcription of one or more polynucleotides, located upstream the polynucleotide sequence(s), and which is structurally identified by the presence of a binding site for DNA-dependent RNA polymerase, transcription initiation sites, and any other DNA sequences including, but not limited to, transcription factor binding sites, repressor, and activator protein binding sites, and any other sequences of nucleotides known in the art to act directly or indirectly to regulate the amount of transcription from the promoter. Any kind of promoters may be used in the disclosure including inducible promoters, constitutive promoters and tissue-specific promoters.

The term “*enhancer*”, as used herein, refers to a DNA sequence element to which transcription factors bind to increase gene transcription. Examples of enhancers may be, without limitation, RSV enhancer, CMV enhancer, HCR enhancer, etc. In another embodiment, the enhancer is a liver-specific enhancer, more preferably a hepatic control region enhancer (HCR).

The term “*operatively linked*”, as used herein, refers to the functional relation and location of a promoter sequence with respect to a polynucleotide of interest (e.g. a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence). Generally, a promoter operatively linked is contiguous to the sequence of interest. However, an enhancer does not have to be contiguous to the sequence of interest to control its expression. In another embodiment, the promoter and the nucleotide sequence encoding ENPP1 or ENPP3 or a functionally equivalent variant thereof.

The term “*effective amount*” refers to a nontoxic but sufficient amount of a viral vector encoding ENPP1 or ENPP3 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.

The term “*Cap protein*”, as used herein, refers to a polypeptide having at least one functional activity of a native AAV Cap protein (e.g. VP1, VP2, VP3). Examples of functional activities of Cap proteins include the ability to induce formation of a capsid,

facilitate accumulation of single-stranded DNA, facilitate AAV DNA packaging into capsids (i.e. encapsidation), bind to cellular receptors, and facilitate entry of the virion into host cells. In principle, any Cap protein can be used in the context of the present disclosure.

The term “*capsid*”, as used herein, refers to the structure in which the viral genome is packaged. A capsid consists of several oligomeric structural subunits made of proteins. For instance, AAV have an icosahedral capsid formed by the interaction of three capsid proteins: VP1, VP2 and VP3.

The term “*Rep protein*”, as used herein, refers to a polypeptide having at least one functional activity of a native AAV Rep protein (e.g. Rep 40, 52, 68, 78). A “functional activity” of a Rep protein is any activity associated with the physiological function of the protein, including facilitating replication of DNA through recognition, binding and nicking of the AAV origin of DNA replication as well as DNA helicase activity.

The term “*adeno-associated virus ITRs*” or “*AAV ITRs*”, as used herein, refers to the inverted terminal repeats present at both ends of the DNA strand of the genome of an adeno-associated virus. The ITR sequences are required for efficient multiplication of the AAV genome. Another property of these sequences is their ability to form a hairpin. This characteristic contributes to its self-priming which allows the primase-independent synthesis of the second DNA strand. Procedures for modifying these ITR sequences are known in the art (Brown T, “*Gene Cloning*”, Chapman & Hall, London, GB, 1995; Watson R, et al., “*Recombinant DNA*”, 2nd Ed. Scientific American Books, New York, N.Y., US, 1992; Alberts B, et al., “*Molecular Biology of the Cell*”, Garland Publishing Inc., New York, N.Y., US, 2008; Innis M, et al., Eds., “*PCR Protocols. A Guide to Methods and Applications*”, Academic Press Inc., San Diego, Calif., US, 1990; and Schleef M, Ed., “*Plasmid for Therapy and Vaccination*”, Wiley-VCH Verlag GmbH, Weinheim, Del., 2001).

The term “*tissue-specific*” promoter is only active in specific types of differentiated cells or tissues. Typically, the downstream gene in a tissue-specific promoter is one which is active to a much higher degree in the tissue(s) for which it is specific than in any other. In this case there may be little or substantially no activity of the promoter in any tissue other than the one(s) for which it is specific.

The term “*inducible promoter*”, as used herein, refers to a promoter that is physiologically or developmentally regulated, e.g. by the application of a chemical inducer. For example, it can be a tetracycline-inducible promoter, a mifepristone (RU-486)-inducible promoter and the like.

The term “*constitutive promoter*”, as used herein, refers to a promoter whose activity is maintained at a relatively constant level in all cells of an organism, or during most developmental stages, with little or no regard to cell environmental conditions. In another embodiment, the transcriptional regulatory region allows constitutive expression of ENPP1. Examples of constitutive promoters include, without limitation, the retroviral Rous sarcoma virus (RSV) LTR promoter (optionally with the RSV enhancer), the cytomegalovirus (CMV) promoter (optionally with the CMV enhancer), the SV40 promoter, the dihydrofolate reductase promoter, the β -actin promoter, the phosphoglycerol kinase (PGK) promoter, and the EF1a promoter (*Boshart M, et al., Cell 1985; 41:521-530*).

The term “*polyadenylation signal*”, as used herein, relates to a nucleic acid sequence that mediates the attachment of a polyadenine stretch to the 3' terminus of the mRNA. Suitable polyadenylation signals include, without limitation, the SV40 early polyadenylation signal, the SV40 late polyadenylation signal, the HSV thymidine kinase polyadenylation signal, the protamine gene polyadenylation signal, the adenovirus 5 E1b polyadenylation signal, the bovine growth hormone polyadenylation signal, the human variant growth hormone polyadenylation signal and the like.

The term “*signal peptide*”, as used herein, refers to a sequence of amino acid residues (ranging in length from 10-30 residues) bound at the amino terminus of a nascent protein of interest during protein translation. The signal peptide is recognized by the signal recognition particle (SRP) and cleaved by the signal peptidase following transport at the endoplasmic reticulum. (*Lodish et al., 2000, Molecular Cell Biology, 4th edition*).

As used herein, the term “*immune response*” or “*immune reaction*” refers to the host's immune system to antigen in an invading (infecting) pathogenic organism, or to introduction or expression of foreign protein. The immune response is generally humoral and local; antibodies produced by B cells combine with antigen in an antigen-antibody complex to inactivate or neutralize antigen. Immune response is often observed when human proteins are injected into mouse model systems. Generally, the mouse model system is made immune tolerant by injecting immune suppressors prior to the introduction of a foreign antigen to ensure better viability.

As used herein, the term “*immunosuppression*” is a deliberate reduction of the activation or efficacy of the host immune system using immunosuppressant drugs to facilitate immune tolerance towards foreign antigens such as foreign proteins, bone marrow and tissue transplantation. Non limiting examples of immunosuppressant drugs include anti-CD4(GK1.5) antibody, Cyclophosphamide, Azathioprine (Imuran), Mycophenolate mofetil

(Cellcept), Cyclosporine (Neoral, Sandimmune, Gengraf), Methotrexate (Rheumatrex), Leflunomide (Arava), Cyclophosphamide (Cytosan) and Chlorambucil (Leukeran).

Ranges: throughout this disclosure, various aspects of the disclosure 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 disclosure. 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.

METHODS OF TREATMENT

The present disclosure relates to administration of an ENPP1 or ENPP3 agent, which includes administering sNPP1 and sNPP3 polypeptides and fusion proteins thereof to a subject, and to administration of nucleic acids encoding such polypeptides. Sequences of such polypeptides include the following, without limitation.

Sequences

SEQ ID NO: 1 - ENPP1 Amino Acid Sequence - Wild Type

	Met	Glu	Arg	Asp	Gly	Cys	Ala	Gly	Gly	Gly	Ser	Arg	Gly	Gly	Glu
Gly	1				5					10					15
	Gly	Arg	Ala	Pro	Arg	Glu	Gly	Pro	Ala	Gly	Asn	Gly	Arg	Asp	Arg
Gly					20					25					30
	Arg	Ser	His	Ala	Ala	Glu	Ala	Pro	Gly	Asp	Pro	Gln	Ala	Ala	Ala
Ser					35					40					45
	Leu	Leu	Ala	Pro	Met	Asp	Val	Gly	Glu	Glu	Pro	Leu	Glu	Lys	Ala
Ala					50					55					60
	Arg	Ala	Arg	Thr	Ala	Lys	Asp	Pro	Asn	Thr	Tyr	Lys	Val	Leu	Ser
Leu															
	65				70					75					80
	Val	Leu	Ser	Val	Cys	Val	Leu	Thr	Thr	Ile	Leu	Gly	Cys	Ile	Phe
Gly															
					85					90					95
	Leu	Lys	Pro	Ser	Cys	Ala	Lys	Glu	Val	Lys	Ser	Cys	Lys	Gly	Arg
Cys															
					100					105					110
	Phe	Glu	Arg	Thr	Phe	Gly	Asn	Cys	Arg	Cys	Asp	Ala	Ala	Cys	Val
Glu															

		115					120					125			
	Leu	Gly	Asn	Cys	Cys	Leu	Asp	Tyr	Gln	Glu	Thr	Cys	Ile	Glu	Pro
Glu															
		130					135					140			
	His	Ile	Trp	Thr	Cys	Asn	Lys	Phe	Arg	Cys	Gly	Glu	Lys	Arg	Leu
Thr															
		145					150					155			
160															
	Arg	Ser	Leu	Cys	Ala	Cys	Ser	Asp	Asp	Cys	Lys	Asp	Lys	Gly	Asp
Cys															
					165					170					175
	Cys	Ile	Asn	Tyr	Ser	Ser	Val	Cys	Gln	Gly	Glu	Lys	Ser	Trp	Val
Glu															
					180				185					190	
	Glu	Pro	Cys	Glu	Ser	Ile	Asn	Glu	Pro	Gln	Cys	Pro	Ala	Gly	Phe
Glu															
		195						200					205		
	Thr	Pro	Pro	Thr	Leu	Leu	Phe	Ser	Leu	Asp	Gly	Phe	Arg	Ala	Glu
Tyr															
		210						215					220		
	Leu	His	Thr	Trp	Gly	Gly	Leu	Leu	Pro	Val	Ile	Ser	Lys	Leu	Lys
Lys															
		225					230					235			
240															
	Cys	Gly	Thr	Tyr	Thr	Lys	Asn	Met	Arg	Pro	Val	Tyr	Pro	Thr	Lys
Thr															
					245				250					255	
	Phe	Pro	Asn	His	Tyr	Ser	Ile	Val	Thr	Gly	Leu	Tyr	Pro	Glu	Ser
His															
				260				265					270		
	Gly	Ile	Ile	Asp	Asn	Lys	Met	Tyr	Asp	Pro	Lys	Met	Asn	Ala	Ser
Phe															
		275						280				285			
	Ser	Leu	Lys	Ser	Lys	Glu	Lys	Phe	Asn	Pro	Glu	Trp	Tyr	Lys	Gly
Glu															
		290					295				300				
	Pro	Ile	Trp	Val	Thr	Ala	Lys	Tyr	Gln	Gly	Leu	Lys	Ser	Gly	Thr
Phe															
		305					310				315				
320															
	Phe	Trp	Pro	Gly	Ser	Asp	Val	Glu	Ile	Asn	Gly	Ile	Phe	Pro	Asp
Ile															
				325				330					335		
	Tyr	Lys	Met	Tyr	Asn	Gly	Ser	Val	Pro	Phe	Glu	Glu	Arg	Ile	Leu
Ala															
			340					345				350			
	Val	Leu	Gln	Trp	Leu	Gln	Leu	Pro	Lys	Asp	Glu	Arg	Pro	His	Phe
Tyr															
		355					360				365				
	Thr	Leu	Tyr	Leu	Glu	Glu	Pro	Asp	Ser	Ser	Gly	His	Ser	Tyr	Gly
Pro															
		370				375				380					
	Val	Ser	Ser	Glu	Val	Ile	Lys	Ala	Leu	Gln	Arg	Val	Asp	Gly	Met
Val															
		385				390				395					
400															

	Gly	Met	Leu	Met	Asp	Gly	Leu	Lys	Glu	Leu	Asn	Leu	His	Arg	Cys
Leu															
				405					410						415
	Asn	Leu	Ile	Leu	Ile	Ser	Asp	His	Gly	Met	Glu	Gln	Gly	Ser	Cys
Lys															
			420						425					430	
	Lys	Tyr	Ile	Tyr	Leu	Asn	Lys	Tyr	Leu	Gly	Asp	Val	Lys	Asn	Ile
Lys															
			435					440					445		
	Val	Ile	Tyr	Gly	Pro	Ala	Ala	Arg	Leu	Arg	Pro	Ser	Asp	Val	Pro
Asp															
		450					455				460				
	Lys	Tyr	Tyr	Ser	Phe	Asn	Tyr	Glu	Gly	Ile	Ala	Arg	Asn	Leu	Ser
Cys															
	465					470					475				
480															
	Arg	Glu	Pro	Asn	Gln	His	Phe	Lys	Pro	Tyr	Leu	Lys	His	Phe	Leu
Pro															
				485						490					495
	Lys	Arg	Leu	His	Phe	Ala	Lys	Ser	Asp	Arg	Ile	Glu	Pro	Leu	Thr
Phe															
			500						505					510	
	Tyr	Leu	Asp	Pro	Gln	Trp	Gln	Leu	Ala	Leu	Asn	Pro	Ser	Glu	Arg
Lys															
			515					520				525			
	Tyr	Cys	Gly	Ser	Gly	Phe	His	Gly	Ser	Asp	Asn	Val	Phe	Ser	Asn
Met															
		530					535				540				
	Gln	Ala	Leu	Phe	Val	Gly	Tyr	Gly	Pro	Gly	Phe	Lys	His	Gly	Ile
Glu															
	545					550					555				
560															
	Ala	Asp	Thr	Phe	Glu	Asn	Ile	Glu	Val	Tyr	Asn	Leu	Met	Cys	Asp
Leu															
				565						570					575
	Leu	Asn	Leu	Thr	Pro	Ala	Pro	Asn	Asn	Gly	Thr	His	Gly	Ser	Leu
Asn															
			580						585					590	
	His	Leu	Leu	Lys	Asn	Pro	Val	Tyr	Thr	Pro	Lys	His	Pro	Lys	Glu
Val															
			595					600				605			
	His	Pro	Leu	Val	Gln	Cys	Pro	Phe	Thr	Arg	Asn	Pro	Arg	Asp	Asn
Leu															
		610					615				620				
	Gly	Cys	Ser	Cys	Asn	Pro	Ser	Ile	Leu	Pro	Ile	Glu	Asp	Phe	Gln
Thr															
	625					630					635				
640															
	Gln	Phe	Asn	Leu	Thr	Val	Ala	Glu	Glu	Lys	Ile	Ile	Lys	His	Glu
Thr															
				645						650					655
	Leu	Pro	Tyr	Gly	Arg	Pro	Arg	Val	Leu	Gln	Lys	Glu	Asn	Thr	Ile
Cys															
			660						665					670	
	Leu	Leu	Ser	Gln	His	Gln	Phe	Met	Ser	Gly	Tyr	Ser	Gln	Asp	Ile
Leu															

		675					680					685			
	Met	Pro	Leu	Trp	Thr	Ser	Tyr	Thr	Val	Asp	Arg	Asn	Asp	Ser	Phe
Ser															
		690					695					700			
	Thr	Glu	Asp	Phe	Ser	Asn	Cys	Leu	Tyr	Gln	Asp	Phe	Arg	Ile	Pro
Leu															
	705					710					715				
720															
	Ser	Pro	Val	His	Lys	Cys	Ser	Phe	Tyr	Lys	Asn	Asn	Thr	Lys	Val
Ser															
				725						730					735
	Tyr	Gly	Phe	Leu	Ser	Pro	Pro	Gln	Leu	Asn	Lys	Asn	Ser	Ser	Gly
Ile															
			740					745					750		
	Tyr	Ser	Glu	Ala	Leu	Leu	Thr	Thr	Asn	Ile	Val	Pro	Met	Tyr	Gln
Ser															
		755					760					765			
	Phe	Gln	Val	Ile	Trp	Arg	Tyr	Phe	His	Asp	Thr	Leu	Leu	Arg	Lys
Tyr															
		770				775					780				
	Ala	Glu	Glu	Arg	Asn	Gly	Val	Asn	Val	Val	Ser	Gly	Pro	Val	Phe
Asp															
	785				790						795				
800															
	Phe	Asp	Tyr	Asp	Gly	Arg	Cys	Asp	Ser	Leu	Glu	Asn	Leu	Arg	Gln
Lys															
				805					810					815	
	Arg	Arg	Val	Ile	Arg	Asn	Gln	Glu	Ile	Leu	Ile	Pro	Thr	His	Phe
Phe															
			820					825					830		
	Ile	Val	Leu	Thr	Ser	Cys	Lys	Asp	Thr	Ser	Gln	Thr	Pro	Leu	His
Cys															
		835					840					845			
	Glu	Asn	Leu	Asp	Thr	Leu	Ala	Phe	Ile	Leu	Pro	His	Arg	Thr	Asp
Asn															
		850				855					860				
	Ser	Glu	Ser	Cys	Val	His	Gly	Lys	His	Asp	Ser	Ser	Trp	Val	Glu
Glu															
	865				870						875				
880															
	Leu	Leu	Met	Leu	His	Arg	Ala	Arg	Ile	Thr	Asp	Val	Glu	His	Ile
Thr															
			885					890					895		
	Gly	Leu	Ser	Phe	Tyr	Gln	Gln	Arg	Lys	Glu	Pro	Val	Ser	Asp	Ile
Leu															
		900					905					910			
	Lys	Leu	Lys	Thr	His	Leu	Pro	Thr	Phe	Ser	Gln	Glu	Asp		
		915					920					925			

SEQ ID No: 2 - Azurocidin-ENPP1-FC

MTRLTVLALLAGLLASSRA**APSCAKEVKSCKGRCFERTFGNCRCDAAVELGNCCLDYQETCIEPE
 HIWTCNKFRGCEKRLTRSLCACSDCKDKGDCCINYSVCQGEKSWVEEPCESINEPQCPAGFETPPT
 LLFSLDGFRAEYLHTWGGLLPVISKLLKCGTYTKNMRPVYPTKTFPNHYSIVTGLYPESHGIIDNKMY
 DPKMNASFSLKSKEKFNPEWYKGEPIWVTAKYQGLKSGTFFWPGSDVEINGIFPDIYKMYNGSVPFEE
 RILAVLQWLQLPKDERPHFYTLYLEEPDSSGHSYGPVSSEVIKALQRVDGMVGMMLMDGLKELNLHRCL

NLILISDHGMEQGSCKKYIYLNKYLGDVKNIKVIYGPAARLRPSDVPDKYYSFNYEGIARNLSCREPN
 QHFKPYLKHFLPKRLHFAKSDRIEPLTFYLDPOWQLALNPSEKRYCGSGFHGSDNVFSNMQALFVGYG
 PGFKHGIEADTFENIEVYNLMCDLLNLTAPANNNGTHGSLNHLLKNPVYTPKHPKEVHPLVQCPFTRNP
 RDNLCSCNPSILPIEDFQTQFNLTVAEEKIIKHETLPYGRPRVLQKENTICLLSQHQFMSGYSQDIL
 MPLWTSYTVDRNDSFSTEDFSNCLYQDFRIPLSPVHKCSFYKNNTKVSYGFLSPPQLNKNSSGIYSEA
 ILTTNTVPMYQSFQVTWRYFHDTLLRKYAEERNGVNVVSGPVFDFDYDGRCDSENLTRQKRRVTRNQF
 ILIPTHFFIVLTSCKDTSQTPLHCENLDTLAFILPHRTDNSESCVHGKHDSSWVEELLMLHRARITDV
 EHITGLSFYQQRKEPVSDILKLKTHLPTFSQEDLINDKTHTCPPCPAPELLGGPSVFLFPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVSCSVMHEALHNHYTQKSLSLSPGK

Single underline - Azurocidin signal sequence, Double underline -
 Beginning and end of ENPP1 sequence, Bold residues- Fc sequence, **
 indicates the cleavage point of the signal sequence.

SEQ ID No: 3 - Azurocidin-ENPP1-Alb

MTRLTVLALLAGLLASSRA**APSCAKEVKSCKGRCFERTFGNCRCDAAACVELGNCCLDYQETCIEPE
 HIWTCNKFRGCEKRLTRSLCACSDDCDKGDCCINYSVCQGEKSWVEEPCESINEPQCPAGFETPPT
 LLFSLDGFRAEYLHTWGGLLPVISKLKCGTYTKNMRPVYPTKTFPNHYSIVTGLYPESHGII DNKMY
 DPKMNASFSLKSKEKFNPEWYKGEPIWVTAKYQGLKSGTFFWPGSDVEINGIFPDIYKMYNGSVPFEE
 RILAVLQWLQLPKDERPHFYTLYLEEPDSSGHSYGPVSSEVIKALQRVDGMVGMMLMDGLKELNLHRCL
 NLILISDHGMEQGSCKKYIYLNKYLGDVKNIKVIYGPAARLRPSDVPDKYYSFNYEGIARNLSCREPN
 QHFKPYLKHFLPKRLHFAKSDRIEPLTFYLDPOWQLALNPSEKRYCGSGFHGSDNVFSNMQALFVGYG
 PGFKHGIEADTFENIEVYNLMCDLLNLTAPANNNGTHGSLNHLLKNPVYTPKHPKEVHPLVQCPFTRNP
 RDNLCSCNPSILPIEDFQTQFNLTVAEEKIIKHETLPYGRPRVLQKENTICLLSQHQFMSGYSQDIL
 MPLWTSYTVDRNDSFSTEDFSNCLYQDFRIPLSPVHKCSFYKNNTKVSYGFLSPPQLNKNSSGIYSEA
 LLTTNTVPMYQSFQVTWRYFHDTLLRKYAEERNGVNVVSGPVFDFDYDGRCDSENLTRQKRRVIRNQF
 ILIPTHFFIVLTSCKDTSQTPLHCENLDTLAFILPHRTDNSESCVHGKHDSSWVEELLMLHRARITDV
 EHITGLSFYQQRKEPVSDILKLKTHLPTFSQEDLINMKWVTFLLLLFVSGSAFSGVFRREAHKSEIA
HRYNDLGEQHFKGLVLIAFSQYLQKCSYDEHAKLVQEVTDFAKTCVADESAANCDKSLHTLFGDKLCA
IPNLRENYGELADCCTKQEPERNECFLOHKDDNPSLPPFERPEAEAMCTSFKENPTTFMGHYLHEVAR
RHPYFYAPELLYYAEQYNEILTQCCAEADKESCLTPKLDGVKEKALVSSVRQRMKCSSMQKFGERAFAK
AWAVARLSQTFPNADFAEITKLATDLTKVNKECCHGDLLECADDRAELAKYMCENQATISSKLQTCDD
KPLLKKAHCLSEVEHDTMPADLPAIAADFVEDQEVCKNYAEAKDVFLGTFLYEYSRRHPDYSVSLLLR
LAKKYEATLEKCCAEANPPACYGTVLAEFQPLVEEPKNLVKTNCDLYEKLGEYGFQNAILVRYTQKAP
QVSTPTLVEAARNLGRVGTKCCTLPEDQRLPCVEDYLSAILNRVCLLHEKTPVSEHVTKCCSGSLVER
RPCFSALTVDETYVPKEFKAETFTFHSDICTLPEKEKQIKKQTALAEVLVHKPKATAEQKLKTMDDFA
QFLDTCCKAADKDTCTFSTEGPNLVTRCKDALARSWSHPQFEK

Single underline - Azurocidin signal sequence, Double underline -
 Beginning and end of ENPP1 sequence, Bold residues- Albumin
 sequence, ** indicates the cleavage point of the signal sequence.

SEQ ID No: 4 - Azurocidin-ENPP1

MTRLTVLALLAGLLASSRA**APSCAKEVKSCKGRCFERTFGNCRCDAAACVELGNCCLDYQETCIEPE
 HIWTCNKFRGCEKRLTRSLCACSDDCDKGDCCINYSVCQGEKSWVEEPCESINEPQCPAGFETPPT
 LLFSLDGFRAEYLHTWGGLLPVISKLKCGTYTKNMRPVYPTKTFPNHYSIVTGLYPESHGII DNKMY
 DPKMNASFSLKSKEKFNPEWYKGEPIWVTAKYQGLKSGTFFWPGSDVEINGIFPDIYKMYNGSVPFEE
 RILAVLQWLQLPKDERPHFYTLYLEEPDSSGHSYGPVSSEVIKALQRVDGMVGMMLMDGLKELNLHRCL
 NLILISDHGMEQGSCKKYIYLNKYLGDVKNIKVIYGPAARLRPSDVPDKYYSFNYEGIARNLSCREPN
 QHFKPYLKHFLPKRLHFAKSDRIEPLTFYLDPOWQLALNPSEKRYCGSGFHGSDNVFSNMQALFVGYG
 PGFKHGIEADTFENIEVYNLMCDLLNLTAPANNNGTHGSLNHLLKNPVYTPKHPKEVHPLVQCPFTRNP
 RDNLCSCNPSILPIEDFQTQFNLTVAEEKIIKHETLPYGRPRVLQKENTICLLSQHQFMSGYSQDIL

MPLWTSYTVDRNDSFSTEDFSNCLYQDFRIPLSPVHKCSFYKNNTKVSYGFLSPPQLNKNSSGIYSEA
 LLTTNIVPMYQSFQVIWRYFHDTLRLKYAEERNGVNVVSGPVDFDFDYDGRCDSENLRQKRRVIRNQE
 ILIPTHFFIVLTSCKDTSQTAPSCAKEVKSCKGRCFERTFGNCRCDAAACVELGNCCLDYQETCIEPEH
 IWTCKNKFRCGEKRLTRSLCACSDDDCKDKGDCCINYSVCQGEKSWVEEPCESINEPQCPAGFETPPTL
 LFSLDGFRAEYLHTWGGLLPVISKLLKCGTYTKNMRPVYPTKTFPNHYSIVTGLYPESHGIIIDNKMYD
 PKMNASFSTLKSKEKFNPFWYKGEPTWVTAKYQGLKSGTFFWPGSDVETNGTFPDIYKMYNGSVPPFEER
 ILAVLQWLQLPKDERPHFYTLYLEEPDSSGHSYGPVSSEVIKALQRVDGMVGMMLMDGLKELNLHRCNL
 LILISDHGMEQGSCKKIYLNKYLGDVKNIKVIYGPAARLRPSDVPDKYYSFNYEGIARNLSCREPNQ
 HFKPYLKHFLPKRLHFAKSDRIEPLTFYLDLPQWQLALNPSEKCYGSGFHGSDNVFSNMQALFVGYP
 GFKHGIEADTFENIEVYNLMCDLLNLTPAPNNGTHGSLNHLLKNPVYTPKHPKEVHPLVQCPFTRNPR
 DNLGCSCNPSILPIEDFQTQFNLTVAEEKI IKHETLPYGRPRVLQKENTICLLSQHQFMSGYSQDILM
 PLWTSYTVDRNDSFSTEDFSNCLYQDFRIPLSPVHKCSFYKNNTKVSYGFLSPPQLNKNSSGIYSEAL
 LT'TNIVPMYQSFQVIWRYFHDTLRLKYAEERNGVNVVSGPVDFDFDYDGRCDSENLRQKRRVIRNQE I
 LIPTHFFIVLTSCKDTSQTPLHCENLDTLAFILPHRTDNSESCVHGKHDSSWVEELLMLHRARITDVE
 HITGLSFYQQRKEPVSDILKLKTHLPTFSQED

Single underline - Azurocidin signal sequence, Double underline -
 Beginning and end of ENPP1 sequence, ** indicates the cleavage point
 of the signal sequence.

SEQ ID NO: 5 - ENPP2 Amino Acid Sequence - Wild Type

Met	Ala	Arg	Arg	Ser	Ser	Phe	Gln	Ser	Cys	Gln	Ile	Ile	Ser	Leu	Phe	1	5	10	15
Thr	Phe	Ala	Val	Gly	Val	Asn	Ile	Cys	Leu	Gly	Phe	Thr	Ala	His	Arg	20	25	30	
Ile	Lys	Arg	Ala	Glu	Gly	Trp	Glu	Glu	Gly	Pro	Pro	Thr	Val	Leu	Ser	35	40	45	
Asp	Ser	Pro	Trp	Thr	Asn	Ile	Ser	Gly	Ser	Cys	Lys	Gly	Arg	Cys	Phe	50	55	60	
Glu	Leu	Gln	Glu	Ala	Gly	Pro	Pro	Asp	Cys	Arg	Cys	Asp	Asn	Leu	Cys	65	70	75	80
Lys	Ser	Tyr	Thr	Ser	Cys	Cys	His	Asp	Phe	Asp	Glu	Leu	Cys	Leu	Lys	85	90	95	
Thr	Ala	Arg	Gly	Trp	Glu	Cys	Thr	Lys	Asp	Arg	Cys	Gly	Glu	Val	Arg	100	105	110	
Asn	Glu	Glu	Asn	Ala	Cys	His	Cys	Ser	Glu	Asp	Cys	Leu	Ala	Arg	Gly	115	120	125	
Asp	Cys	Cys	Thr	Asn	Tyr	Gln	Val	Val	Cys	Lys	Gly	Glu	Ser	His	Trp	130	135	140	
Val	Asp	Asp	Asp	Cys	Glu	Glu	Ile	Lys	Ala	Ala	Glu	Cys	Pro	Ala	Gly	145	150	155	160
Phe	Val	Arg	Pro	Pro	Leu	Ile	Ile	Phe	Ser	Val	Asp	Gly	Phe	Arg	Ala	165	170	175	
Ser	Tyr	Met	Lys	Lys	Gly	Ser	Lys	Val	Met	Pro	Asn	Ile	Glu	Lys	Leu	180	185	190	
Arg	Ser	Cys	Gly	Thr	His	Ser	Pro	Tyr	Met	Arg	Pro	Val	Tyr	Pro	Thr	195	200	205	
Lys	Thr	Phe	Pro	Asn	Leu	Tyr	Thr	Leu	Ala	Thr	Gly	Leu	Tyr	Pro	Glu	210	215	220	
Ser	His	Gly	Ile	Val	Gly	Asn	Ser	Met	Tyr	Asp	Pro	Val	Phe	Asp	Ala	225	230	235	240
Thr	Phe	His	Leu	Arg	Gly	Arg	Glu	Lys	Phe	Asn	His	Arg	Trp	Trp	Gly	245	250	255	
Gly	Gln	Pro	Leu	Trp	Ile	Thr	Ala	Thr	Lys	Gln	Gly	Val	Lys	Ala	Gly	260	265	270	

Thr	Phe	Phe	Trp	Ser	Val	Val	Ile	Pro	His	Glu	Arg	Arg	Ile	Leu	Thr	275	280	285
Ile	Leu	Gln	Trp	Leu	Thr	Leu	Pro	Asp	His	Glu	Arg	Pro	Ser	Val	Tyr	290	295	300
Ala	Phe	Tyr	Ser	Glu	Gln	Pro	Asp	Phe	Ser	Gly	His	Lys	Tyr	Gly	Pro	305	310	315
Phe	Gly	Pro	Glu	Met	Thr	Asn	Pro	Leu	Arg	Glu	Ile	Asp	Lys	Ile	Val	325	330	335
Gly	Gln	Leu	Met	Asp	Gly	Leu	Lys	Gln	Leu	Lys	Leu	His	Arg	Cys	Val	340	345	350
Asn	Val	Ile	Phe	Val	Gly	Asp	His	Gly	Met	Glu	Asp	Val	Thr	Cys	Asp	355	360	365
Arg	Thr	Glu	Phe	Leu	Ser	Asn	Tyr	Leu	Thr	Asn	Val	Asp	Asp	Ile	Thr	370	375	380
Leu	Val	Pro	Gly	Thr	Leu	Gly	Arg	Ile	Arg	Ser	Lys	Phe	Ser	Asn	Asn	385	390	395
Ala	Lys	Tyr	Asp	Pro	Lys	Ala	Ile	Ile	Ala	Asn	Leu	Thr	Cys	Lys	Lys	405	410	415
Pro	Asp	Gln	His	Phe	Lys	Pro	Tyr	Leu	Lys	Gln	His	Leu	Pro	Lys	Arg	420	425	430
Leu	His	Tyr	Ala	Asn	Asn	Arg	Arg	Ile	Glu	Asp	Ile	His	Leu	Leu	Val	435	440	445
Glu	Arg	Arg	Trp	His	Val	Ala	Arg	Lys	Pro	Leu	Asp	Val	Tyr	Lys	Lys	450	455	460
Pro	Ser	Gly	Lys	Cys	Phe	Phe	Gln	Gly	Asp	His	Gly	Phe	Asp	Asn	Lys	465	470	475
Val	Asn	Ser	Met	Gln	Thr	Val	Phe	Val	Gly	Tyr	Gly	Ser	Thr	Phe	Lys	485	490	495
Tyr	Lys	Thr	Lys	Val	Pro	Pro	Phe	Glu	Asn	Ile	Glu	Leu	Tyr	Asn	Val	500	505	510
Met	Cys	Asp	Leu	Leu	Gly	Leu	Lys	Pro	Ala	Pro	Asn	Asn	Gly	Thr	His	515	520	525
Gly	Ser	Leu	Asn	His	Leu	Leu	Arg	Thr	Asn	Thr	Phe	Arg	Pro	Thr	Met	530	535	540
Pro	Glu	Glu	Val	Thr	Arg	Pro	Asn	Tyr	Pro	Gly	Ile	Met	Tyr	Leu	Gln	545	550	555
Ser	Asp	Phe	Asp	Leu	Gly	Cys	Thr	Cys	Asp	Asp	Lys	Val	Glu	Pro	Lys	565	570	575
Asn	Lys	Leu	Asp	Glu	Leu	Asn	Lys	Arg	Leu	His	Thr	Lys	Gly	Ser	Thr	580	585	590
Glu	Ala	Glu	Thr	Arg	Lys	Phe	Arg	Gly	Ser	Arg	Asn	Glu	Asn	Lys	Glu	595	600	605
Asn	Ile	Asn	Gly	Asn	Phe	Glu	Pro	Arg	Lys	Glu	Arg	His	Leu	Leu	Tyr	610	615	620
Gly	Arg	Pro	Ala	Val	Leu	Tyr	Arg	Thr	Arg	Tyr	Asp	Ile	Leu	Tyr	His	625	630	635
Thr	Asp	Phe	Glu	Ser	Gly	Tyr	Ser	Glu	Ile	Phe	Leu	Met	Pro	Leu	Trp	645	650	655
Thr	Ser	Tyr	Thr	Val	Ser	Lys	Gln	Ala	Glu	Val	Ser	Ser	Val	Pro	Asp	660	665	670
His	Leu	Thr	Ser	Cys	Val	Arg	Pro	Asp	Val	Arg	Val	Ser	Pro	Ser	Phe	675	680	685
Ser	Gln	Asn	Cys	Leu	Ala	Tyr	Lys	Asn	Asp	Lys	Gln	Met	Ser	Tyr	Gly	690	695	700
Phe	Leu	Phe	Pro	Pro	Tyr	Leu	Ser	Ser	Ser	Pro	Glu	Ala	Lys	Tyr	Asp	705	710	715
																		720

Ala Phe Leu Val Thr Asn Met Val Pro Met Tyr Pro Ala Phe Lys Arg
725 730 735
Val Trp Asn Tyr Phe Gln Arg Val Leu Val Lys Lys Tyr Ala Ser Glu
740 745 750
Arg Asn Gly Val Asn Val Ile Ser Gly Pro Ile Phe Asp Tyr Asp Tyr
755 760 765
Asp Gly Leu His Asp Thr Glu Asp Lys Ile Lys Gln Tyr Val Glu Gly
770 775 780
Ser Ser Ile Pro Val Pro Thr His Tyr Tyr Ser Ile Ile Thr Ser Cys
785 790 795 800
Leu Asp Phe Thr Gln Pro Ala Asp Lys Cys Asp Gly Pro Leu Ser Val
805 810 815
Ser Ser Phe Ile Leu Pro His Arg Pro Asp Asn Glu Glu Ser Cys Asn
820 825 830
Ser Ser Glu Asp Glu Ser Lys Trp Val Glu Glu Leu Met Lys Met His
835 840 845
Thr Ala Arg Val Arg Asp Ile Glu His Leu Thr Ser Leu Asp Phe Phe
850 855 860
Arg Lys Thr Ser Arg Ser Tyr Pro Glu Ile Leu Thr Leu Lys Thr Tyr
865 870 875 880
Leu His Thr Tyr Glu Ser Glu Ile
885

SEQ. ID NO:6 - Extracellular Domain of ENPP3:

Glu Lys Gln Gly Ser Cys Arg Lys Lys Cys Phe Asp Ala Ser Phe Arg
1 5 10 15
Gly Leu Glu Asn Cys Arg Cys Asp Val Ala Cys Lys Asp Arg Gly Asp
20 25 30
Cys Cys Trp Asp Phe Glu Asp Thr Cys Val Glu Ser Thr Arg Ile Trp
35 40 45
Met Cys Asn Lys Phe Arg Cys Gly Glu Thr Arg Leu Glu Ala Ser Leu
50 55 60
Cys Ser Cys Ser Asp Asp Cys Leu Gln Arg Lys Asp Cys Cys Ala Asp
65 70 75 80
Tyr Lys Ser Val Cys Gln Gly Glu Thr Ser Trp Leu Glu Glu Asn Cys
85 90 95
Asp Thr Ala Gln Gln Ser Gln Cys Pro Glu Gly Phe Asp Leu Pro Pro
100 105 110
Val Ile Leu Phe Ser Met Asp Gly Phe Arg Ala Glu Tyr Leu Tyr Thr
115 120 125
Trp Asp Thr Leu Met Pro Asn Ile Asn Lys Leu Lys Thr Cys Gly Ile
130 135 140
His Ser Lys Tyr Met Arg Ala Met Tyr Pro Thr Lys Thr Phe Pro Asn
145 150 155 160
His Tyr Thr Ile Val Thr Gly Leu Tyr Pro Glu Ser His Gly Ile Ile
165 170 175
Asp Asn Asn Met Tyr Asp Val Asn Leu Asn Lys Asn Phe Ser Leu Ser
180 185 190
Ser Lys Glu Gln Asn Asn Pro Ala Trp Trp His Gly Gln Pro Met Trp
195 200 205
Leu Thr Ala Met Tyr Gln Gly Leu Lys Ala Ala Thr Tyr Phe Trp Pro
210 215 220
Gly Ser Glu Val Ala Ile Asn Gly Ser Phe Pro Ser Ile Tyr Met Pro

225					230					235					240
Tyr	Asn	Gly	Ser	Val	Pro	Phe	Glu	Glu	Arg	Ile	Ser	Thr	Leu	Leu	Lys
				245					250					255	
Trp	Leu	Asp	Leu	Pro	Lys	Ala	Glu	Arg	Pro	Arg	Phe	Tyr	Thr	Met	Tyr
			260					265					270		
Phe	Glu	Glu	Pro	Asp	Ser	Ser	Gly	His	Ala	Gly	Gly	Pro	Val	Ser	Ala
		275					280					285			
Arg	Val	Ile	Lys	Ala	Leu	Gln	Val	Val	Asp	His	Ala	Phe	Gly	Met	Leu
	290					295					300				
Met	Glu	Gly	Leu	Lys	Gln	Arg	Asn	Leu	His	Asn	Cys	Val	Asn	Ile	Ile
305					310					315				320	
Leu	Leu	Ala	Asp	His	Gly	Met	Asp	Gln	Thr	Tyr	Cys	Asn	Lys	Met	Glu
				325						330				335	
Tyr	Met	Thr	Asp	Tyr	Phe	Pro	Arg	Ile	Asn	Phe	Phe	Tyr	Met	Tyr	Glu
			340					345					350		
Gly	Pro	Ala	Pro	Arg	Ile	Arg	Ala	His	Asn	Ile	Pro	His	Asp	Phe	Phe
		355					360					365			
Ser	Phe	Asn	Ser	Glu	Glu	Ile	Val	Arg	Asn	Leu	Ser	Cys	Arg	Lys	Pro
	370					375					380				
Asp	Gln	His	Phe	Lys	Pro	Tyr	Leu	Thr	Pro	Asp	Leu	Pro	Lys	Arg	Leu
385					390					395					400
His	Tyr	Ala	Lys	Asn	Val	Arg	Ile	Asp	Lys	Val	His	Leu	Phe	Val	Asp
				405					410					415	
Gln	Gln	Trp	Leu	Ala	Val	Arg	Ser	Lys	Ser	Asn	Thr	Asn	Cys	Gly	Gly
			420					425					430		
Gly	Asn	His	Gly	Tyr	Asn	Asn	Glu	Phe	Arg	Ser	Met	Glu	Ala	Ile	Phe
		435					440					445			
Leu	Ala	His	Gly	Pro	Ser	Phe	Lys	Glu	Lys	Thr	Glu	Val	Glu	Pro	Phe
	450					455					460				
Glu	Asn	Ile	Glu	Val	Tyr	Asn	Leu	Met	Cys	Asp	Leu	Leu	Arg	Ile	Gln
465					470					475					480
Pro	Ala	Pro	Asn	Asn	Gly	Thr	His	Gly	Ser	Leu	Asn	His	Leu	Leu	Lys
				485					490						495
Val	Pro	Phe	Tyr	Glu	Pro	Ser	His	Ala	Glu	Glu	Val	Ser	Lys	Phe	Ser
			500					505					510		
Val	Cys	Gly	Phe	Ala	Asn	Pro	Leu	Pro	Thr	Glu	Ser	Leu	Asp	Cys	Phe
		515					520					525			
Cys	Pro	His	Leu	Gln	Asn	Ser	Thr	Gln	Leu	Glu	Gln	Val	Asn	Gln	Met
	530					535						540			
Leu	Asn	Leu	Thr	Gln	Glu	Ile	Thr	Ala	Thr	Val	Lys	Val	Asn	Leu	
545					550				555						560
Pro	Phe	Gly	Arg	Pro	Arg	Val	Leu	Gln	Lys	Asn	Val	Asp	His	Cys	Leu
				565					570					575	
Leu	Tyr	His	Arg	Glu	Tyr	Val	Ser	Gly	Phe	Gly	Lys	Ala	Met	Arg	Met
			580					585					590		
Pro	Met	Trp	Ser	Ser	Tyr	Thr	Val	Pro	Gln	Leu	Gly	Asp	Thr	Ser	Pro
		595					600					605			
Leu	Pro	Pro	Thr	Val	Pro	Asp	Cys	Leu	Arg	Ala	Asp	Val	Arg	Val	Pro
	610					615					620				
Pro	Ser	Glu	Ser	Gln	Lys	Cys	Ser	Phe	Tyr	Leu	Ala	Asp	Lys	Asn	Ile
625					630					635					640
Thr	His	Gly	Phe	Leu	Tyr	Pro	Pro	Ala	Ser	Asn	Arg	Thr	Ser	Asp	Ser
				645					650					655	
Gln	Tyr	Asp	Ala	Leu	Ile	Thr	Ser	Asn	Leu	Val	Pro	Met	Tyr	Glu	Glu
			660					665					670		
Phe	Arg	Lys	Met	Trp	Asp	Tyr	Phe	His	Ser	Val	Leu	Leu	Ile	Lys	His

675	680	685
Ala Thr Glu Arg Asn Gly Val Asn Val Val Ser Gly Pro Ile Phe Asp		
690	695	700
Tyr Asn Tyr Asp Gly His Phe Asp Ala Pro Asp Glu Ile Thr Lys His		
705	710	715
Leu Ala Asn Thr Asp Val Pro Ile Pro Thr His Tyr Phe Val Val Leu		
725	730	735
Thr Ser Cys Lys Asn Lys Ser His Thr Pro Glu Asn Cys Pro Gly Trp		
740	745	750
Leu Asp Val Leu Pro Phe Ile Ile Pro His Arg Pro Thr Asn Val Glu		
755	760	765
Ser Cys Pro Glu Gly Lys Pro Glu Ala Leu Trp Val Glu Glu Arg Phe		
770	775	780
Thr Ala His Ile Ala Arg Val Arg Asp Val Glu Leu Leu Thr Gly Leu		
785	790	795
Asp Phe Tyr Gln Asp Lys Val Gln Pro Val Ser Glu Ile Leu Gln Leu		
805	810	815
Lys Thr Tyr Leu Pro Thr Phe Glu Thr Thr Ile		
820	825	

SEQ. ID NO: 7 - NPP3 Amino Acid Sequence:

Met Glu Ser Thr Leu Thr Leu Ala Thr Glu Gln Pro Val Lys Lys Asn	
1	5
Thr Leu Lys Lys Tyr Lys Ile Ala Cys Ile Val Leu Leu Ala Leu Leu	
20	25
Val Ile Met Ser Leu Gly Leu Gly Leu Gly Leu Gly Leu Arg Lys Leu	
35	40
Glu Lys Gln Gly Ser Cys Arg Lys Lys Cys Phe Asp Ala Ser Phe Arg	
50	55
Gly Leu Glu Asn Cys Arg Cys Asp Val Ala Cys Lys Asp Arg Gly Asp	
65	70
Cys Cys Trp Asp Phe Glu Asp Thr Cys Val Glu Ser Thr Arg Ile Trp	
85	90
Met Cys Asn Lys Phe Arg Cys Gly Glu Thr Arg Leu Glu Ala Ser Leu	
100	105
Cys Ser Cys Ser Asp Asp Cys Leu Gln Arg Lys Asp Cys Ala Asp	
115	120
Tyr Lys Ser Val Cys Gln Gly Glu Thr Ser Trp Leu Glu Glu Asn Cys	
130	135
Asp Thr Ala Gln Gln Ser Gln Cys Pro Glu Gly Phe Asp Leu Pro Pro	
145	150
Val Ile Leu Phe Ser Met Asp Gly Phe Arg Ala Glu Tyr Leu Tyr Thr	
165	170
Trp Asp Thr Leu Met Pro Asn Ile Asn Lys Leu Lys Thr Cys Gly Ile	
180	185
His Ser Lys Tyr Met Arg Ala Met Tyr Pro Thr Lys Thr Phe Pro Asn	
195	200
His Tyr Thr Ile Val Thr Gly Leu Tyr Pro Glu Ser His Gly Ile Ile	
210	215
Asp Asn Asn Met Tyr Asp Val Asn Leu Asn Lys Asn Phe Ser Leu Ser	
225	230
Ser Lys Glu Gln Asn Asn Pro Ala Trp Trp His Gly Gln Pro Met Trp	
245	250
Leu Thr Ala Met Tyr Gln Gly Leu Lys Ala Ala Thr Tyr Phe Trp Pro	

			260					265				270				
Gly	Ser	Glu	Val	Ala	Ile	Asn	Gly	Ser	Phe	Pro	Ser	Ile	Tyr	Met	Pro	
		275					280					285				
Tyr	Asn	Gly	Ser	Val	Pro	Phe	Glu	Glu	Arg	Ile	Ser	Thr	Leu	Leu	Lys	
	290					295					300					
Trp	Leu	Asp	Leu	Pro	Lys	Ala	Glu	Arg	Pro	Arg	Phe	Tyr	Thr	Met	Tyr	
305					310					315					320	
Phe	Glu	Glu	Pro	Asp	Ser	Ser	Gly	His	Ala	Gly	Gly	Pro	Val	Ser	Ala	
				325					330					335		
Arg	Val	Ile	Lys	Ala	Leu	Gln	Val	Val	Asp	His	Ala	Phe	Gly	Met	Leu	
			340					345					350			
Met	Glu	Gly	Leu	Lys	Gln	Arg	Asn	Leu	His	Asn	Cys	Val	Asn	Ile	Ile	
	355						360					365				
Leu	Leu	Ala	Asp	His	Gly	Met	Asp	Gln	Thr	Tyr	Cys	Asn	Lys	Met	Glu	
	370					375					380					
Tyr	Met	Thr	Asp	Tyr	Phe	Pro	Arg	Ile	Asn	Phe	Phe	Tyr	Met	Tyr	Glu	
385					390					395					400	
Gly	Pro	Ala	Pro	Arg	Ile	Arg	Ala	His	Asn	Ile	Pro	His	Asp	Phe	Phe	
				405					410					415		
Ser	Phe	Asn	Ser	Glu	Glu	Ile	Val	Arg	Asn	Leu	Ser	Cys	Arg	Lys	Pro	
			420					425					430			
Asp	Gln	His	Phe	Lys	Pro	Tyr	Leu	Thr	Pro	Asp	Leu	Pro	Lys	Arg	Leu	
		435					440					445				
His	Tyr	Ala	Lys	Asn	Val	Arg	Ile	Asp	Lys	Val	His	Leu	Phe	Val	Asp	
	450					455					460					
Gln	Gln	Trp	Leu	Ala	Val	Arg	Ser	Lys	Ser	Asn	Thr	Asn	Cys	Gly	Gly	
465					470					475					480	
Gly	Asn	His	Gly	Tyr	Asn	Asn	Glu	Phe	Arg	Ser	Met	Glu	Ala	Ile	Phe	
			485						490					495		
Leu	Ala	His	Gly	Pro	Ser	Phe	Lys	Glu	Lys	Thr	Glu	Val	Glu	Pro	Phe	
			500					505					510			
Glu	Asn	Ile	Glu	Val	Tyr	Asn	Leu	Met	Cys	Asp	Leu	Leu	Arg	Ile	Gln	
	515						520					525				
Pro	Ala	Pro	Asn	Asn	Gly	Thr	His	Gly	Ser	Leu	Asn	His	Leu	Leu	Lys	
	530					535					540					
Val	Pro	Phe	Tyr	Glu	Pro	Ser	His	Ala	Glu	Glu	Val	Ser	Lys	Phe	Ser	
545					550					555					560	
Val	Cys	Gly	Phe	Ala	Asn	Pro	Leu	Pro	Thr	Glu	Ser	Leu	Asp	Cys	Phe	
			565						570					575		
Cys	Pro	His	Leu	Gln	Asn	Ser	Thr	Gln	Leu	Glu	Gln	Val	Asn	Gln	Met	
			580					585					590			
Leu	Asn	Leu	Thr	Gln	Glu	Glu	Ile	Thr	Ala	Thr	Val	Lys	Val	Asn	Leu	
	595						600					605				
Pro	Phe	Gly	Arg	Pro	Arg	Val	Leu	Gln	Lys	Asn	Val	Asp	His	Cys	Leu	
	610					615						620				
Leu	Tyr	His	Arg	Glu	Tyr	Val	Ser	Gly	Phe	Gly	Lys	Ala	Met	Arg	Met	
625					630					635					640	
Pro	Met	Trp	Ser	Ser	Tyr	Thr	Val	Pro	Gln	Leu	Gly	Asp	Thr	Ser	Pro	
			645						650					655		
Leu	Pro	Pro	Thr	Val	Pro	Asp	Cys	Leu	Arg	Ala	Asp	Val	Arg	Val	Pro	
			660					665					670			
Pro	Ser	Glu	Ser	Gln	Lys	Cys	Ser	Phe	Tyr	Leu	Ala	Asp	Lys	Asn	Ile	
	675						680					685				
Thr	His	Gly	Phe	Leu	Tyr	Pro	Pro	Ala	Ser	Asn	Arg	Thr	Ser	Asp	Ser	
	690						695					700				
Gln	Tyr	Asp	Ala	Leu	Ile	Thr	Ser	Asn	Leu	Val	Pro	Met	Tyr	Glu	Glu	

705					710					715				720
Phe	Arg	Lys	Met	Trp	Asp	Tyr	Phe	His	Ser	Val	Leu	Leu	Ile	Lys
					725					730				735
Ala	Thr	Glu	Arg	Asn	Gly	Val	Asn	Val	Val	Ser	Gly	Pro	Ile	Phe
					740					745				750
Tyr	Asn	Tyr	Asp	Gly	His	Phe	Asp	Ala	Pro	Asp	Glu	Ile	Thr	Lys
					755					760				765
Leu	Ala	Asn	Thr	Asp	Val	Pro	Ile	Pro	Thr	His	Tyr	Phe	Val	Val
					770					775				780
Thr	Ser	Cys	Lys	Asn	Lys	Ser	His	Thr	Pro	Glu	Asn	Cys	Pro	Gly
					785					790				795
Leu	Asp	Val	Leu	Pro	Phe	Ile	Ile	Pro	His	Arg	Pro	Thr	Asn	Val
					805					810				815
Ser	Cys	Pro	Glu	Gly	Lys	Pro	Glu	Ala	Leu	Trp	Val	Glu	Glu	Arg
					820					825				830
Thr	Ala	His	Ile	Ala	Arg	Val	Arg	Asp	Val	Glu	Leu	Leu	Thr	Gly
					835					840				845
Asp	Phe	Tyr	Gln	Asp	Lys	Val	Gln	Pro	Val	Ser	Glu	Ile	Leu	Gln
					850					855				860
Lys	Thr	Tyr	Leu	Pro	Thr	Phe	Glu	Thr	Thr	Ile				
					865					870				875

SEQ ID No: 8 - Azurocidin-ENPP3-FC

MTRLTVLALLAGLLASSRA**AKQGSCRKKCFDASFRGLENCRCDVACKDRGDCCWDFEDTC
 VESTRIWMCNKFRCGETRLEASLCSCSDCLQRKDCCADYKSVCQGETSWLEENCDAQQSQCEGFD
 LPPVILFSMDGFRAEYLYTWDTLMPNINKLKTGCIHISKYMRAMYPTKTFPNHYTIVTGLYPESHGIID
 NNMYDVNLNKNFSLSSKEQNNPAWWHGQPMNLTAMYQGLKAATYFWPGSEVAINGSFPSIYMPYNGSV
 PFEERISTLLKWLDPKAERPRFYTMYPFEEPSSGHAGGPVSARVIKALQVVDHAFGMLMEGLKQRNL
 HNCVNIILLADHGMDQTYCNKMEYMTDYFPRINFFYMYEGPAPRIRAHNIPHDFFSFNSEIIVRNLS
 RKPQDQHFKEPYLTPDLPKRLHYAKNVRIDKVHLFVDQQLAVRSKSNNTNCGGGNHGYNNEFRSMEAI
 FL AHGPSFKEKTEVEPFENIEVYNLMCDLLRIQPAPNNGTHGSLNHLKVPFYEP SHAEVSKFSVCGFA
 NPLPTESLDCFCPHLQNSTQLEQVNQMLNLTQEEITATVKVNLFPGRPRVLQKNVDHCLLYHREYVSG
 FGKAMRMPMWSSYTVPQLGDTSPLPPTVPDCLRADVRVPPSESQKCSFYLDKNITHGFLYPASNRT
 SDSQYDALITSNLVPMYEEFRKMWDYFHSVLLIKHATERNGVNVVSGPIFDYNYDGHFDAPDEITKHL
 ANTVDVPIPTHYFVVLTSCKNKSHTPENCPGWLVDVLPFIIPHRPTNVESCEPGKEALWVEERFTHIA
 RVRDVELLTGLDFYQDKVQPVSEILQLKTYLPTFETIDKTHTCPPCPAPELLGGPSVFLFPPKPKDT
LMISRTPEVT**CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE**
YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFC**SVMH****EALHNHYTQKSLSLSPGK**

Single underline - Azurocidin signal sequence, Double underline - Beginning and end of ENPP3 sequence, Bold residues- Fc sequence, ** indicates the cleavage point of the signal sequence.

SEQ ID No: 9 - Azurocidin-ENPP3-Albumin

MTRLTVLALLAGLLASSRA**AKQGSCRKKCFDASFRGLENCRCDVACKDRGDCCWDFEDTC
 VESTRIWMCNKFRCGETRLEASLCSCSDCLQRKDCCADYKSVCQGETSWLEENCDAQQSQCEGFD
 LPPVILFSMDGFRAEYLYTWDTLMPNINKLKTGCIHISKYMRAMYPTKTFPNHYTIVTGLYPESHGIID
 NNMYDVNLNKNFSLSSKEQNNPAWWHGQPMNLTAMYQGLKAATYFWPGSEVAINGSFPSIYMPYNGSV
 PFEERISTLLKWLDPKAERPRFYTMYPFEEPSSGHAGGPVSARVIKALQVVDHAFGMLMEGLKQRNL
 HNCVNIILLADHGMDQTYCNKMEYMTDYFPRINFFYMYEGPAPRIRAHNIPHDFFSFNSEIIVRNLS
 RKPQDQHFKEPYLTPDLPKRLHYAKNVRIDKVHLFVDQQLAVRSKSNNTNCGGGNHGYNNEFRSMEAI
 FL

AHGSPFKEKTEVEPFENIEVYNLMCDLLRIQPAPNNGTHGSLNHLLKVPFYEP SHAEEVSKFSVCGFA
 NPLPTESLDCFCPHLQNSTQLEQVNQMLNLTQEEITATVKVNLFPGRPRVLQKNVDHCLLYHREYVSG
 FGKAMRMPMWSSYTVPQLGDTSPLPPTVPDCLRADVRVPPSESQKCSFYLDKNITHGFLYPPASNRT
 SDSQYDALITSNLVPMYEEFRKMWDYFHSVLLIKHATERNGVNVVSGPIFDYNYDGHFDAPDEITKHL
 ANTDPVPIPTHYFVVLTSCKNKSHTPENCPGWLDVLPFIIPHRPTNVESCPGKPEALWVEERFTHAIA
 RVRDVELLTGLDFYQDKVQPVSEITLQIKTYLPTFETTIMKWVTFLLLLFVSGSAFSRGVFRREAHKSE
IAHRYNDLGEQHFKGLVLIAFSQYLQKCSYDEHAKLVQEVTDFAKTCVADESAANCDKSLHTLFGDKL
CAIPNLRENYGELADCCTKQEPERNECFLOHKDDNPSPFPFERPEAEAMCTSFKENPTTFMGHYLHEV
ARRHPYFYAPELLYYAEQYNEILTQCCAADKESCLTPKLDGVKEKALVSSVRQRMKCSSMQKFGERA
FKAWAVARLSQTFPNADFAEITKLATDLTKVNKECCHGDLLECADDRAELAKYMCENQATISSKLQTC
CDKPLLKKAHCLSEVEHDTMPADLPAIAADFVEDQEVCKNYAEAKDVFLGTFLYEYSRRHPDYSVSL
LRLAKKYEATLEKCCAEANPPACYGTVLAEFQPLVEEPKNLVKTNCDLYEKLGEYGFQNAILVRYTQK
APQVSTPTLVEAARNLGRVGTKCTLPEDQRLPCVEDYLSAILNRVCLLHEKTPVSEHVTKCCSGSLV
ERRPCFSALTVDETYVPKEFKAETFTFHSIDICTLPEKEKQIKKQTALAELVKHKPKATAEQKLTVMDD
FAQFLDTCCKAADKDTCTFSTEGPNLVTRCKDALARSWSHPQFEK

Single underline - Azurocidin signal sequence, Double underline - Beginning and end of ENPP3 sequence, Bold residues - Albumin sequence, ** indicates the cleavage point of the signal sequence.

SEQ ID No: 10 - Azurocidin-ENPP3

MTRLTVLALLAGLLASSRA**AKQGSCRKKCFDASFRGLENCRCDVACKDRGDCCWDFEDTC
 VESTRIWMCNKFRGGETRLEASLSCSDCLQRKDCCADYKSVQGETSWLENCDTAQSQCPGFD
 LPPVILFSMDGFRAEYLYTWDTLMPNINKLKTCGIHISKYMRAMYPTKTFPNHYTIVTGLYPESHGIID
 NNMYDVNLNKNFSLSSKEQNNPAWWHGQPMNLTAMYQGLKAATYFWPGSEVAINGSFPSIYMPYNGSV
 PFEERISTLLKWLDPKAERPRFYTYMFEEDSSGHAGGPVSARVIKALQVVDHAFGMLMEGLKQRNL
 HNCVNIILLADHGMDQTYCNKMEYMTDYFPRINFFYMEGPAPRIRAHNIPHDFFSFNSEEIVRNLS
 RKPQDQHFKPYPYLPDLPKRLHYAKNVRIDKVHLFVDQQLAVRSKSNNTNCGGNGHYNNEFRSMEAI
 FL AHGSPFKEKTEVEPFENIEVYNLMCDLLRIQPAPNNGTHGSLNHLLKVPFYEP SHAEEVSKFSVCGFA
 NPLPTESLDCFCPHLQNSTQLEQVNQMLNLTQEEITATVKVNLFPGRPRVLQKNVDHCLLYHREYVSG
 FGKAMRMPMWSSYTVPQLGDTSPLPPTVPDCLRADVRVPPSESQKCSFYLDKNITHGFLYPPASNRT
 SDSQYDALITSNLVPMYEEFRKMWDYFHSVLLIKHATERNGVNVVSGPIFDYNYDGHFDAPDEITKHL
 ANTDPVPIPTHYFVVLTSCKNKSHTPENCPGWLDVLPFIIPHRPTNVESCPGKPEALWVEERFTHAIA
 RVRDVELLTGLDFYQDKVQPVSEILQLKTYLPTFETTI

Single underline - Azurocidin signal sequence, Double underline - Beginning and end of ENPP3 sequence, ** indicates the cleavage point of the signal sequence.

SEQ. ID NO:11 - ENPP4 Amino Acid Sequence - Wild Type

	Met	Lys	Leu	Leu	Val	Ile	Leu	Leu	Phe	Ser	Gly	Leu	Ile	Thr	Gly
Phe	1				5					10					15
	Arg	Ser	Asp	Ser	Ser	Ser	Ser	Leu	Pro	Pro	Lys	Leu	Leu	Leu	Val
Ser					20					25					30
	Phe	Asp	Gly	Phe	Arg	Ala	Asp	Tyr	Leu	Lys	Asn	Tyr	Glu	Phe	Pro
His															
					35					40					45
	Leu	Gln	Asn	Phe	Ile	Lys	Glu	Gly	Val	Leu	Val	Glu	His	Val	Lys
Asn															

	50		55		60										
	Val	Phe	Ile	Thr	Lys	Thr	Phe	Pro	Asn	His	Tyr	Ser	Ile	Val	Thr
Gly															
	65					70					75				80
	Leu	Tyr	Glu	Glu	Ser	His	Gly	Ile	Val	Ala	Asn	Ser	Met	Tyr	Asp
Ala															
					85					90					95
	Val	Thr	Lys	Lys	His	Phe	Ser	Asp	Ser	Asn	Asp	Lys	Asp	Pro	Phe
Trp															
				100					105					110	
	Trp	Asn	Glu	Ala	Val	Pro	Ile	Trp	Val	Thr	Asn	Gln	Leu	Gln	Glu
Asn															
		115					120				125				
	Arg	Ser	Ser	Ala	Ala	Ala	Met	Trp	Pro	Gly	Thr	Asp	Val	Pro	Ile
His															
		130					135				140				
	Asp	Thr	Ile	Ser	Ser	Tyr	Phe	Met	Asn	Tyr	Asn	Ser	Ser	Val	Ser
Phe															
	145					150					155				
160															
	Glu	Glu	Arg	Leu	Asn	Asn	Ile	Thr	Met	Trp	Leu	Asn	Asn	Ser	Asn
Pro															
				165					170					175	
	Pro	Val	Thr	Phe	Ala	Thr	Leu	Tyr	Trp	Glu	Glu	Pro	Asp	Ala	Ser
Gly															
			180					185					190		
	His	Lys	Tyr	Gly	Pro	Glu	Asp	Lys	Glu	Asn	Met	Ser	Arg	Val	Leu
Lys															
		195					200					205			
	Lys	Ile	Asp	Asp	Leu	Ile	Gly	Asp	Leu	Val	Gln	Arg	Leu	Lys	Met
Leu															
		210					215					220			
	Gly	Leu	Trp	Glu	Asn	Leu	Asn	Val	Ile	Ile	Thr	Ser	Asp	His	Gly
Met															
	225					230						235			
240															
	Thr	Gln	Cys	Ser	Gln	Asp	Arg	Leu	Ile	Asn	Leu	Asp	Ser	Cys	Ile
Asp															
				245					250					255	
	His	Ser	Tyr	Tyr	Thr	Leu	Ile	Asp	Leu	Ser	Pro	Val	Ala	Ala	Ile
Leu															
			260					265					270		
	Pro	Lys	Ile	Asn	Arg	Thr	Glu	Val	Tyr	Asn	Lys	Leu	Lys	Asn	Cys
Ser															
		275					280				285				
	Pro	His	Met	Asn	Val	Tyr	Leu	Lys	Glu	Asp	Ile	Pro	Asn	Arg	Phe
Tyr															
		290					295				300				
	Tyr	Gln	His	Asn	Asp	Arg	Ile	Gln	Pro	Ile	Ile	Leu	Val	Ala	Asp
Glu															
	305					310					315				
320															
	Gly	Trp	Thr	Ile	Val	Leu	Asn	Glu	Ser	Ser	Gln	Lys	Leu	Gly	Asp
His															
				325					330					335	

Gly Tyr Asp Asn Ser Leu Pro Ser Met His Pro Phe Leu Ala Ala
 His
 340 345 350
 Gly Pro Ala Phe His Lys Gly Tyr Lys His Ser Thr Ile Asn Ile
 Val
 355 360 365
 Asp Ile Tyr Pro Met Met Cys His Ile Leu Gly Leu Lys Pro His
 Pro
 370 375 380
 Asn Asn Gly Thr Phe Gly His Thr Lys Cys Leu Leu Val Asp Gln
 Trp
 385 390 395
 400
 Cys Ile Asn Leu Pro Glu Ala Ile Ala Ile Val Ile Gly Ser Leu
 Leu
 405 410 415
 Val Leu Thr Met Leu Thr Cys Leu Ile Ile Ile Met Gln Asn Arg
 Leu
 420 425 430
 Ser Val Pro Arg Pro Phe Ser Arg Leu Gln Leu Gln Glu Asp Asp
 Asp
 435 440 445
 Asp Pro Leu Ile Gly
 450

SEQ. ID NO: 12 - ENPP51 Amino Acid Sequence

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 Leu Gln**Pro Ser Cys Ala Lys Glu Val
 Lys
 20 25 30
 Ser Cys Lys Gly Arg Cys Phe Glu Arg Thr Phe Ser Asn Cys Arg
 Cys
 35 40 45
 Asp Ala Cys Val Ser Leu Gly Asn Cys Cys Leu Asp Phe Gln
 Glu
 50 55 60
 Thr Cys Val Glu Pro Thr His Ile Trp Thr Cys Asn Lys Phe Arg
 Cys
 65 70 75 80
 Gly Glu Lys Arg Leu Ser Arg Phe Val Cys Ser Cys Ala Asp Asp
 Cys
 85 90 95
 Lys Thr His Asn Asp Cys Cys Ile Asn Tyr Ser Ser Val Cys Gln
 Asp
 100 105 110
 Lys Lys Ser Trp Val Glu Glu Thr Cys Glu Ser Ile Asp Thr Pro
 Glu
 115 120 125
 Cys Pro Ala Glu Phe Glu Ser Pro Pro Thr Leu Leu Phe Ser Leu
 Asp

		130				135				140					
	Gly	Phe	Arg	Ala	Glu	Tyr	Leu	His	Thr	Trp	Gly	Gly	Leu	Leu	Pro
Val															
	145					150				155					
160															
	Tle	Ser	Lys	Leu	Lys	Asn	Cys	Gly	Thr	Tyr	Thr	Lys	Asn	Met	Arg
Pro															
					165					170					175
	Met	Tyr	Pro	Thr	Lys	Thr	Phe	Pro	Asn	His	Tyr	Ser	Ile	Val	Thr
Gly															
				180					185					190	
	Leu	Tyr	Pro	Glu	Ser	His	Gly	Ile	Ile	Asp	Asn	Lys	Met	Tyr	Asp
Pro															
		195					200					205			
	Lys	Met	Asn	Ala	Ser	Phe	Ser	Leu	Lys	Ser	Lys	Glu	Lys	Phe	Asn
Pro															
	210						215				220				
	Leu	Trp	Tyr	Lys	Gly	Gln	Pro	Ile	Trp	Val	Thr	Ala	Asn	His	Gln
Glu															
	225					230					235				
240															
	Val	Lys	Ser	Gly	Thr	Tyr	Phe	Trp	Pro	Gly	Ser	Asp	Val	Glu	Ile
Asp															
				245					250					255	
	Gly	Ile	Leu	Pro	Asp	Ile	Tyr	Lys	Val	Tyr	Asn	Gly	Ser	Val	Pro
Phe															
			260					265					270		
	Glu	Glu	Arg	Ile	Leu	Ala	Val	Leu	Glu	Trp	Leu	Gln	Leu	Pro	Ser
His															
		275					280				285				
	Glu	Arg	Pro	His	Phe	Tyr	Thr	Leu	Tyr	Leu	Glu	Glu	Pro	Asp	Ser
Ser															
	290						295				300				
	Gly	His	Ser	His	Gly	Pro	Val	Ser	Ser	Glu	Val	Ile	Lys	Ala	Leu
Gln															
	305					310					315				
320															
	Lys	Val	Asp	Arg	Leu	Val	Gly	Met	Leu	Met	Asp	Gly	Leu	Lys	Asp
Leu															
				325					330					335	
	Gly	Leu	Asp	Lys	Cys	Leu	Asn	Leu	Ile	Leu	Ile	Ser	Asp	His	Gly
Met															
			340					345				350			
	Glu	Gln	Gly	Ser	Cys	Lys	Lys	Tyr	Val	Tyr	Leu	Asn	Lys	Tyr	Leu
Gly															
		355					360			365					
	Asp	Val	Asn	Asn	Val	Lys	Val	Val	Tyr	Gly	Pro	Ala	Ala	Arg	Leu
Arg															
	370					375				380					
	Pro	Thr	Asp	Val	Pro	Glu	Thr	Tyr	Tyr	Ser	Phe	Asn	Tyr	Glu	Ala
Leu															
	385				390					395					
400															
	Ala	Lys	Asn	Leu	Ser	Cys	Arg	Glu	Pro	Asn	Gln	His	Phe	Arg	Pro
Tyr															
				405					410					415	

	Leu	Lys	Pro	Phe	Leu	Pro	Lys	Arg	Leu	His	Phe	Ala	Lys	Ser	Asp
Arg															
				420					425					430	
	Ile	Glu	Pro	Leu	Thr	Phe	Tyr	Leu	Asp	Pro	Gln	Trp	Gln	Leu	Ala
Leu															
			435					440					445		
	Asn	Pro	Ser	Glu	Arg	Lys	Tyr	Cys	Gly	Ser	Gly	Phe	His	Gly	Ser
Asp															
	450						455				460				
	Asn	Leu	Phe	Ser	Asn	Met	Gln	Ala	Leu	Phe	Ile	Gly	Tyr	Gly	Pro
Ala															
	465					470					475				
480															
	Phe	Lys	His	Gly	Ala	Glu	Val	Asp	Ser	Phe	Glu	Asn	Ile	Glu	Val
Tyr															
				485						490				495	
	Asn	Leu	Met	Cys	Asp	Leu	Leu	Gly	Leu	Ile	Pro	Ala	Pro	Asn	Asn
Gly															
			500						505					510	
	Ser	His	Gly	Ser	Leu	Asn	His	Leu	Leu	Lys	Lys	Pro	Ile	Tyr	Asn
Pro															
			515					520					525		
	Ser	His	Pro	Lys	Glu	Glu	Gly	Phe	Leu	Ser	Gln	Cys	Pro	Ile	Lys
Ser															
	530						535				540				
	Thr	Ser	Asn	Asp	Leu	Gly	Cys	Thr	Cys	Asp	Pro	Trp	Ile	Val	Pro
Ile															
	545					550					555				
560															
	Lys	Asp	Phe	Glu	Lys	Gln	Leu	Asn	Leu	Thr	Thr	Glu	Asp	Val	Asp
Asp															
				565					570					575	
	Ile	Tyr	His	Met	Thr	Val	Pro	Tyr	Gly	Arg	Pro	Arg	Ile	Leu	Leu
Lys															
			580						585					590	
	Gln	His	Arg	Val	Cys	Leu	Leu	Gln	Gln	Gln	Gln	Phe	Leu	Thr	Gly
Tyr															
			595					600					605		
	Ser	Leu	Asp	Leu	Leu	Met	Pro	Leu	Trp	Ala	Ser	Tyr	Thr	Phe	Leu
Ser															
	610						615					620			
	Asn	Asp	Gln	Phe	Ser	Arg	Asp	Asp	Phe	Ser	Asn	Cys	Leu	Tyr	Gln
Asp															
	625					630					635				
640															
	Leu	Arg	Ile	Pro	Leu	Ser	Pro	Val	His	Lys	Cys	Ser	Tyr	Tyr	Lys
Ser															
				645					650					655	
	Asn	Ser	Lys	Leu	Ser	Tyr	Gly	Phe	Leu	Thr	Pro	Pro	Arg	Leu	Asn
Arg															
			660					665					670		
	Val	Ser	Asn	His	Ile	Tyr	Ser	Glu	Ala	Leu	Leu	Thr	Ser	Asn	Ile
Val															
			675					680				685			
	Pro	Met	Tyr	Gln	Ser	Phe	Gln	Val	Ile	Trp	His	Tyr	Leu	His	Asp
Thr															

	690		695		700
	Leu Leu Gln Arg Tyr Ala His Glu Arg Asn Gly Ile Asn Val Val				
Ser	705		710		715
720	Gly Pro Val Phe Asp Phe Asp Tyr Asp Gly Arg Tyr Asp Ser Leu				
Glu		725		730	735
	Ile Leu Lys Gln Asn Ser Arg Val Ile Arg Ser Gln Glu Ile Leu				
Ile		740		745	750
	Pro Thr His Phe Phe Ile Val Leu Thr Ser Cys Lys Gln Leu Ser				
Glu		755		760	765
	Thr Pro Leu Glu Cys Ser Ala Leu Glu Ser Ser Ala Tyr Ile Leu				
Pro		770		775	780
	His Arg Pro Asp Asn Ile Glu Ser Cys Thr His Gly Lys Arg Glu				
Ser	785		790		795
800	Ser Trp Val Glu Glu Leu Leu Thr Leu His Arg Ala Arg Val Thr				
Asp		805		810	815
	Val Glu Leu Ile Thr Gly Leu Ser Phe Tyr Gln Asp Arg Gln Glu				
Ser		820		825	830
	Val Ser Glu Leu Leu Arg Leu Lys Thr His Leu Pro Ile Phe Ser				
<u>Gln</u>		835		840	845
	<u>Glu Asp</u>				
	850				

Singly underlined: signal peptide sequence; double-underlined: beginning and end of NPP1; ** = cleavage position at the signal peptide sequence

SEQ. ID NO: 13 - ENPP51 - ALB Amino Acid Sequence:

	Met Thr Ser Lys Phe Leu Leu Val Ser Phe Ile Leu Ala Ala Leu
<u>Ser</u>	
	1 5 10 15
	<u>Leu Ser Thr Thr Phe Ser Leu Gln**<u>Pro Ser Cys Ala Lys</u> Glu Val</u>
Lys	
	20 25 30
	Ser Cys Lys Gly Arg Cys Phe Glu Arg Thr Phe Ser Asn Cys Arg
Cys	
	35 40 45
	Asp Ala Ala Cys Val Ser Leu Gly Asn Cys Cys Leu Asp Phe Gln
Glu	
	50 55 60
	Thr Cys Val Glu Pro Thr His Ile Trp Thr Cys Asn Lys Phe Arg
Cys	

	65				70				75					80
Cys	Gly	Glu	Lys	Arg	Leu	Ser	Arg	Phe	Val	Cys	Ser	Cys	Ala	Asp
					85				90					95
Asp	Lys	Thr	His	Asn	Asp	Cys	Cys	Ile	Asn	Tyr	Ser	Ser	Val	Cys
				100				105					110	
Glu	Lys	Lys	Ser	Trp	Val	Glu	Glu	Thr	Cys	Glu	Ser	Ile	Asp	Thr
			115				120					125		
Asp	Cys	Pro	Ala	Glu	Phe	Glu	Ser	Pro	Pro	Thr	Leu	Leu	Phe	Ser
		130				135				140				
Val	Gly	Phe	Arg	Ala	Glu	Tyr	Leu	His	Thr	Trp	Gly	Gly	Leu	Leu
	145				150					155				
160														
Pro	Ile	Ser	Lys	Leu	Lys	Asn	Cys	Gly	Thr	Tyr	Thr	Lys	Asn	Met
				165				170					175	
Gly	Met	Tyr	Pro	Thr	Lys	Thr	Phe	Pro	Asn	His	Tyr	Ser	Ile	Val
			180				185					190		
Pro	Leu	Tyr	Pro	Glu	Ser	His	Gly	Ile	Ile	Asp	Asn	Lys	Met	Tyr
		195				200					205			
Pro	Lys	Met	Asn	Ala	Ser	Phe	Ser	Leu	Lys	Ser	Lys	Glu	Lys	Phe
		210				215					220			
Glu	Leu	Trp	Tyr	Lys	Gly	Gln	Pro	Ile	Trp	Val	Thr	Ala	Asn	His
	225				230					235				
240														
Asp	Val	Lys	Ser	Gly	Thr	Tyr	Phe	Trp	Pro	Gly	Ser	Asp	Val	Glu
			245				250					255		
Phe	Gly	Ile	Leu	Pro	Asp	Ile	Tyr	Lys	Val	Tyr	Asn	Gly	Ser	Val
			260				265					270		
His	Glu	Glu	Arg	Ile	Leu	Ala	Val	Leu	Glu	Trp	Leu	Gln	Leu	Pro
		275				280					285			
Ser	Glu	Arg	Pro	His	Phe	Tyr	Thr	Leu	Tyr	Leu	Glu	Glu	Pro	Asp
		290				295					300			
Gln	Gly	His	Ser	His	Gly	Pro	Val	Ser	Ser	Glu	Val	Ile	Lys	Ala
	305				310					315				
320														
Leu	Lys	Val	Asp	Arg	Leu	Val	Gly	Met	Leu	Met	Asp	Gly	Leu	Lys
			325				330					335		
Met	Gly	Leu	Asp	Lys	Cys	Leu	Asn	Leu	Ile	Leu	Ile	Ser	Asp	His
			340				345					350		

	Glu	Gln	Gly	Ser	Cys	Lys	Lys	Tyr	Val	Tyr	Leu	Asn	Lys	Tyr	Leu
Gly			355					360				365			
	Asp	Val	Asn	Asn	Val	Lys	Val	Val	Tyr	Gly	Pro	Ala	Ala	Arg	Leu
Arg		370					375				380				
	Pro	Thr	Asp	Val	Pro	Glu	Thr	Tyr	Tyr	Ser	Phe	Asn	Tyr	Glu	Ala
Leu	385					390					395				
400															
	Ala	Lys	Asn	Leu	Ser	Cys	Arg	Glu	Pro	Asn	Gln	His	Phe	Arg	Pro
Tyr				405						410				415	
	Leu	Lys	Pro	Phe	Leu	Pro	Lys	Arg	Leu	His	Phe	Ala	Lys	Ser	Asp
Arg				420				425						430	
	Ile	Glu	Pro	Leu	Thr	Phe	Tyr	Leu	Asp	Pro	Gln	Trp	Gln	Leu	Ala
Leu			435					440				445			
	Asn	Pro	Ser	Glu	Arg	Lys	Tyr	Cys	Gly	Ser	Gly	Phe	His	Gly	Ser
Asp															
	Asn	Leu	Phe	Ser	Asn	Met	Gln	Ala	Leu	Phe	Ile	Gly	Tyr	Gly	Pro
Ala	450					455					460				
480															
	Phe	Lys	His	Gly	Ala	Glu	Val	Asp	Ser	Phe	Glu	Asn	Ile	Glu	Val
Tyr				485				490						495	
	Asn	Leu	Met	Cys	Asp	Leu	Leu	Gly	Leu	Ile	Pro	Ala	Pro	Asn	Asn
Gly															
	Ser	His	Gly	Ser	Leu	Asn	His	Leu	Leu	Lys	Lys	Pro	Ile	Tyr	Asn
Pro															
	Ser	His	Pro	Lys	Glu	Glu	Gly	Phe	Leu	Ser	Gln	Cys	Pro	Ile	Lys
Ser															
	Thr	Ser	Asn	Asp	Leu	Gly	Cys	Thr	Cys	Asp	Pro	Trp	Ile	Val	Pro
Ile	530					535					540				
560															
	Lys	Asp	Phe	Glu	Lys	Gln	Leu	Asn	Leu	Thr	Thr	Glu	Asp	Val	Asp
Asp															
	Ile	Tyr	His	Met	Thr	Val	Pro	Tyr	Gly	Arg	Pro	Arg	Ile	Leu	Leu
Lys				565				570						575	
	Gln	His	Arg	Val	Cys	Leu	Leu	Gln	Gln	Gln	Gln	Phe	Leu	Thr	Gly
Tyr				580				585					590		
	Ser	Leu	Asp	Leu	Leu	Met	Pro	Leu	Trp	Ala	Ser	Tyr	Thr	Phe	Leu
Ser															
	Asn	Asp	Gln	Phe	Ser	Arg	Asp	Asp	Phe	Ser	Asn	Cys	Leu	Tyr	Gln
Asp	610					615					620				

	625		630		635										
640	Leu	Arg	Ile	Pro	Leu	Ser	Pro	Val	His	Lys	Cys	Ser	Tyr	Tyr	Lys
Ser															
					645					650					655
Arg	Asn	Ser	Tys	Leu	Ser	Tyr	Gly	Phe	Leu	Thr	Pro	Pro	Arg	Leu	Asn
				660					665					670	
Val	Val	Ser	Asn	His	Ile	Tyr	Ser	Glu	Ala	Leu	Leu	Thr	Ser	Asn	Ile
				675				680					685		
Thr	Pro	Met	Tyr	Gln	Ser	Phe	Gln	Val	Ile	Trp	His	Tyr	Leu	His	Asp
		690					695					700			
Ser	Leu	Leu	Gln	Arg	Tyr	Ala	His	Glu	Arg	Asn	Gly	Ile	Asn	Val	Val
720	705						710						715		
Glu	Gly	Pro	Val	Phe	Asp	Phe	Asp	Tyr	Asp	Gly	Arg	Tyr	Asp	Ser	Leu
					725				730						735
Ile	Ile	Leu	Lys	Gln	Asn	Ser	Arg	Val	Ile	Arg	Ser	Gln	Glu	Ile	Leu
				740				745					750		
Glu	Pro	Thr	His	Phe	Phe	Ile	Val	Leu	Thr	Ser	Cys	Lys	Gln	Leu	Ser
		755					760					765			
Pro	Thr	Pro	Leu	Glu	Cys	Ser	Ala	Leu	Glu	Ser	Ser	Ala	Tyr	Ile	Leu
		770				775					780				
Ser	His	Arg	Pro	Asp	Asn	Ile	Glu	Ser	Cys	Thr	His	Gly	Lys	Arg	Glu
800	785					790					795				
Asp	Ser	Trp	Val	Glu	Glu	Leu	Leu	Thr	Leu	His	Arg	Ala	Arg	Val	Thr
				805					810						815
Ser	Val	Glu	Leu	Ile	Thr	Gly	Leu	Ser	Phe	Tyr	Gln	Asp	Arg	Gln	Glu
			820					825					830		
Gln	Val	Ser	Glu	Leu	Leu	Arg	Leu	Lys	Thr	His	Leu	Pro	Ile	Phe	Ser
		835				840					845				
Leu	Glu	Asp	<u>Gly</u>	<u>Gly</u>	<u>Ser</u>	<u>Gly</u>	<u>Gly</u>	<u>Ser</u>	Met	Lys	Trp	Val	Thr	Phe	Leu
		850				855					860				
Arg	Leu	Leu	Phe	Val	Ser	Gly	Ser	Ala	Phe	Ser	Arg	Gly	Val	Phe	Arg
880	865					870					875				
Glu	Glu	Ala	His	Lys	Ser	Glu	Ile	Ala	His	Arg	Tyr	Asn	Asp	Leu	Gly
				885				890					895		
Gln	Gln	His	Phe	Lys	Gly	Leu	Val	Leu	Ile	Ala	Phe	Ser	Gln	Tyr	Leu
			900					905					910		

	Lys	Cys	Ser	Tyr	Asp	Glu	His	Ala	Lys	Leu	Val	Gln	Glu	Val	Thr
Asp															
		915						920				925			
	Phe	Ala	Lys	Thr	Cys	Val	Ala	Asp	Glu	Ser	Ala	Ala	Asn	Cys	Asp
Lys															
		930						935				940			
	Ser	Leu	His	Thr	Leu	Phe	Gly	Asp	Lys	Leu	Cys	Ala	Ile	Pro	Asn
Leu															
	945					950					955				
960															
	Arg	Glu	Asn	Tyr	Gly	Glu	Leu	Ala	Asp	Cys	Cys	Thr	Lys	Gln	Glu
Pro															
					965				970				975		
	Glu	Arg	Asn	Glu	Cys	Phe	Leu	Gln	His	Lys	Asp	Asp	Asn	Pro	Ser
Leu															
					980				985				990		
	Pro	Pro	Phe	Glu	Arg	Pro	Glu	Ala	Glu	Ala	Met	Cys	Thr	Ser	Phe
Lys															
					995				1000				1005		
	Glu	Asn	Pro	Thr	Thr	Phe	Met	Gly	His	Tyr	Leu	His	Glu	Val	Ala
	1010						1015					1020			
	Arg	Arg	His	Pro	Tyr	Phe	Tyr	Ala	Pro	Glu	Leu	Leu	Tyr	Tyr	Ala
	1025						1030					1035			
	Glu	Gln	Tyr	Asn	Glu	Ile	Leu	Thr	Gln	Cys	Cys	Ala	Glu	Ala	Asp
	1040						1045					1050			
	Lys	Glu	Ser	Cys	Leu	Thr	Pro	Lys	Leu	Asp	Gly	Val	Lys	Glu	Lys
	1055						1060					1065			
	Ala	Leu	Val	Ser	Ser	Val	Arg	Gln	Arg	Met	Lys	Cys	Ser	Ser	Met
	1070						1075					1080			
	Gln	Lys	Phe	Gly	Glu	Arg	Ala	Phe	Lys	Ala	Trp	Ala	Val	Ala	Arg
	1085						1090					1095			
	Leu	Ser	Gln	Thr	Phe	Pro	Asn	Ala	Asp	Phe	Ala	Glu	Ile	Thr	Lys
	1100						1105					1110			
	Leu	Ala	Thr	Asp	Leu	Thr	Lys	Val	Asn	Lys	Glu	Cys	Cys	His	Gly
	1115						1120					1125			
	Asp	Leu	Leu	Glu	Cys	Ala	Asp	Asp	Arg	Ala	Glu	Leu	Ala	Lys	Tyr
	1130						1135					1140			
	Met	Cys	Glu	Asn	Gln	Ala	Thr	Ile	Ser	Ser	Lys	Leu	Gln	Thr	Cys
	1145						1150					1155			
	Cys	Asp	Lys	Pro	Leu	Leu	Lys	Lys	Ala	His	Cys	Leu	Ser	Glu	Val
	1160						1165					1170			
	Glu	His	Asp	Thr	Met	Pro	Ala	Asp	Leu	Pro	Ala	Ile	Ala	Ala	Asp
	1175						1180					1185			
	Phe	Val	Glu	Asp	Gln	Glu	Val	Cys	Lys	Asn	Tyr	Ala	Glu	Ala	Lys
	1190						1195					1200			
	Asp	Val	Phe	Leu	Gly	Thr	Phe	Leu	Tyr	Glu	Tyr	Ser	Arg	Arg	His
	1205						1210					1215			
	Pro	Asp	Tyr	Ser	Val	Ser	Leu	Leu	Leu	Arg	Leu	Ala	Lys	Lys	Tyr
	1220						1225					1230			
	Glu	Ala	Thr	Leu	Glu	Lys	Cys	Cys	Ala	Glu	Ala	Asn	Pro	Pro	Ala
	1235						1240					1245			
	Cys	Tyr	Gly	Thr	Val	Leu	Ala	Glu	Phe	Gln	Pro	Leu	Val	Glu	Glu
	1250						1255					1260			
	Pro	Lys	Asn	Leu	Val	Lys	Thr	Asn	Cys	Asp	Leu	Tyr	Glu	Lys	Leu
	1265						1270					1275			
	Gly	Glu	Tyr	Gly	Phe	Gln	Asn	Ala	Ile	Leu	Val	Arg	Tyr	Thr	Gln

1280	Lys	Ala	Pro	Gln	Val	Ser	1285	Thr	Pro	Thr	Leu	Val	1290	Glu	Ala	Ala	Arg
1295	Asn	Leu	Gly	Arg	Val	Gly	1300	Thr	Lys	Cys	Cys	Thr	1305	Leu	Pro	Glu	Asp
1310	Gln	Arg	Leu	Pro	Cys	Val	1315	Glu	Asp	Tyr	Leu	Ser	1320	Ala	Ile	Leu	Asn
1325	Arg	Val	Cys	Leu	Leu	His	1330	Glu	Lys	Thr	Pro	Val	1335	Ser	Glu	His	Val
1340	Thr	Lys	Cys	Cys	Ser	Gly	1345	Ser	Leu	Val	Glu	Arg	1350	Arg	Pro	Cys	Phe
1355	Ser	Ala	Leu	Thr	Val	Asp	1375	Glu	Thr	Tyr	Val	Pro	1380	Lys	Glu	Phe	Lys
1385	Ala	Glu	Thr	Phe	Thr	Phe	1390	His	Ser	Asp	Ile	Cys	1395	Thr	Leu	Pro	Glu
1400	Lys	Glu	Lys	Gln	Ile	Lys	1405	Lys	Gln	Thr	Ala	Leu	1410	Ala	Glu	Leu	Val
1415	Lys	His	Lys	Pro	Lys	Ala	1420	Thr	Ala	Glu	Gln	Leu	1425	Lys	Thr	Val	Met
1430	Asp	Asp	Phe	Ala	Gln	Phe	1435	Leu	Asp	Thr	Cys	Cys	1440	Lys	Ala	Ala	Asp
1445	Lys	Asp	Thr	Cys	Phe	Ser	1450	Thr	Glu	Gly	Pro	Asn	1455	Leu	Val	Thr	Arg
1460	Cys	Lys	Asp	Ala	Leu	Ala	1465	Arg	Ser	Trp	Ser	His	1470	Pro	Gln	Phe	Glu
	Lys																

Singly underlined: signal peptide sequence; double-underlined: beginning and end of NPP1; ** = cleavage position at the signal peptide sequence; bold residues indicate albumin sequence

SEQ. ID NO: 14 - ENPP5-NPP3-Fc sequence

	Met	Thr	Ser	Lys	Phe	Leu	Leu	Val	Ser	Phe	Ile	Leu	Ala	Ala	Leu
<u>Ser</u>	1			5					10					15	
	Leu	Ser	Thr	Thr	Phe	Ser	**	<u>Lys</u>	<u>Gln</u>	<u>Gly</u>	Ser	Cys	Arg	Lys	Lys
Phe															
				20					25				30		
	Asp	Ala	Ser	Phe	Arg	Gly	Leu	Glu	Asn	Cys	Arg	Cys	Asp	Val	Ala
Cys															
				35					40				45		
	Lys	Asp	Arg	Gly	Asp	Cys	Cys	Trp	Asp	Phe	Glu	Asp	Thr	Cys	Val
Glu															
				50				55				60			
	Ser	Thr	Arg	Ile	Trp	Met	Cys	Asn	Lys	Phe	Arg	Cys	Gly	Glu	Arg
Leu															
	65				70				75				80		
	Glu	Ala	Ser	Leu	Cys	Ser	Cys	Ser	Asp	Asp	Cys	Leu	Gln	Arg	Lys
Asp															
				85					90				95		

	Cys	Cys	Ala	Asp	Tyr	Lys	Ser	Val	Cys	Gln	Gly	Glu	Thr	Ser	Trp
Leu															
				100					105					110	
	Glu	Glu	Asn	Cys	Asp	Thr	Ala	Gln	Gln	Ser	Gln	Cys	Pro	Glu	Gly
Phe															
			115					120					125		
	Asp	Leu	Pro	Pro	Val	Ile	Leu	Phe	Ser	Met	Asp	Gly	Phe	Arg	Ala
Glu															
		130					135					140			
	Tyr	Leu	Tyr	Thr	Trp	Asp	Thr	Leu	Met	Pro	Asn	Ile	Asn	Lys	Leu
Lys															
	145					150					155				
160															
	Thr	Cys	Gly	Ile	His	Ser	Lys	Tyr	Met	Arg	Ala	Met	Tyr	Pro	Thr
Lys															
				165					170					175	
	Thr	Phe	Pro	Asn	His	Tyr	Thr	Ile	Val	Thr	Gly	Leu	Tyr	Pro	Glu
Ser															
			180					185					190		
	His	Gly	Ile	Ile	Asp	Asn	Asn	Met	Tyr	Asp	Val	Asn	Leu	Asn	Lys
Asn															
		195					200					205			
	Phe	Ser	Leu	Ser	Ser	Lys	Glu	Gln	Asn	Asn	Pro	Ala	Trp	Trp	His
Gly															
		210					215				220				
	Gln	Pro	Met	Trp	Leu	Thr	Ala	Met	Tyr	Gln	Gly	Leu	Lys	Ala	Ala
Thr															
	225					230					235				
240															
	Tyr	Phe	Trp	Pro	Gly	Ser	Glu	Val	Ala	Ile	Asn	Gly	Ser	Phe	Pro
Ser															
				245					250					255	
	Ile	Tyr	Met	Pro	Tyr	Asn	Gly	Ser	Val	Pro	Phe	Glu	Glu	Arg	Ile
Ser															
			260					265					270		
	Thr	Leu	Leu	Lys	Trp	Leu	Asp	Leu	Pro	Lys	Ala	Glu	Arg	Pro	Arg
Phe															
		275						280					285		
	Tyr	Thr	Met	Tyr	Phe	Glu	Glu	Pro	Asp	Ser	Ser	Gly	His	Ala	Gly
Gly															
		290					295				300				
	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	Tys	Pro	Asp	Gln	His	Phe	Tys	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				
	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	<u>Thr</u>	<u>Thr</u>	<u>Ile</u>
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															

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          930          935          940
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
Glu
945          950          955
960
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
Tyr
          965          970          975
Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
Leu
          980          985          990
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
Trp
          995          1000          1005
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
1010          1015          1020
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
1025          1030          1035
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
1040          1045          1050
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
1055          1060          1065
Ser Leu Ser Pro Gly Lys
1070

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Singly underlined: signal peptide sequence; double-underlined: beginning and end of NPP33; ** = cleavage position at the signal peptide sequence; bold residues indicate albumin sequence

SEQ. ID NO: 15- ENPP5-NPP3-Albumin sequence

```

Met Thr Ser Lys Phe Leu Leu Val Ser Phe Ile Leu Ala Ala Leu
Ser
1          5          10          15
Leu Ser Thr Thr Phe Ser**Lys Gln Gly Ser Cys Arg Lys Lys Cys
Phe
          20          25          30
Asp Ala Ser Phe Arg Gly Leu Glu Asn Cys Arg Cys Asp Val Ala
Cys
          35          40          45
Lys Asp Arg Gly Asp Cys Cys Trp Asp Phe Glu Asp Thr Cys Val
Glu
          50          55          60
Ser Thr Arg Ile Trp Met Cys Asn Lys Phe Arg Cys Gly Glu Arg
Leu
65          70          75          80
Glu Ala Ser Leu Cys Ser Cys Ser Asp Asp Cys Leu Gln Arg Lys
Asp
          85          90          95
Cys Cys Ala Asp Tyr Lys Ser Val Cys Gln Gly Glu Thr Ser Trp
Leu
          100          105          110

```

	Glu	Glu	Asn	Cys	Asp	Thr	Ala	Gln	Gln	Ser	Gln	Cys	Pro	Glu	Gly
Phe															
			115					120				125			
	Asp	Leu	Pro	Pro	Val	Ile	Leu	Phe	Ser	Met	Asp	Gly	Phe	Arg	Ala
Glu															
		130					135					140			
	Tyr	Leu	Tyr	Thr	Trp	Asp	Thr	Leu	Met	Pro	Asn	Ile	Asn	Lys	Leu
Lys															
	145					150					155				
160															
	Thr	Cys	Gly	Ile	His	Ser	Lys	Tyr	Met	Arg	Ala	Met	Tyr	Pro	Thr
Lys															
				165					170					175	
	Thr	Phe	Pro	Asn	His	Tyr	Thr	Ile	Val	Thr	Gly	Leu	Tyr	Pro	Glu
Ser															
			180					185					190		
	His	Gly	Ile	Ile	Asp	Asn	Asn	Met	Tyr	Asp	Val	Asn	Leu	Asn	Lys
Asn															
		195						200					205		
	Phe	Ser	Leu	Ser	Ser	Lys	Glu	Gln	Asn	Asn	Pro	Ala	Trp	Trp	His
Gly															
		210					215					220			
	Gln	Pro	Met	Trp	Leu	Thr	Ala	Met	Tyr	Gln	Gly	Leu	Lys	Ala	Ala
Thr															
	225					230					235				
240															
	Tyr	Phe	Trp	Pro	Gly	Ser	Glu	Val	Ala	Ile	Asn	Gly	Ser	Phe	Pro
Ser															
				245					250					255	
	Ile	Tyr	Met	Pro	Tyr	Asn	Gly	Ser	Val	Pro	Phe	Glu	Glu	Arg	Ile
Ser															
			260					265					270		
	Thr	Leu	Leu	Lys	Trp	Leu	Asp	Leu	Pro	Lys	Ala	Glu	Arg	Pro	Arg
Phe															
		275						280					285		
	Tyr	Thr	Met	Tyr	Phe	Glu	Glu	Pro	Asp	Ser	Ser	Gly	His	Ala	Gly
Gly															
		290					295					300			
	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
His	Pro	Lys	Arg	Leu	His	Tyr	Ala	Lys	Asn	Val	Arg	Ile	Asp	Lys	Val
			420						425						430
Thr	Leu	Phe	Val	Asp	Gln	Gln	Trp	Leu	Ala	Val	Arg	Ser	Lys	Ser	Asn
		435						440					445		
Met	Asn	Cys	Gly	Gly	Gly	Asn	His	Gly	Tyr	Asn	Asn	Glu	Phe	Arg	Ser
	450						455					460			
Glu	Glu	Ala	Ile	Phe	Leu	Ala	His	Gly	Pro	Ser	Phe	Lys	Glu	Lys	Thr
480	465					470					475				
Leu	Val	Glu	Pro	Phe	Glu	Asn	Ile	Glu	Val	Tyr	Asn	Leu	Met	Cys	Asp
				485						490					495
Asn	Leu	Arg	Ile	Gln	Pro	Ala	Pro	Asn	Asn	Gly	Thr	His	Gly	Ser	Leu
			500						505					510	
Val	His	Leu	Leu	Lys	Val	Pro	Phe	Tyr	Glu	Pro	Ser	His	Ala	Glu	Glu
		515						520					525		
Ser	Ser	Lys	Phe	Ser	Val	Cys	Gly	Phe	Ala	Asn	Pro	Leu	Pro	Thr	Glu
	530						535					540			
Gln	Leu	Asp	Cys	Phe	Cys	Pro	His	Leu	Gln	Asn	Ser	Thr	Gln	Leu	Glu
560	545					550						555			
Val	Val	Asn	Gln	Met	Leu	Asn	Leu	Thr	Gln	Glu	Glu	Ile	Thr	Ala	Thr
				565						570					575
Val	Lys	Val	Asn	Leu	Pro	Phe	Gly	Arg	Pro	Arg	Val	Leu	Gln	Lys	Asn
			580						585					590	
Lys	Asp	His	Cys	Leu	Leu	Tyr	His	Arg	Glu	Tyr	Val	Ser	Gly	Phe	Gly
		595						600					605		
Gly	Ala	Met	Arg	Met	Pro	Met	Trp	Ser	Ser	Tyr	Thr	Val	Pro	Gln	Leu
	610						615					620			
Asp	Asp	Thr	Ser	Pro	Leu	Pro	Pro	Thr	Val	Pro	Asp	Cys	Leu	Arg	Ala
640	625					630						635			
Ala	Val	Arg	Val	Pro	Pro	Ser	Glu	Ser	Gln	Lys	Cys	Ser	Phe	Tyr	Leu
				645					650					655	
Arg	Asp	Lys	Asn	Ile	Thr	His	Gly	Phe	Leu	Tyr	Pro	Pro	Ala	Ser	Asn
			660						665					670	

Pro	Thr	Ser	Asp	Ser	Gln	Tyr	Asp	Ala	Leu	Ile	Thr	Ser	Asn	Leu	Val
			675					680					685		
Leu	Met	Tyr	Glu	Glu	Phe	Arg	Lys	Met	Trp	Asp	Tyr	Phe	His	Ser	Val
		690					695					700			
Gly	Leu	Ile	Lys	His	Ala	Thr	Glu	Arg	Asn	Gly	Val	Asn	Val	Val	Ser
720	705					710					715				
Glu	Pro	Ile	Phe	Asp	Tyr	Asn	Tyr	Asp	Gly	His	Phe	Asp	Ala	Pro	Asp
				725						730					735
Tyr	Ile	Thr	Lys	His	Leu	Ala	Asn	Thr	Asp	Val	Pro	Ile	Pro	Thr	His
			740					745					750		
Asn	Phe	Val	Val	Leu	Thr	Ser	Cys	Lys	Asn	Lys	Ser	His	Thr	Pro	Glu
		755					760					765			
Pro	Cys	Pro	Gly	Trp	Leu	Asp	Val	Leu	Pro	Phe	Ile	Ile	Pro	His	Arg
		770				775					780				
Val	Thr	Asn	Val	Glu	Ser	Cys	Pro	Glu	Gly	Lys	Pro	Glu	Ala	Leu	Trp
800	785					790					795				
Leu	Glu	Glu	Arg	Phe	Thr	Ala	His	Ile	Ala	Arg	Val	Arg	Asp	Val	Glu
				805					810					815	
Glu	Leu	Thr	Gly	Leu	Asp	Phe	Tyr	Gln	Asp	Lys	Val	Gln	Pro	Val	Ser
			820					825				830			
Gly	Ile	Leu	Gln	Leu	Lys	Thr	Tyr	Leu	Pro	Thr	Phe	Glu	<u>Thr</u>	<u>Thr</u>	<u>Ile</u>
		835					840				845				
Trp	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Met	Lys
		850				855					860				
Arg	Val	Thr	Phe	Leu	Leu	Leu	Leu	Phe	Val	Ser	Gly	Ser	Ala	Phe	Ser
880	865					870					875				
Tyr	Gly	Val	Phe	Arg	Arg	Glu	Ala	His	Lys	Ser	Glu	Ile	Ala	His	Arg
			885					890					895		
Phe	Asn	Asp	Leu	Gly	Glu	Gln	His	Phe	Lys	Gly	Leu	Val	Leu	Ile	Ala
			900					905					910		
Val	Ser	Gln	Tyr	Leu	Gln	Lys	Cys	Ser	Tyr	Asp	Glu	His	Ala	Lys	Leu
		915						920					925		
Ala	Gln	Glu	Val	Thr	Asp	Phe	Ala	Lys	Thr	Cys	Val	Ala	Asp	Glu	Ser
		930						935					940		
Cys	Ala	Asn	Cys	Asp	Lys	Ser	Leu	His	Thr	Leu	Phe	Gly	Asp	Lys	Leu

	945						950						955					
960	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							
Pro	Leu	Val	Glu	Glu	Pro	Lys	Asn	Leu	Val	Lys	Thr	Asn	Cys	Asp				
	1265					1270					1275							
Leu	Tyr	Glu	Lys	Leu	Gly	Glu	Tyr	Gly	Phe	Gln	Asn	Ala	Ile	Leu				
	1280					1285					1290							
Val	Arg	Tyr</																

Singly underlined: signal peptide sequence; double-underlined: beginning and end of NPP3; ** = cleavage position at the signal peptide sequence; bold residues indicate albumin sequence

[illegible]

65

	Cys	Lys	Asp	Lys	Gly	Asp	Cys	Cys	Ile	Asn	Tyr	Ser	Ser	Val	Cys
Gln															
				100					105					110	
	Gly	Glu	Lys	Ser	Trp	Val	Glu	Glu	Pro	Cys	Glu	Ser	Ile	Asn	Glu
Pro															
			115					120					125		
	Gln	Cys	Pro	Ala	Gly	Phe	Glu	Thr	Pro	Pro	Thr	Leu	Leu	Phe	Ser
Leu															
		130					135					140			
	Asp	Gly	Phe	Arg	Ala	Glu	Tyr	Leu	His	Thr	Trp	Gly	Gly	Leu	Leu
Pro															
	145					150					155				
160															
	Val	Ile	Ser	Lys	Leu	Lys	Lys	Cys	Gly	Thr	Tyr	Thr	Lys	Asn	Met
Arg															
				165					170					175	
	Pro	Val	Tyr	Pro	Thr	Lys	Thr	Phe	Pro	Asn	His	Tyr	Ser	Ile	Val
Thr															
			180					185						190	
	Gly	Leu	Tyr	Pro	Glu	Ser	His	Gly	Ile	Ile	Asp	Asn	Lys	Met	Tyr
Asp															
		195						200					205		
	Pro	Lys	Met	Asn	Ala	Ser	Phe	Ser	Leu	Lys	Ser	Lys	Glu	Lys	Phe
Asn															
		210					215					220			
	Pro	Glu	Trp	Tyr	Lys	Gly	Glu	Pro	Ile	Trp	Val	Thr	Ala	Lys	Tyr
Gln															
	225					230					235				
240															
	Gly	Leu	Lys	Ser	Gly	Thr	Phe	Phe	Trp	Pro	Gly	Ser	Asp	Val	Glu
Ile															
			245					250						255	
	Asn	Gly	Ile	Phe	Pro	Asp	Ile	Tyr	Lys	Met	Tyr	Asn	Gly	Ser	Val
Pro															
			260					265						270	
	Phe	Glu	Glu	Arg	Ile	Leu	Ala	Val	Leu	Gln	Trp	Leu	Gln	Leu	Pro
Lys															
		275					280						285		
	Asp	Glu	Arg	Pro	His	Phe	Tyr	Thr	Leu	Tyr	Leu	Glu	Glu	Pro	Asp
Ser															
		290					295					300			
	Ser	Gly	His	Ser	Tyr	Gly	Pro	Val	Ser	Ser	Glu	Val	Ile	Lys	Ala
Leu															
	305					310					315				
320															
	Gln	Arg	Val	Asp	Gly	Met	Val	Gly	Met	Leu	Met	Asp	Gly	Leu	Lys
Glu															
			325					330						335	
	Leu	Asn	Leu	His	Arg	Cys	Leu	Asn	Leu	Ile	Leu	Ile	Ser	Asp	His
Gly															
			340					345					350		
	Met	Glu	Gln	Gly	Ser	Cys	Lys	Lys	Tyr	Ile	Tyr	Leu	Asn	Lys	Tyr
Leu															
		355					360					365			
	Gly	Asp	Val	Lys	Asn	Ile	Lys	Val	Ile	Tyr	Gly	Pro	Ala	Ala	Arg
Leu															

	370		375		380														
	Arg	Pro	Ser	Asp	Val	Pro	Asp	Lys	Tyr	Tyr	Ser	Phe	Asn	Tyr	Glu				
Gly																			
	385					390					395								
400																			
	Tle	Ala	Arg	Asn	Leu	Ser	Cys	Arg	Glu	Pro	Asn	Gln	His	Phe	Tys				
Pro																			
				405					410						415				
	Tyr	Leu	Lys	His	Phe	Leu	Pro	Lys	Arg	Leu	His	Phe	Ala	Lys	Ser				
Asp																			
			420					425						430					
	Arg	Ile	Glu	Pro	Leu	Thr	Phe	Tyr	Leu	Asp	Pro	Gln	Trp	Gln	Leu				
Ala																			
		435					440					445							
	Leu	Asn	Pro	Ser	Glu	Arg	Lys	Tyr	Cys	Gly	Ser	Gly	Phe	His	Gly				
Ser																			
	450					455					460								
	Asp	Asn	Val	Phe	Ser	Asn	Met	Gln	Ala	Leu	Phe	Val	Gly	Tyr	Gly				
Pro																			
	465					470					475								
480																			
	Gly	Phe	Lys	His	Gly	Ile	Glu	Ala	Asp	Thr	Phe	Glu	Asn	Ile	Glu				
Val																			
			485					490						495					
	Tyr	Asn	Leu	Met	Cys	Asp	Leu	Leu	Asn	Leu	Thr	Pro	Ala	Pro	Asn				
Asn																			
		500					505					510							
	Gly	Thr	His	Gly	Ser	Leu	Asn	His	Leu	Leu	Lys	Asn	Pro	Val	Tyr				
Thr																			
		515					520					525							
	Pro	Lys	His	Pro	Lys	Glu	Val	His	Pro	Leu	Val	Gln	Cys	Pro	Phe				
Thr																			
	530						535					540							
	Arg	Asn	Pro	Arg	Asp	Asn	Leu	Gly	Cys	Ser	Cys	Asn	Pro	Ser	Ile				
Leu																			
	545					550					555								
560																			
	Pro	Ile	Glu	Asp	Phe	Gln	Thr	Gln	Phe	Asn	Leu	Thr	Val	Ala	Glu				
Glu																			
			565					570						575					
	Lys	Ile	Ile	Lys	His	Glu	Thr	Leu	Pro	Tyr	Gly	Arg	Pro	Arg	Val				
Leu																			
		580						585					590						
	Gln	Lys	Glu	Asn	Thr	Ile	Cys	Leu	Leu	Ser	Gln	His	Gln	Phe	Met				
Ser																			
		595						600				605							
	Gly	Tyr	Ser	Gln	Asp	Ile	Leu	Met	Pro	Leu	Trp	Thr	Ser	Tyr	Thr				
Val																			
	610						615					620							
	Asp	Arg	Asn	Asp	Ser	Phe	Ser	Thr	Glu	Asp	Phe	Ser	Asn	Cys	Leu				
Tyr																			
	625					630					635								
640																			
	Gln	Asp	Phe	Arg	Ile	Pro	Leu	Ser	Pro	Val	His	Lys	Cys	Ser	Phe				
Tyr																			
					645						650				655				

Lys Asn Asn Thr Lys Val Ser Tyr Gly Phe Leu Ser Pro Pro Gln
 Leu
 660 665 670
 Asn Lys Asn Ser Ser Gly Ile Tyr Ser Glu Ala Leu Leu Thr Thr
 Asn
 675 680 685
 Ile Val Pro Met Tyr Gln Ser Phe Gln Val Ile Trp Arg Tyr Phe
 His
 690 695 700
 Asp Thr Leu Leu Arg Lys Tyr Ala Glu Glu Arg Asn Gly Val Asn
 Val
 705 710 715
 720
 Val Ser Gly Pro Val Phe Asp Phe Asp Tyr Asp Gly Arg Cys Asp
 Ser
 725 730 735
 Leu Glu Asn Leu Arg Gln Lys Arg Arg Val Ile Arg Asn Gln Glu
 Ile
 740 745 750
 Leu Ile Pro Thr His Phe Phe Ile Val Leu Thr Ser Cys Lys Asp
 Thr
 755 760 765
 Ser Gln Thr Pro Leu His Cys Glu Asn Leu Asp Thr Leu Ala Phe
 Ile
 770 775 780
 Leu Pro His Arg Thr Asp Asn Ser Glu Ser Cys Val His Gly Lys
 His
 785 790 795
 800
 Asp Ser Ser Trp Val Glu Glu Leu Leu Met Leu His Arg Ala Arg
 Ile
 805 810 815
 Thr Asp Val Glu His Ile Thr Gly Leu Ser Phe Tyr Gln Gln Arg
 Lys
 820 825 830
 Glu Pro Val Ser Asp Ile Leu Lys Leu Lys Thr His Leu Pro Thr
 Phe
 835 840 845
 Ser Gln Glu Asp **Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala**
 Pro
 850 855 860
 Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
 Lys
 865 870 875
 880
 Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
 Val
 885 890 895
 Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
 Asp
 900 905 910
 Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
 Tyr
 915 920 925

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Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
Asp      930                      935                      940
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
Leu      945                      950                      955
960
Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
Arg
      965                      970                      975
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr
Lys
      980                      985                      990
Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
Asp
      995                      1000                      1005
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
      1010                      1015                      1020
Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
      1025                      1030                      1035
Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
      1040                      1045                      1050
Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
      1055                      1060                      1065
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
      1070                      1075

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Singly underlined: signal peptide sequence; double-underlined: beginning and end of NPP3; ** = cleavage position at the signal peptide sequence; bold residues indicate Fc sequence

SEQ. ID NO: 18 - ENPP71-Fc Amino Acid Sequence

```

Met Arg Gly Pro Ala Val Leu Leu Thr Val Ala Leu Ala Thr Leu
Leu
1           5           10           15
Ala Pro Gly Ala Gly Ala**Gly Leu Lys Pro Ser Cys Ala Lys Glu
Val
      20           25           30
Lys Ser Cys Lys Gly Arg Cys Phe Glu Arg Thr Phe Gly Asn Cys
Arg
      35           40           45
Cys Asp Ala Ala Cys Val Glu Leu Gly Asn Cys Cys Leu Asp Tyr
Gln
      50           55           60
Glu Thr Cys Ile Glu Pro Glu His Ile Trp Thr Cys Asn Lys Phe
Arg
      65           70           75           80
Cys Gly Glu Lys Arg Leu Thr Arg Ser Leu Cys Ala Cys Ser Asp
Asp
      85           90           95

```

	Cys	Lys	Asp	Lys	Gly	Asp	Cys	Cys	Ile	Asn	Tyr	Ser	Ser	Val	Cys
Gln															
				100					105					110	
	Gly	Glu	Lys	Ser	Trp	Val	Glu	Glu	Pro	Cys	Glu	Ser	Ile	Asn	Glu
Pro															
			115						120					125	
	Gln	Cys	Pro	Ala	Gly	Phe	Glu	Thr	Pro	Pro	Thr	Leu	Leu	Phe	Ser
Leu															
		130					135					140			
	Asp	Gly	Phe	Arg	Ala	Glu	Tyr	Leu	His	Thr	Trp	Gly	Gly	Leu	Leu
Pro															
	145					150					155				
160															
	Val	Ile	Ser	Lys	Leu	Lys	Lys	Cys	Gly	Thr	Tyr	Thr	Lys	Asn	Met
Arg															
				165					170					175	
	Pro	Val	Tyr	Pro	Thr	Lys	Thr	Phe	Pro	Asn	His	Tyr	Ser	Ile	Val
Thr															
			180						185					190	
	Gly	Leu	Tyr	Pro	Glu	Ser	His	Gly	Ile	Ile	Asp	Asn	Lys	Met	Tyr
Asp															
		195						200					205		
	Pro	Lys	Met	Asn	Ala	Ser	Phe	Ser	Leu	Lys	Ser	Lys	Glu	Lys	Phe
Asn															
		210					215					220			
	Pro	Glu	Trp	Tyr	Lys	Gly	Glu	Pro	Ile	Trp	Val	Thr	Ala	Lys	Tyr
Gln															
	225					230					235				
240															
	Gly	Leu	Lys	Ser	Gly	Thr	Phe	Phe	Trp	Pro	Gly	Ser	Asp	Val	Glu
Ile															
			245						250					255	
	Asn	Gly	Ile	Phe	Pro	Asp	Ile	Tyr	Lys	Met	Tyr	Asn	Gly	Ser	Val
Pro															
			260						265					270	
	Phe	Glu	Glu	Arg	Ile	Leu	Ala	Val	Leu	Gln	Trp	Leu	Gln	Leu	Pro
Lys															
		275						280					285		
	Asp	Glu	Arg	Pro	His	Phe	Tyr	Thr	Leu	Tyr	Leu	Glu	Glu	Pro	Asp
Ser															
		290					295				300				
	Ser	Gly	His	Ser	Tyr	Gly	Pro	Val	Ser	Ser	Glu	Val	Ile	Lys	Ala
Leu															
	305					310					315				
320															
	Gln	Arg	Val	Asp	Gly	Met	Val	Gly	Met	Leu	Met	Asp	Gly	Leu	Lys
Glu															
			325						330					335	
	Leu	Asn	Leu	His	Arg	Cys	Leu	Asn	Leu	Ile	Leu	Ile	Ser	Asp	His
Gly															
			340						345				350		
	Met	Glu	Gln	Gly	Ser	Cys	Lys	Lys	Tyr	Ile	Tyr	Leu	Asn	Lys	Tyr
Leu															
		355						360				365			
	Gly	Asp	Val	Lys	Asn	Ile	Lys	Val	Ile	Tyr	Gly	Pro	Ala	Ala	Arg
Leu															

71

Lys Asn Asn Thr Lys Val Ser Tyr Gly Phe Leu Ser Pro Pro Gln
 Leu
 660 665 670
 Asn Lys Asn Ser Ser Gly Ile Tyr Ser Glu Ala Leu Leu Thr Thr
 Asn
 675 680 685
 Ile Val Pro Met Tyr Gln Ser Phe Gln Val Ile Trp Arg Tyr Phe
 His
 690 695 700
 Asp Thr Leu Leu Arg Lys Tyr Ala Glu Glu Arg Asn Gly Val Asn
 Val
 705 710 715
 720
 Val Ser Gly Pro Val Phe Asp Phe Asp Tyr Asp Gly Arg Cys Asp
 Ser
 725 730 735
 Leu Glu Asn Leu Arg Gln Lys Arg Arg Val Ile Arg Asn Gln Glu
 Ile
 740 745 750
 Leu Ile Pro Thr His Phe Phe Ile Val Leu Thr Ser Cys Lys Asp
 Thr
 755 760 765
 Ser Gln Thr Pro Leu His Cys Glu Asn Leu Asp Thr Leu Ala Phe
 Ile
 770 775 780
 Leu Pro His Arg Thr Asp Asn Ser Glu Ser Cys Val His Gly Lys
 His
 785 790 795
 800
 Asp Ser Ser Trp Val Glu Glu Leu Leu Met Leu His Arg Ala Arg
 Ile
 805 810 815
 Thr Asp Val Glu His Ile Thr Gly Leu Ser Phe Tyr Gln Gln Arg
 Lys
 820 825 830
 Glu Pro Val Ser Asp Ile Leu Lys Leu Lys Thr His Leu Pro Thr
 Phe
 835 840 845
 Ser Gln Glu Asp Leu Ile Asn **Asp Lys Thr His Thr Cys Pro Pro**
Cys
 850 855 860
Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
Pro
 865 870 875
 880
Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
Cys
 885 890 895
Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
Trp
 900 905 910
Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
Glu
 915 920 925
Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
Leu

```

          930          935          940
Asn  His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
945          950          955
960  Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
Gly
          965          970          975
Glu  Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu
          980          985          990
Tyr  Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
          995          1000          1005
Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
1010          1015          1020
Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
1025          1030          1035
Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
1040          1045          1050
Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
1055          1060          1065
Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
1070          1075          1080

```

Singly underlined: signal peptide sequence; double-underlined: beginning and end of NPP1; ** = cleavage position at the signal peptide sequence; bold residues indicate Fc sequence

SEQ. ID NO: 19 - ENPP71 (lacking NPP1 N-Terminus GLK) Amino Acid Sequence:

```

Met Arg Gly Pro Ala Val Leu Leu Thr Val Ala Leu Ala Thr Leu
Leu
1          5          10          15
Ala Pro Gly Ala Gly Ala**Pro Ser Cys Ala Lys Glu Val Lys Ser
Cys
          20          25          30
Lys Gly Arg Cys Phe Glu Arg Thr Phe Gly Asn Cys Arg Cys Asp
Ala
          35          40          45
Ala Cys Val Glu Leu Gly Asn Cys Cys Leu Asp Tyr Gln Glu Thr
Cys
          50          55          60
Ile Glu Pro Glu His Ile Trp Thr Cys Asn Lys Phe Arg Cys Gly
Glu
65          70          75          80
Lys Arg Leu Thr Arg Ser Leu Cys Ala Cys Ser Asp Asp Cys Lys
Asp
          85          90          95
Lys Gly Asp Cys Cys Ile Asn Tyr Ser Ser Val Cys Gln Gly Glu
Lys
          100          105          110

```

	Ser	Trp	Val	Glu	Glu	Pro	Cys	Glu	Ser	Ile	Asn	Glu	Pro	Gln	Cys
Pro															
			115					120					125		
	Ala	Gly	Phe	Glu	Thr	Pro	Pro	Thr	Leu	Leu	Phe	Ser	Leu	Asp	Gly
Phe															
		130					135						140		
	Arg	Ala	Glu	Tyr	Leu	His	Thr	Trp	Gly	Gly	Leu	Leu	Pro	Val	Ile
Ser															
	145					150						155			
160															
	Lys	Leu	Lys	Lys	Cys	Gly	Thr	Tyr	Thr	Lys	Asn	Met	Arg	Pro	Val
Tyr															
				165					170					175	
	Pro	Thr	Lys	Thr	Phe	Pro	Asn	His	Tyr	Ser	Ile	Val	Thr	Gly	Leu
Tyr															
			180					185					190		
	Pro	Glu	Ser	His	Gly	Ile	Ile	Asp	Asn	Lys	Met	Tyr	Asp	Pro	Lys
Met															
		195					200					205			
	Asn	Ala	Ser	Phe	Ser	Leu	Lys	Ser	Lys	Glu	Lys	Phe	Asn	Pro	Glu
Trp															
		210					215					220			
	Tyr	Lys	Gly	Glu	Pro	Ile	Trp	Val	Thr	Ala	Lys	Tyr	Gln	Gly	Leu
Lys															
	225					230						235			
240															
	Ser	Gly	Thr	Phe	Phe	Trp	Pro	Gly	Ser	Asp	Val	Glu	Ile	Asn	Gly
Ile															
				245					250					255	
	Phe	Pro	Asp	Ile	Tyr	Lys	Met	Tyr	Asn	Gly	Ser	Val	Pro	Phe	Glu
Glu															
			260					265					270		
	Arg	Ile	Leu	Ala	Val	Leu	Gln	Trp	Leu	Gln	Leu	Pro	Lys	Asp	Glu
Arg															
		275						280					285		
	Pro	His	Phe	Tyr	Thr	Leu	Tyr	Leu	Glu	Glu	Pro	Asp	Ser	Ser	Gly
His															
		290					295					300			
	Ser	Tyr	Gly	Pro	Val	Ser	Ser	Glu	Val	Ile	Lys	Ala	Leu	Gln	Arg
Val															
	305					310						315			
320															
	Asp	Gly	Met	Val	Gly	Met	Leu	Met	Asp	Gly	Leu	Lys	Glu	Leu	Asn
Leu															
				325					330					335	
	His	Arg	Cys	Leu	Asn	Leu	Ile	Leu	Ile	Ser	Asp	His	Gly	Met	Glu
Gln															
			340					345					350		
	Gly	Ser	Cys	Lys	Lys	Tyr	Ile	Tyr	Leu	Asn	Lys	Tyr	Leu	Gly	Asp
Val															
		355						360					365		
	Lys	Asn	Ile	Lys	Val	Ile	Tyr	Gly	Pro	Ala	Ala	Arg	Leu	Arg	Pro
Ser															
		370					375					380			
	Asp	Val	Pro	Asp	Lys	Tyr	Tyr	Ser	Phe	Asn	Tyr	Glu	Gly	Ile	Ala
Arg															

385 390 395
 400 Asn Leu Ser Cys Arg Glu Pro Asn Gln His Phe Lys Pro Tyr Leu
 Lys
 405 410 415
 Glu His Phe Leu Pro Lys Arg Leu His Phe Ala Lys Ser Asp Arg Ile
 420 425 430
 Pro Leu Thr Phe Tyr Leu Asp Pro Gln Trp Gln Leu Ala Leu Asn
 Pro
 435 440 445
 Val Ser Glu Arg Lys Tyr Cys Gly Ser Gly Phe His Gly Ser Asp Asn
 450 455 460
 Lys Phe Ser Asn Met Gln Ala Leu Phe Val Gly Tyr Gly Pro Gly Phe
 465 470 475
 480 His Gly Ile Glu Ala Asp Thr Phe Glu Asn Ile Glu Val Tyr Asn
 Leu
 485 490 495
 His Met Cys Asp Leu Leu Asn Leu Thr Pro Ala Pro Asn Asn Gly Thr
 500 505 510
 His Gly Ser Leu Asn His Leu Leu Lys Asn Pro Val Tyr Thr Pro Lys
 515 520 525
 Pro Lys Glu Val His Pro Leu Val Gln Cys Pro Phe Thr Arg Asn
 Pro
 530 535 540
 Glu Arg Asp Asn Leu Gly Cys Ser Cys Asn Pro Ser Ile Leu Pro Ile
 545 550 555
 560 Asp Phe Gln Thr Gln Phe Asn Leu Thr Val Ala Glu Glu Lys Ile
 Ile
 565 570 575
 Glu Lys His Glu Thr Leu Pro Tyr Gly Arg Pro Arg Val Leu Gln Lys
 580 585 590
 Ser Asn Thr Ile Cys Leu Leu Ser Gln His Gln Phe Met Ser Gly Tyr
 595 600 605
 Asn Gln Asp Ile Leu Met Pro Leu Trp Thr Ser Tyr Thr Val Asp Arg
 610 615 620
 Phe Asp Ser Phe Ser Thr Glu Asp Phe Ser Asn Cys Leu Tyr Gln Asp
 625 630 635
 640 Arg Ile Pro Leu Ser Pro Val His Lys Cys Ser Phe Tyr Lys Asn
 Asn
 645 650 655
 Asn Thr Lys Val Ser Tyr Gly Phe Leu Ser Pro Pro Gln Leu Asn Lys
 660 665 670

Ser Ser Gly Ile Tyr Ser Glu Ala Leu Leu Thr Thr Asn Ile Val
 Pro
 675 680 685
 Met Tyr Gln Ser Phe Gln Val Ile Trp Arg Tyr Phe His Asp Thr
 Leu
 690 695 700
 Leu Arg Lys Tyr Ala Glu Glu Arg Asn Gly Val Asn Val Val Ser
 Gly
 705 710 715
 720
 Pro Val Phe Asp Phe Asp Tyr Asp Gly Arg Cys Asp Ser Leu Glu
 Asn
 725 730 735
 Leu Arg Gln Lys Arg Arg Val Ile Arg Asn Gln Glu Ile Leu Ile
 Pro
 740 745 750
 Thr His Phe Phe Ile Val Leu Thr Ser Cys Lys Asp Thr Ser Gln
 Thr
 755 760 765
 Pro Leu His Cys Glu Asn Leu Asp Thr Leu Ala Phe Ile Leu Pro
 His
 770 775 780
 Arg Thr Asp Asn Ser Glu Ser Cys Val His Gly Lys His Asp Ser
 Ser
 785 790 795
 800
 Trp Val Glu Glu Leu Leu Met Leu His Arg Ala Arg Ile Thr Asp
 Val
 805 810 815
 Glu His Ile Thr Gly Leu Ser Phe Tyr Gln Gln Arg Lys Glu Pro
 Val
 820 825 830
 Ser Asp Ile Leu Lys Leu Lys Thr His Leu Pro Thr Phe Ser Gln
Glu
 835 840 845
Asp

Singly underlined: signal peptide sequence; double-underlined:
 beginning and end of NPP3; ** = cleavage position at the signal
 peptide sequence

SEQ. ID NO: 20 -ENPP71 (lacking NPP1 N-Terminus GLK) - Fc
Amino Acid Sequence:

Met Arg Gly Pro Ala Val Leu Leu Thr Val Ala Leu Ala Thr Leu
Leu
 1 5 10 15
Ala Pro Gly Ala Gly Ala**Pro Ser Cys Ala Lys Glu Val Lys Ser
 Cys
 20 25 30
 Lys Gly Arg Cys Phe Glu Arg Thr Phe Gly Asn Cys Arg Cys Asp
 Ala
 35 40 45

	Ala	Cys	Val	Glu	Leu	Gly	Asn	Cys	Cys	Leu	Asp	Tyr	Gln	Glu	Thr
Cys		50					55					60			
	Ile	Glu	Pro	Glu	His	Ile	Trp	Thr	Cys	Asn	Lys	Phe	Arg	Cys	Gly
Glu		65				70					75				80
	Lys	Arg	Leu	Thr	Arg	Ser	Leu	Cys	Ala	Cys	Ser	Asp	Asp	Cys	Lys
Asp					85					90					95
	Lys	Gly	Asp	Cys	Cys	Ile	Asn	Tyr	Ser	Ser	Val	Cys	Gln	Gly	Glu
Lys				100					105				110		
	Ser	Trp	Val	Glu	Glu	Pro	Cys	Glu	Ser	Ile	Asn	Glu	Pro	Gln	Cys
Pro			115					120					125		
	Ala	Gly	Phe	Glu	Thr	Pro	Pro	Thr	Leu	Leu	Phe	Ser	Leu	Asp	Gly
Phe		130					135					140			
	Arg	Ala	Glu	Tyr	Leu	His	Thr	Trp	Gly	Gly	Leu	Leu	Pro	Val	Ile
Ser															
160	145					150					155				
	Lys	Leu	Lys	Lys	Cys	Gly	Thr	Tyr	Thr	Lys	Asn	Met	Arg	Pro	Val
Tyr					165					170				175	
	Pro	Thr	Lys	Thr	Phe	Pro	Asn	His	Tyr	Ser	Ile	Val	Thr	Gly	Leu
Tyr					180					185				190	
	Pro	Glu	Ser	His	Gly	Ile	Ile	Asp	Asn	Lys	Met	Tyr	Asp	Pro	Lys
Met															
	Asn	Ala	Ser	Phe	Ser	Leu	Lys	Ser	Lys	Glu	Lys	Phe	Asn	Pro	Glu
Trp															
	Tyr	Lys	Gly	Glu	Pro	Ile	Trp	Val	Thr	Ala	Lys	Tyr	Gln	Gly	Leu
Lys		210					215					220			
	225					230						235			
240															
	Ser	Gly	Thr	Phe	Phe	Trp	Pro	Gly	Ser	Asp	Val	Glu	Ile	Asn	Gly
Ile															
				245						250				255	
	Phe	Pro	Asp	Ile	Tyr	Lys	Met	Tyr	Asn	Gly	Ser	Val	Pro	Phe	Glu
Glu															
			260						265					270	
	Arg	Ile	Leu	Ala	Val	Leu	Gln	Trp	Leu	Gln	Leu	Pro	Lys	Asp	Glu
Arg															
		275						280					285		
	Pro	His	Phe	Tyr	Thr	Leu	Tyr	Leu	Glu	Glu	Pro	Asp	Ser	Ser	Gly
His															
		290					295					300			
	Ser	Tyr	Gly	Pro	Val	Ser	Ser	Glu	Val	Ile	Lys	Ala	Leu	Gln	Arg
Val															
	305					310						315			
320															
	Asp	Gly	Met	Val	Gly	Met	Leu	Met	Asp	Gly	Leu	Lys	Glu	Leu	Asn
Leu															

					325					330				335	
Gln	His	Arg	Cys	Leu	Asn	Leu	Ile	Leu	Ile	Ser	Asp	His	Gly	Met	Glu
					340					345				350	
Val	Gly	Ser	Cys	Lys	Lys	Tyr	Ile	Tyr	Leu	Asn	Lys	Tyr	Leu	Gly	Asp
					355					360				365	
Ser	Lys	Asn	Ile	Lys	Val	Ile	Tyr	Gly	Pro	Ala	Ala	Arg	Leu	Arg	Pro
					370					375				380	
Arg	Asp	Val	Pro	Asp	Lys	Tyr	Tyr	Ser	Phe	Asn	Tyr	Glu	Gly	Ile	Ala
400	385					390					395				
Lys	Asn	Leu	Ser	Cys	Arg	Glu	Pro	Asn	Gln	His	Phe	Lys	Pro	Tyr	Leu
					405					410				415	
Glu	His	Phe	Leu	Pro	Lys	Arg	Leu	His	Phe	Ala	Lys	Ser	Asp	Arg	Ile
					420					425				430	
Pro	Pro	Leu	Thr	Phe	Tyr	Leu	Asp	Pro	Gln	Trp	Gln	Leu	Ala	Leu	Asn
					435					440				445	
Val	Ser	Glu	Arg	Lys	Tyr	Cys	Gly	Ser	Gly	Phe	His	Gly	Ser	Asp	Asn
					450					455				460	
Lys	Phe	Ser	Asn	Met	Gln	Ala	Leu	Phe	Val	Gly	Tyr	Gly	Pro	Gly	Phe
480	465					470					475				
Leu	His	Gly	Ile	Glu	Ala	Asp	Thr	Phe	Glu	Asn	Ile	Glu	Val	Tyr	Asn
					485					490				495	
His	Met	Cys	Asp	Leu	Leu	Asn	Leu	Thr	Pro	Ala	Pro	Asn	Asn	Gly	Thr
					500					505				510	
His	Gly	Ser	Leu	Asn	His	Leu	Leu	Lys	Asn	Pro	Val	Tyr	Thr	Pro	Lys
					515					520				525	
Pro	Pro	Lys	Glu	Val	His	Pro	Leu	Val	Gln	Cys	Pro	Phe	Thr	Arg	Asn
					530					535				540	
Glu	Arg	Asp	Asn	Leu	Gly	Cys	Ser	Cys	Asn	Pro	Ser	Ile	Leu	Pro	Ile
560	545					550					555				
Ile	Asp	Phe	Gln	Thr	Gln	Phe	Asn	Leu	Thr	Val	Ala	Glu	Glu	Lys	Ile
					565					570				575	
Glu	Lys	His	Glu	Thr	Leu	Pro	Tyr	Gly	Arg	Pro	Arg	Val	Leu	Gln	Lys
					580					585				590	
Ser	Asn	Thr	Ile	Cys	Leu	Leu	Ser	Gln	His	Gln	Phe	Met	Ser	Gly	Tyr
					595					600				605	

	Gln	Asp	Ile	Leu	Met	Pro	Leu	Trp	Thr	Ser	Tyr	Thr	Val	Asp	Arg
Asn															
		610					615					620			
	Asp	Ser	Phe	Ser	Thr	Glu	Asp	Phe	Ser	Asn	Cys	Leu	Tyr	Gln	Asp
Phe															
	625					630					635				
640															
	Arg	Ile	Pro	Leu	Ser	Pro	Val	His	Lys	Cys	Ser	Phe	Tyr	Lys	Asn
Asn															
				645					650					655	
	Thr	Lys	Val	Ser	Tyr	Gly	Phe	Leu	Ser	Pro	Pro	Gln	Leu	Asn	Lys
Asn															
			660					665					670		
	Ser	Ser	Gly	Ile	Tyr	Ser	Glu	Ala	Leu	Leu	Thr	Thr	Asn	Ile	Val
Pro															
		675					680					685			
	Met	Tyr	Gln	Ser	Phe	Gln	Val	Ile	Trp	Arg	Tyr	Phe	His	Asp	Thr
Leu															
		690				695					700				
	Leu	Arg	Lys	Tyr	Ala	Glu	Glu	Arg	Asn	Gly	Val	Asn	Val	Val	Ser
Gly															
	705					710					715				
720															
	Pro	Val	Phe	Asp	Phe	Asp	Tyr	Asp	Gly	Arg	Cys	Asp	Ser	Leu	Glu
Asn															
				725				730					735		
	Leu	Arg	Gln	Lys	Arg	Arg	Val	Ile	Arg	Asn	Gln	Glu	Ile	Leu	Ile
Pro															
			740					745				750			
	Thr	His	Phe	Phe	Ile	Val	Leu	Thr	Ser	Cys	Lys	Asp	Thr	Ser	Gln
Thr															
		755					760				765				
	Pro	Leu	His	Cys	Glu	Asn	Leu	Asp	Thr	Leu	Ala	Phe	Ile	Leu	Pro
His															
		770				775				780					
	Arg	Thr	Asp	Asn	Ser	Glu	Ser	Cys	Val	His	Gly	Lys	His	Asp	Ser
Ser															
	785					790				795					
800															
	Trp	Val	Glu	Glu	Leu	Leu	Met	Leu	His	Arg	Ala	Arg	Ile	Thr	Asp
Val															
			805					810					815		
	Glu	His	Ile	Thr	Gly	Leu	Ser	Phe	Tyr	Gln	Gln	Arg	Lys	Glu	Pro
Val															
			820					825				830			
	Ser	Asp	Ile	Leu	Lys	Leu	Lys	Thr	His	Leu	Pro	Thr	Phe	Ser	<u>Gln</u>
<u>Glu</u>															
		835					840				845				
	<u>Asp</u>	Leu	Ile	Asn	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala
Pro															
		850				855				860					
	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro
Lys															
	865					870				875					
880															

```

Val    Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
      885                               890                               895
Asp    Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
      900                               905                               910
Tyr    Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
      915                               920                               925
Asp    Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
      930                               935                               940
Leu    Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
      945                               950                               955
960    Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
Arg     Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr
      965                               970                               975
Lys    Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr
      980                               985                               990
Asp    Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
      995                               1000                              1005
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
      1010                              1015                              1020
Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
      1025                              1030                              1035
Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
      1040                              1045                              1050
Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
      1055                              1060                              1065
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
      1070                              1075

```

Singly underlined: signal peptide sequence; double-underlined: beginning and end of NPP1; ** = cleavage position at the signal peptide sequence; bold residues indicate Fc sequence

SEQ. ID NO: 21 - ENPP71 (lacking NPP1 N-Terminus GLK) - ALB
Amino Acid Sequence

```

Leu    Met Arg Gly Pro Ala Val Leu Leu Thr Val Ala Leu Ala Thr Leu
      1           5           10           15
Cys    Ala Pro Gly Ala Gly Ala**Pro Ser Cys Ala Lys Glu Val Lys Ser
      20           25           30
Ala    Lys Gly Arg Cys Phe Glu Arg Thr Phe Gly Asn Cys Arg Cys Asp
      35           40           45
Cys    Ala Cys Val Glu Leu Gly Asn Cys Cys Leu Asp Tyr Gln Glu Thr
      50           55           60

```

	Ile	Glu	Pro	Glu	His	Ile	Trp	Thr	Cys	Asn	Lys	Phe	Arg	Cys	Gly	
Glu	65					70					75					80
	Lys	Arg	Leu	Thr	Arg	Ser	Leu	Cys	Ala	Cys	Ser	Asp	Asp	Cys	Lys	
Asp					85					90					95	
	Lys	Gly	Asp	Cys	Cys	Ile	Asn	Tyr	Ser	Ser	Val	Cys	Gln	Gly	Glu	
Lys					100				105					110		
	Ser	Trp	Val	Glu	Glu	Pro	Cys	Glu	Ser	Ile	Asn	Glu	Pro	Gln	Cys	
Pro					115				120					125		
	Ala	Gly	Phe	Glu	Thr	Pro	Pro	Thr	Leu	Leu	Phe	Ser	Leu	Asp	Gly	
Phe					130				135					140		
	Arg	Ala	Glu	Tyr	Leu	His	Thr	Trp	Gly	Gly	Leu	Leu	Pro	Val	Ile	
Ser																
145						150					155					
160																
	Lys	Leu	Lys	Lys	Cys	Gly	Thr	Tyr	Thr	Lys	Asn	Met	Arg	Pro	Val	
Tyr																
					165					170					175	
	Pro	Thr	Lys	Thr	Phe	Pro	Asn	His	Tyr	Ser	Ile	Val	Thr	Gly	Leu	
Tyr																
					180					185					190	
	Pro	Glu	Ser	His	Gly	Ile	Ile	Asp	Asn	Lys	Met	Tyr	Asp	Pro	Lys	
Met																
					195				200					205		
	Asn	Ala	Ser	Phe	Ser	Leu	Lys	Ser	Lys	Glu	Lys	Phe	Asn	Pro	Glu	
Trp																
					210				215					220		
	Tyr	Lys	Gly	Glu	Pro	Ile	Trp	Val	Thr	Ala	Lys	Tyr	Gln	Gly	Leu	
Lys																
225																
240						230						235				
	Ser	Gly	Thr	Phe	Phe	Trp	Pro	Gly	Ser	Asp	Val	Glu	Ile	Asn	Gly	
Ile																
					245					250					255	
	Phe	Pro	Asp	Ile	Tyr	Lys	Met	Tyr	Asn	Gly	Ser	Val	Pro	Phe	Glu	
Glu																
					260					265					270	
	Arg	Ile	Leu	Ala	Val	Leu	Gln	Trp	Leu	Gln	Leu	Pro	Lys	Asp	Glu	
Arg																
					275					280				285		
	Pro	His	Phe	Tyr	Thr	Leu	Tyr	Leu	Glu	Glu	Pro	Asp	Ser	Ser	Gly	
His																
					290					295				300		
	Ser	Tyr	Gly	Pro	Val	Ser	Ser	Glu	Val	Ile	Lys	Ala	Leu	Gln	Arg	
Val																
305																
320						310						315				
	Asp	Gly	Met	Val	Gly	Met	Leu	Met	Asp	Gly	Leu	Lys	Glu	Leu	Asn	
Leu																
					325					330					335	
	His	Arg	Cys	Leu	Asn	Leu	Ile	Leu	Ile	Ser	Asp	His	Gly	Met	Glu	
Gln																

				340					345				350		
	Gly	Ser	Cys	Lys	Lys	Tyr	Ile	Tyr	Leu	Asn	Lys	Tyr	Leu	Gly	Asp
Val															
			355					360					365		
	Lys	Asn	Ile	Lys	Val	Ile	Tyr	Gly	Pro	Ala	Ala	Arg	Leu	Arg	Pro
Ser															
		370				375						380			
	Asp	Val	Pro	Asp	Lys	Tyr	Tyr	Ser	Phe	Asn	Tyr	Glu	Gly	Ile	Ala
Arg															
	385					390						395			
400															
	Asn	Leu	Ser	Cys	Arg	Glu	Pro	Asn	Gln	His	Phe	Lys	Pro	Tyr	Leu
Lys															
				405					410					415	
	His	Phe	Leu	Pro	Lys	Arg	Leu	His	Phe	Ala	Lys	Ser	Asp	Arg	Ile
Glu															
			420					425					430		
	Pro	Leu	Thr	Phe	Tyr	Leu	Asp	Pro	Gln	Trp	Gln	Leu	Ala	Leu	Asn
Pro															
		435					440					445			
	Ser	Glu	Arg	Lys	Tyr	Cys	Gly	Ser	Gly	Phe	His	Gly	Ser	Asp	Asn
Val															
		450				455					460				
	Phe	Ser	Asn	Met	Gln	Ala	Leu	Phe	Val	Gly	Tyr	Gly	Pro	Gly	Phe
Lys															
	465					470					475				
480															
	His	Gly	Ile	Glu	Ala	Asp	Thr	Phe	Glu	Asn	Ile	Glu	Val	Tyr	Asn
Leu															
			485				490						495		
	Met	Cys	Asp	Leu	Leu	Asn	Leu	Thr	Pro	Ala	Pro	Asn	Asn	Gly	Thr
His															
			500				505						510		
	Gly	Ser	Leu	Asn	His	Leu	Leu	Lys	Asn	Pro	Val	Tyr	Thr	Pro	Lys
His															
		515				520						525			
	Pro	Lys	Glu	Val	His	Pro	Leu	Val	Gln	Cys	Pro	Phe	Thr	Arg	Asn
Pro															
		530				535						540			
	Arg	Asp	Asn	Leu	Gly	Cys	Ser	Cys	Asn	Pro	Ser	Ile	Leu	Pro	Ile
Glu															
	545					550						555			
560															
	Asp	Phe	Gln	Thr	Gln	Phe	Asn	Leu	Thr	Val	Ala	Glu	Glu	Lys	Ile
Ile															
			565				570						575		
	Lys	His	Glu	Thr	Leu	Pro	Tyr	Gly	Arg	Pro	Arg	Val	Leu	Gln	Lys
Glu															
			580				585						590		
	Asn	Thr	Ile	Cys	Leu	Leu	Ser	Gln	His	Gln	Phe	Met	Ser	Gly	Tyr
Ser															
		595				600						605			
	Gln	Asp	Ile	Leu	Met	Pro	Leu	Trp	Thr	Ser	Tyr	Thr	Val	Asp	Arg
Asn															
		610				615						620			

	Asp	Ser	Phe	Ser	Thr	Glu	Asp	Phe	Ser	Asn	Cys	Leu	Tyr	Gln	Asp
Phe	625					630					635				
640	Arg	Ile	Pro	Leu	Ser	Pro	Val	His	Lys	Cys	Ser	Phe	Tyr	Lys	Asn
Asn					645					650					655
	Thr	Lys	Val	Ser	Tyr	Gly	Phe	Leu	Ser	Pro	Pro	Gln	Leu	Asn	Lys
Asn				660					665					670	
	Ser	Ser	Gly	Ile	Tyr	Ser	Glu	Ala	Leu	Leu	Thr	Thr	Asn	Ile	Val
Pro			675					680					685		
	Met	Tyr	Gln	Ser	Phe	Gln	Val	Ile	Trp	Arg	Tyr	Phe	His	Asp	Thr
Leu															
		690					695					700			
	Leu	Arg	Lys	Tyr	Ala	Glu	Glu	Arg	Asn	Gly	Val	Asn	Val	Val	Ser
Gly															
	705					710						715			
720	Pro	Val	Phe	Asp	Phe	Asp	Tyr	Asp	Gly	Arg	Cys	Asp	Ser	Leu	Glu
Asn															
				725					730					735	
	Leu	Arg	Gln	Lys	Arg	Arg	Val	Ile	Arg	Asn	Gln	Glu	Ile	Leu	Ile
Pro															
			740						745					750	
	Thr	His	Phe	Phe	Ile	Val	Leu	Thr	Ser	Cys	Lys	Asp	Thr	Ser	Gln
Thr															
		755						760					765		
	Pro	Leu	His	Cys	Glu	Asn	Leu	Asp	Thr	Leu	Ala	Phe	Ile	Leu	Pro
His															
		770				775					780				
	Arg	Thr	Asp	Asn	Ser	Glu	Ser	Cys	Val	His	Gly	Lys	His	Asp	Ser
Ser															
	785					790						795			
800	Trp	Val	Glu	Glu	Leu	Leu	Met	Leu	His	Arg	Ala	Arg	Ile	Thr	Asp
Val															
				805					810					815	
	Glu	His	Ile	Thr	Gly	Leu	Ser	Phe	Tyr	Gln	Gln	Arg	Lys	Glu	Pro
Val															
			820					825					830		
	Ser	Asp	Ile	Leu	Lys	Leu	Lys	Thr	His	Leu	Pro	Thr	Phe	Ser	Gln
Glu															
		835					840					845			
	Asp	<u>Arg</u>	<u>Ser</u>	<u>Gly</u>	<u>Ser</u>	<u>Gly</u>	<u>Gly</u>	<u>Ser</u>	Met	Lys	Trp	Val	Thr	Phe	Leu
Leu															
		850				855						860			
	Leu	Leu	Phe	Val	Ser	Gly	Ser	Ala	Phe	Ser	Arg	Gly	Val	Phe	Arg
Arg															
	865					870						875			
880	Glu	Ala	His	Lys	Ser	Glu	Ile	Ala	His	Arg	Tyr	Asn	Asp	Leu	Gly
Glu															
				885					890					895	

Gln	Gln	His	Phe	Lys	Gly	Leu	Val	Leu	Ile	Ala	Phe	Ser	Gln	Tyr	Leu
				900				905					910		
Asp	Lys	Cys	Ser	Tyr	Asp	Glu	His	Ala	Lys	Leu	Val	Gln	Glu	Val	Thr
			915					920					925		
Lys	Phe	Ala	Lys	Thr	Cys	Val	Ala	Asp	Glu	Ser	Ala	Ala	Asn	Cys	Asp
		930						935					940		
Leu	Ser	Leu	His	Thr	Leu	Phe	Gly	Asp	Lys	Leu	Cys	Ala	Ile	Pro	Asn
	945					950						955			
960															
Pro	Arg	Glu	Asn	Tyr	Gly	Glu	Leu	Ala	Asp	Cys	Cys	Thr	Lys	Gln	Glu
				965					970				975		
Leu	Glu	Arg	Asn	Glu	Cys	Phe	Leu	Gln	His	Lys	Asp	Asp	Asn	Pro	Ser
			980						985				990		
Lys	Pro	Pro	Phe	Glu	Arg	Pro	Glu	Ala	Glu	Ala	Met	Cys	Thr	Ser	Phe
			995					1000					1005		
	Glu	Asn	Pro	Thr	Thr	Phe	Met	Gly	His	Tyr	Leu	His	Glu	Val	Ala
	1010						1015					1020			
	Arg	Arg	His	Pro	Tyr	Phe	Tyr	Ala	Pro	Glu	Leu	Leu	Tyr	Tyr	Ala
	1025						1030					1035			
	Glu	Gln	Tyr	Asn	Glu	Ile	Leu	Thr	Gln	Cys	Cys	Ala	Glu	Ala	Asp
	1040						1045					1050			
	Lys	Glu	Ser	Cys	Leu	Thr	Pro	Lys	Leu	Asp	Gly	Val	Lys	Glu	Lys
	1055						1060					1065			
	Ala	Leu	Val	Ser	Ser	Val	Arg	Gln	Arg	Met	Lys	Cys	Ser	Ser	Met
	1070						1075					1080			
	Gln	Lys	Phe	Gly	Glu	Arg	Ala	Phe	Lys	Ala	Trp	Ala	Val	Ala	Arg
	1085						1090					1095			
	Leu	Ser	Gln	Thr	Phe	Pro	Asn	Ala	Asp	Phe	Ala	Glu	Ile	Thr	Lys
	1100						1105					1110			
	Leu	Ala	Thr	Asp	Leu	Thr	Lys	Val	Asn	Lys	Glu	Cys	Cys	His	Gly
	1115						1120					1125			
	Asp	Leu	Leu	Glu	Cys	Ala	Asp	Asp	Arg	Ala	Glu	Leu	Ala	Lys	Tyr
	1130						1135					1140			
	Met	Cys	Glu	Asn	Gln	Ala	Thr	Ile	Ser	Ser	Lys	Leu	Gln	Thr	Cys
	1145						1150					1155			
	Cys	Asp	Lys	Pro	Leu	Leu	Lys	Lys	Ala	His	Cys	Leu	Ser	Glu	Val
	1160						1165					1170			
	Glu	His	Asp	Thr	Met	Pro	Ala	Asp	Leu	Pro	Ala	Ile	Ala	Ala	Asp
	1175						1180					1185			
	Phe	Val	Glu	Asp	Gln	Glu	Val	Cys	Lys	Asn	Tyr	Ala	Glu	Ala	Lys
	1190						1195					1200			
	Asp	Val	Phe	Leu	Gly	Thr	Phe	Leu	Tyr	Glu	Tyr	Ser	Arg	Arg	His
	1205						1210					1215			
	Pro	Asp	Tyr	Ser	Val	Ser	Leu	Leu	Leu	Arg	Leu	Ala	Lys	Lys	Tyr
	1220						1225					1230			
	Glu	Ala	Thr	Leu	Glu	Lys	Cys	Cys	Ala	Glu	Ala	Asn	Pro	Pro	Ala
	1235						1240					1245			
	Cys	Tyr	Gly	Thr	Val	Leu	Ala	Glu	Phe	Gln	Pro	Leu	Val	Glu	Glu
	1250						1255					1260			

Pro	Lys	Asn	Leu	Val	Lys	Thr	Asn	Cys	Asp	Leu	Tyr	Glu	Lys	Leu
	1265					1270					1275			
Gly	Glu	Tyr	Gly	Phe	Gln	Asn	Ala	Ile	Leu	Val	Arg	Tyr	Thr	Gln
	1280					1285					1290			
Lys	Ala	Pro	Gln	Val	Ser	Thr	Pro	Thr	Leu	Val	Glu	Ala	Ala	Arg
	1295					1300					1305			
Asn	Leu	Gly	Arg	Val	Gly	Thr	Lys	Cys	Cys	Thr	Leu	Pro	Glu	Asp
	1310					1315					1320			
Gln	Arg	Leu	Pro	Cys	Val	Glu	Asp	Tyr	Leu	Ser	Ala	Ile	Leu	Asn
	1325					1330					1335			
Arg	Val	Cys	Leu	Leu	His	Glu	Lys	Thr	Pro	Val	Ser	Glu	His	Val
	1340					1345					1350			
Thr	Lys	Cys	Cys	Ser	Gly	Ser	Leu	Val	Glu	Arg	Arg	Pro	Cys	Phe
	1355					1360					1365			
Ser	Ala	Leu	Thr	Val	Asp	Glu	Thr	Tyr	Val	Pro	Lys	Glu	Phe	Lys
	1370					1375					1380			
Ala	Glu	Thr	Phe	Thr	Phe	His	Ser	Asp	Ile	Cys	Thr	Leu	Pro	Glu
	1385					1390					1395			
Lys	Glu	Lys	Gln	Ile	Lys	Lys	Gln	Thr	Ala	Leu	Ala	Glu	Leu	Val
	1400					1405					1410			
Lys	His	Lys	Pro	Lys	Ala	Thr	Ala	Glu	Gln	Leu	Lys	Thr	Val	Met
	1415					1420					1425			
Asp	Asp	Phe	Ala	Gln	Phe	Leu	Asp	Thr	Cys	Cys	Lys	Ala	Ala	Asp
	1430					1435					1440			
Lys	Asp	Thr	Cys	Phe	Ser	Thr	Glu	Gly	Pro	Asn	Leu	Val	Thr	Arg
	1445					1450					1455			
Cys	Lys	Asp	Ala	Leu	Ala	Arg	Ser	Trp	Ser	His	Pro	Gln	Phe	Glu
	1460					1465					1470			

Lys

Singly underlined: signal peptide sequence; double-underlined: beginning and end of NPP1; ** = cleavage position at the signal peptide sequence; bold residues indicate albumin sequence

SEQ. ID NO: 22 - ENPP7-NPP3-Fc sequence:

	Met	Arg	Gly	Pro	Ala	Val	Leu	Leu	Thr	Val	Ala	Leu	Ala	Thr	Leu
<u>Leu</u>															
	1				5					10				15	
	Ala	Pro	Gly	Ala	**	<u>Lys</u>	<u>Gln</u>	<u>Gly</u>	Ser	Cys	Arg	Lys	Lys	Cys	Phe
Ala															Asp
				20					25					30	
	Ser	Phe	Arg	Gly	Leu	Glu	Asn	Cys	Arg	Cys	Asp	Val	Ala	Cys	Lys
Asp															
			35						40				45		
	Arg	Gly	Asp	Cys	Cys	Trp	Asp	Phe	Glu	Asp	Thr	Cys	Val	Glu	Ser
Thr															
		50					55					60			
	Arg	Ile	Trp	Met	Cys	Asn	Lys	Phe	Arg	Cys	Gly	Glu	Arg	Leu	Glu
Ala															
	65					70					75				80
	Ser	Leu	Cys	Ser	Cys	Ser	Asp	Asp	Cys	Leu	Gln	Arg	Lys	Asp	Cys
Cys															

					85					90				95	
	Ala	Asp	Tyr	Lys	Ser	Val	Cys	Gln	Gly	Glu	Thr	Ser	Trp	Leu	Glu
Glu															
					100					105				110	
	Asn	Cys	Asp	Thr	Ala	Gln	Gln	Ser	Gln	Cys	Pro	Glu	Gly	Phe	Asp
Leu															
					115					120				125	
	Pro	Pro	Val	Ile	Leu	Phe	Ser	Met	Asp	Gly	Phe	Arg	Ala	Glu	Tyr
Leu															
					130					135				140	
	Tyr	Thr	Trp	Asp	Thr	Leu	Met	Pro	Asn	Ile	Asn	Lys	Leu	Lys	Thr
Cys															
	145						150					155			
160															
	Gly	Ile	His	Ser	Lys	Tyr	Met	Arg	Ala	Met	Tyr	Pro	Thr	Lys	Thr
Phe															
					165					170					175
	Pro	Asn	His	Tyr	Thr	Ile	Val	Thr	Gly	Leu	Tyr	Pro	Glu	Ser	His
Gly															
					180					185				190	
	Ile	Ile	Asp	Asn	Asn	Met	Tyr	Asp	Val	Asn	Leu	Asn	Lys	Asn	Phe
Ser															
					195					200				205	
	Leu	Ser	Ser	Lys	Glu	Gln	Asn	Asn	Pro	Ala	Trp	Trp	His	Gly	Gln
Pro															
					210					215				220	
	Met	Trp	Leu	Thr	Ala	Met	Tyr	Gln	Gly	Leu	Lys	Ala	Ala	Thr	Tyr
Phe															
	225						230					235			
240															
	Trp	Pro	Gly	Ser	Glu	Val	Ala	Ile	Asn	Gly	Ser	Phe	Pro	Ser	Ile
Tyr															
					245					250					255
	Met	Pro	Tyr	Asn	Gly	Ser	Val	Pro	Phe	Glu	Glu	Arg	Ile	Ser	Thr
Leu															
					260					265				270	
	Leu	Lys	Trp	Leu	Asp	Leu	Pro	Lys	Ala	Glu	Arg	Pro	Arg	Phe	Tyr
Thr															
					275					280				285	
	Met	Tyr	Phe	Glu	Glu	Pro	Asp	Ser	Ser	Gly	His	Ala	Gly	Gly	Pro
Val															
					290					295				300	
	Ser	Ala	Arg	Val	Ile	Lys	Ala	Leu	Gln	Val	Val	Asp	His	Ala	Phe
Gly															
	305						310					315			
320															
	Met	Leu	Met	Glu	Gly	Leu	Lys	Gln	Arg	Asn	Leu	His	Asn	Cys	Val
Asn															
					325					330					335
	Ile	Ile	Leu	Leu	Ala	Asp	His	Gly	Met	Asp	Gln	Thr	Tyr	Cys	Asn
Lys															
					340					345				350	
	Met	Glu	Tyr	Met	Thr	Asp	Tyr	Phe	Pro	Arg	Ile	Asn	Phe	Phe	Tyr
Met															
					355					360				365	

	Tyr	Glu	Gly	Pro	Ala	Pro	Arg	Ile	Arg	Ala	His	Asn	Ile	Pro	His
Asp															
	370						375					380			
	Phe	Phe	Ser	Phe	Asn	Ser	Glu	Glu	Ile	Val	Arg	Asn	Leu	Ser	Cys
Arg															
	385						390					395			
400															
	Lys	Pro	Asp	Gln	His	Phe	Lys	Pro	Tyr	Leu	Thr	Pro	Asp	Leu	Pro
Lys															
					405					410				415	
	Arg	Leu	His	Tyr	Ala	Lys	Asn	Val	Arg	Ile	Asp	Lys	Val	His	Leu
Phe															
				420					425					430	
	Val	Asp	Gln	Gln	Trp	Leu	Ala	Val	Arg	Ser	Lys	Ser	Asn	Thr	Asn
Cys															
		435						440					445		
	Gly	Gly	Gly	Asn	His	Gly	Tyr	Asn	Asn	Glu	Phe	Arg	Ser	Met	Glu
Ala															
		450						455					460		
	Ile	Phe	Leu	Ala	His	Gly	Pro	Ser	Phe	Lys	Glu	Lys	Thr	Glu	Val
Glu															
	465						470					475			
480															
	Pro	Phe	Glu	Asn	Ile	Glu	Val	Tyr	Asn	Leu	Met	Cys	Asp	Leu	Leu
Arg															
					485					490				495	
	Ile	Gln	Pro	Ala	Pro	Asn	Asn	Gly	Thr	His	Gly	Ser	Leu	Asn	His
Leu															
				500					505				510		
	Leu	Lys	Val	Pro	Phe	Tyr	Glu	Pro	Ser	His	Ala	Glu	Glu	Val	Ser
Lys															
		515						520				525			
	Phe	Ser	Val	Cys	Gly	Phe	Ala	Asn	Pro	Leu	Pro	Thr	Glu	Ser	Leu
Asp															
		530						535				540			
	Cys	Phe	Cys	Pro	His	Leu	Gln	Asn	Ser	Thr	Gln	Leu	Glu	Gln	Val
Asn															
	545						550					555			
560															
	Gln	Met	Leu	Asn	Leu	Thr	Gln	Glu	Glu	Ile	Thr	Ala	Thr	Val	Lys
Val															
					565					570				575	
	Asn	Leu	Pro	Phe	Gly	Arg	Pro	Arg	Val	Leu	Gln	Lys	Asn	Val	Asp
His															
				580					585				590		
	Cys	Leu	Leu	Tyr	His	Arg	Glu	Tyr	Val	Ser	Gly	Phe	Gly	Lys	Ala
Met															
				595					600				605		
	Arg	Met	Pro	Met	Trp	Ser	Ser	Tyr	Thr	Val	Pro	Gln	Leu	Gly	Asp
Thr															
		610						615				620			
	Ser	Pro	Leu	Pro	Pro	Thr	Val	Pro	Asp	Cys	Leu	Arg	Ala	Asp	Val
Arg															
	625						630					635			
640															

	Val	Pro	Pro	Ser	Glu	Ser	Gln	Lys	Cys	Ser	Phe	Tyr	Leu	Ala	Asp
Lys					645					650					655
	Asn	Ile	Thr	His	Gly	Phe	Leu	Tyr	Pro	Pro	Ala	Ser	Asn	Arg	Thr
Ser				660					665					670	
	Asp	Ser	Gln	Tyr	Asp	Ala	Leu	Ile	Thr	Ser	Asn	Leu	Val	Pro	Met
Tyr			675					680					685		
	Glu	Glu	Phe	Arg	Lys	Met	Trp	Asp	Tyr	Phe	His	Ser	Val	Leu	Leu
Ile		690					695					700			
	Lys	His	Ala	Thr	Glu	Arg	Asn	Gly	Val	Asn	Val	Val	Ser	Gly	Pro
Ile															
720	705					710					715				
	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															
800	785					790					795				
	Arg	Phe	Thr	Ala	His	Ile	Ala	Arg	Val	Arg	Asp	Val	Glu	Leu	Leu
Thr															
				805						810					815
	Gly	Leu	Asp	Phe	Tyr	Gln	Asp	Lys	Val	Gln	Pro	Val	Ser	Glu	Ile
Leu															
			820						825					830	
	Gln	Leu	Lys	Thr	Tyr	Leu	Pro	Thr	<u>Phe</u>	<u>Glu</u>	<u>Thr</u>	<u>Thr</u>	<u>Ile</u>	Asp	Lys
Thr															
		835						840					845		
	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro
Ser															
		850					855					860			
	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser
Arg															
880	865					870					875				
	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp
Pro															
				885					890					895	
	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn
Ala															
			900					905						910	
	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val
Val															

```

          915          920          925
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
Tyr
          930          935          940
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
Thr
          945          950          955
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

```

Singly underlined: signal peptide sequence; double-underlined: beginning and end of NPP3; ** = cleavage position at the signal peptide sequence; bold residues indicate Fc sequence

SEQ. ID NO: 23 - ENPP71-Albumin

```

Met Arg Gly Pro Ala Val Leu Leu Thr Val Ala Leu Ala Thr Leu
Leu
1      5      10      15
Ala Pro Gly Ala Gly Leu Lys**Pro Ser Cys Ala Lys Glu Val Lys
Ser
20      25      30
Cys Lys Gly Arg Cys Phe Glu Arg Thr Phe Gly Asn Cys Arg Cys
Asp
35      40      45
Ala Ala Cys Val Glu Leu Gly Asn Cys Cys Leu Asp Tyr Gln Glu
Thr
50      55      60
Cys Ile Glu Pro Glu His Ile Trp Thr Cys Asn Lys Phe Arg Cys
Gly
65      70      75      80
Glu Lys Arg Leu Thr Arg Ser Leu Cys Ala Cys Ser Asp Asp Cys
Lys
85      90      95
Asp Lys Gly Asp Cys Cys Ile Asn Tyr Ser Ser Val Cys Gln Gly
Glu
100      105      110

```

	Lys	Ser	Trp	Val	Glu	Glu	Pro	Cys	Glu	Ser	Ile	Asn	Glu	Pro	Gln
Cys															
			115					120					125		
	Pro	Ala	Gly	Phe	Glu	Thr	Pro	Pro	Thr	Leu	Leu	Phe	Ser	Leu	Asp
Gly															
		130					135					140			
	Phe	Arg	Ala	Glu	Tyr	Leu	His	Thr	Trp	Gly	Gly	Leu	Leu	Pro	Val
Ile															
	145						150					155			
160															
	Ser	Lys	Leu	Lys	Lys	Cys	Gly	Thr	Tyr	Thr	Lys	Asn	Met	Arg	Pro
Val															
				165						170					175
	Tyr	Pro	Thr	Lys	Thr	Phe	Pro	Asn	His	Tyr	Ser	Ile	Val	Thr	Gly
Leu															
			180						185					190	
	Tyr	Pro	Glu	Ser	His	Gly	Ile	Ile	Asp	Asn	Lys	Met	Tyr	Asp	Pro
Lys															
		195						200					205		
	Met	Asn	Ala	Ser	Phe	Ser	Leu	Lys	Ser	Lys	Glu	Lys	Phe	Asn	Pro
Glu															
		210					215					220			
	Trp	Tyr	Lys	Gly	Glu	Pro	Ile	Trp	Val	Thr	Ala	Lys	Tyr	Gln	Gly
Leu															
	225						230					235			
240															
	Lys	Ser	Gly	Thr	Phe	Phe	Trp	Pro	Gly	Ser	Asp	Val	Glu	Ile	Asn
Gly															
				245						250					255
	Ile	Phe	Pro	Asp	Ile	Tyr	Lys	Met	Tyr	Asn	Gly	Ser	Val	Pro	Phe
Glu															
			260						265					270	
	Glu	Arg	Ile	Leu	Ala	Val	Leu	Gln	Trp	Leu	Gln	Leu	Pro	Lys	Asp
Glu															
		275						280					285		
	Arg	Pro	His	Phe	Tyr	Thr	Leu	Tyr	Leu	Glu	Glu	Pro	Asp	Ser	Ser
Gly															
		290					295					300			
	His	Ser	Tyr	Gly	Pro	Val	Ser	Ser	Glu	Val	Ile	Lys	Ala	Leu	Gln
Arg															
	305						310					315			
320															
	Val	Asp	Gly	Met	Val	Gly	Met	Leu	Met	Asp	Gly	Leu	Lys	Glu	Leu
Asn															
				325						330					335
	Leu	His	Arg	Cys	Leu	Asn	Leu	Ile	Leu	Ile	Ser	Asp	His	Gly	Met
Glu															
			340						345					350	
	Gln	Gly	Ser	Cys	Lys	Lys	Tyr	Ile	Tyr	Leu	Asn	Lys	Tyr	Leu	Gly
Asp															
		355						360					365		
	Val	Lys	Asn	Ile	Lys	Val	Ile	Tyr	Gly	Pro	Ala	Ala	Arg	Leu	Arg
Pro															
		370					375						380		
	Ser	Asp	Val	Pro	Asp	Lys	Tyr	Tyr	Ser	Phe	Asn	Tyr	Glu	Gly	Ile
Ala															

	385		390		395										
400	Arg	Asn	Leu	Ser	Cys	Arg	Glu	Pro	Asn	Gln	His	Phe	Lys	Pro	Tyr
Leu															
					405					410					415
	Tys	His	Phe	Leu	Pro	Tys	Arg	Leu	His	Phe	Ala	Tys	Ser	Asp	Arg
Ile															
				420					425					430	
	Glu	Pro	Leu	Thr	Phe	Tyr	Leu	Asp	Pro	Gln	Trp	Gln	Leu	Ala	Leu
Asn															
			435					440				445			
	Pro	Ser	Glu	Arg	Lys	Tyr	Cys	Gly	Ser	Gly	Phe	His	Gly	Ser	Asp
Asn															
		450					455				460				
	Val	Phe	Ser	Asn	Met	Gln	Ala	Leu	Phe	Val	Gly	Tyr	Gly	Pro	Gly
Phe															
	465					470					475				
480															
	Lys	His	Gly	Ile	Glu	Ala	Asp	Thr	Phe	Glu	Asn	Ile	Glu	Val	Tyr
Asn															
				485					490						495
	Leu	Met	Cys	Asp	Leu	Leu	Asn	Leu	Thr	Pro	Ala	Pro	Asn	Asn	Gly
Thr															
				500				505					510		
	His	Gly	Ser	Leu	Asn	His	Leu	Leu	Lys	Asn	Pro	Val	Tyr	Thr	Pro
Lys															
		515					520			525					
	His	Pro	Lys	Glu	Val	His	Pro	Leu	Val	Gln	Cys	Pro	Phe	Thr	Arg
Asn															
		530				535			540						
	Pro	Arg	Asp	Asn	Leu	Gly	Cys	Ser	Cys	Asn	Pro	Ser	Ile	Leu	Pro
Ile															
	545				550				555						
560															
	Glu	Asp	Phe	Gln	Thr	Gln	Phe	Asn	Leu	Thr	Val	Ala	Glu	Glu	Lys
Ile															
				565				570						575	
	Ile	Lys	His	Glu	Thr	Leu	Pro	Tyr	Gly	Arg	Pro	Arg	Val	Leu	Gln
Lys															
			580				585					590			
	Glu	Asn	Thr	Ile	Cys	Leu	Leu	Ser	Gln	His	Gln	Phe	Met	Ser	Gly
Tyr															
		595				600			605						
	Ser	Gln	Asp	Ile	Leu	Met	Pro	Leu	Trp	Thr	Ser	Tyr	Thr	Val	Asp
Arg															
		610				615			620						
	Asn	Asp	Ser	Phe	Ser	Thr	Glu	Asp	Phe	Ser	Asn	Cys	Leu	Tyr	Gln
Asp															
	625				630				635						
640															
	Phe	Arg	Ile	Pro	Leu	Ser	Pro	Val	His	Lys	Cys	Ser	Phe	Tyr	Lys
Asn															
			645				650						655		
	Asn	Thr	Lys	Val	Ser	Tyr	Gly	Phe	Leu	Ser	Pro	Pro	Gln	Leu	Asn
Lys															
			660				665						670		

Val	Asn	Ser	Ser	Gly	Ile	Tyr	Ser	Glu	Ala	Leu	Leu	Thr	Thr	Asn	Ile
			675					680					685		
Thr	Pro	Met	Tyr	Gln	Ser	Phe	Gln	Val	Ile	Trp	Arg	Tyr	Phe	His	Asp
		690					695					700			
Ser	Leu	Leu	Arg	Lys	Tyr	Ala	Glu	Glu	Arg	Asn	Gly	Val	Asn	Val	Val
	705					710					715				
720	Gly	Pro	Val	Phe	Asp	Phe	Asp	Tyr	Asp	Gly	Arg	Cys	Asp	Ser	Leu
Glu					725					730				735	
Ile	Asn	Leu	Arg	Gln	Lys	Arg	Arg	Val	Ile	Arg	Asn	Gln	Glu	Ile	Leu
			740						745				750		
Gln	Pro	Thr	His	Phe	Phe	Ile	Val	Leu	Thr	Ser	Cys	Lys	Asp	Thr	Ser
		755						760				765			
Pro	Thr	Pro	Leu	His	Cys	Glu	Asn	Leu	Asp	Thr	Leu	Ala	Phe	Ile	Leu
		770					775				780				
Ser	His	Arg	Thr	Asp	Asn	Ser	Glu	Ser	Cys	Val	His	Gly	Lys	His	Asp
	785					790					795				
800	Ser	Trp	Val	Glu	Glu	Leu	Leu	Met	Leu	His	Arg	Ala	Arg	Ile	Thr
Asp					805				810					815	
Pro	Val	Glu	His	Ile	Thr	Gly	Leu	Ser	Phe	Tyr	Gln	Gln	Arg	Lys	Glu
			820						825				830		
<u>Gln</u>	Val	Ser	Asp	Ile	Leu	Lys	Leu	Lys	Thr	His	Leu	Pro	Thr	Phe	Ser
		835						840				845			
<u>Leu</u>	<u>Glu Asp</u>	Gly	Gly	Ser	Gly	Gly	Ser	Met	Lys	Trp	Val	Thr	Phe	Leu	
		850					855				860				
Arg	Leu	Leu	Phe	Val	Ser	Gly	Ser	Ala	Phe	Ser	Arg	Gly	Val	Phe	Arg
	865					870					875				
880	Glu	Ala	His	Lys	Ser	Glu	Ile	Ala	His	Arg	Tyr	Asn	Asp	Leu	Gly
Glu					885				890					895	
Gln	Gln	His	Phe	Lys	Gly	Leu	Val	Leu	Ile	Ala	Phe	Ser	Gln	Tyr	Leu
			900					905					910		
Asp	Lys	Cys	Ser	Tyr	Asp	Glu	His	Ala	Lys	Leu	Val	Gln	Glu	Val	Thr
		915					920				925				
Lys	Phe	Ala	Lys	Thr	Cys	Val	Ala	Asp	Glu	Ser	Ala	Ala	Asn	Cys	Asp
		930					935				940				
Leu	Ser	Leu	His	Thr	Leu	Phe	Gly	Asp	Lys	Leu	Cys	Ala	Ile	Pro	Asn

960	945	950	955	
Pro	Arg Glu Asn Tyr Gly Glu Leu Ala Asp Cys Cys Thr Lys Gln Glu			
		965	970	975
Leu	Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Ser			
		980	985	990
Lys	Pro Pro Phe Glu Arg Pro Glu Ala Glu Ala Met Cys Thr Ser Phe			
	995	1000	1005	
	Glu Asn Pro Thr Thr Phe Met Gly His Tyr Leu His Glu Val Ala			
	1010	1015	1020	
	Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Tyr Tyr Ala			
	1025	1030	1035	
	Glu Gln Tyr Asn Glu Ile Leu Thr Gln Cys Cys Ala Glu Ala Asp			
	1040	1045	1050	
	Lys Glu Ser Cys Leu Thr Pro Lys Leu Asp Gly Val Lys Glu Lys			
	1055	1060	1065	
	Ala Leu Val Ser Ser Val Arg Gln Arg Met Lys Cys Ser Ser Met			
	1070	1075	1080	
	Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg			
	1085	1090	1095	
	Leu Ser Gln Thr Phe Pro Asn Ala Asp Phe Ala Glu Ile Thr Lys			
	1100	1105	1110	
	Leu Ala Thr Asp Leu Thr Lys Val Asn Lys Glu Cys Cys His Gly			
	1115	1120	1125	
	Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Glu Leu Ala Lys Tyr			
	1130	1135	1140	
	Met Cys Glu Asn Gln Ala Thr Ile Ser Ser Lys Leu Gln Thr Cys			
	1145	1150	1155	
	Cys Asp Lys Pro Leu Leu Lys Lys Ala His Cys Leu Ser Glu Val			
	1160	1165	1170	
	Glu His Asp Thr Met Pro Ala Asp Leu Pro Ala Ile Ala Ala Asp			
	1175	1180	1185	
	Phe Val Glu Asp Gln Glu Val Cys Lys Asn Tyr Ala Glu Ala Lys			
	1190	1195	1200	
	Asp Val Phe Leu Gly Thr Phe Leu Tyr Glu Tyr Ser Arg Arg His			
	1205	1210	1215	
	Pro Asp Tyr Ser Val Ser Leu Leu Leu Arg Leu Ala Lys Lys Tyr			
	1220	1225	1230	
	Glu Ala Thr Leu Glu Lys Cys Cys Ala Glu Ala Asn Pro Pro Ala			
	1235	1240	1245	
	Cys Tyr Gly Thr Val Leu Ala Glu Phe Gln Pro Leu Val Glu Glu			
	1250	1255	1260	
	Pro Lys Asn Leu Val Lys Thr Asn Cys Asp Leu Tyr Glu Lys Leu			
	1265	1270	1275	
	Gly Glu Tyr Gly Phe Gln Asn Ala Ile Leu Val Arg Tyr Thr Gln			
	1280	1285	1290	
	Lys Ala Pro Gln Val Ser Thr Pro Thr Leu Val Glu Ala Ala Arg			
	1295	1300	1305	
	Asn Leu Gly Arg Val Gly Thr Lys Cys Cys Thr Leu Pro Glu Asp			
	1310	1315	1320	
	Gln Arg Leu Pro Cys Val Glu Asp Tyr Leu Ser Ala Ile Leu Asn			
	1325	1330	1335	
	Arg Val Cys Leu Leu His Glu Lys Thr Pro Val Ser Glu His Val			

1340	1345	1350
Thr Lys Cys Cys Ser Gly	Ser Leu Val Glu Arg	Arg Pro Cys Phe
1355	1360	1365
Ser Ala Leu Thr Val Asp	Glu Thr Tyr Val Pro	Lys Glu Phe Lys
1370	1375	1380
Ala Glu Thr Phe Thr Phe	His Ser Asp Ile Cys	Thr Leu
1385	1390	1395

Singly underlined: signal peptide sequence; double-underlined: beginning and end of NPP3; ** = cleavage position at the signal peptide sequence; bold residues indicate Fc sequence

SEQ. ID NO: 24 - ENPP7-NPP3-Albumin

	Met Arg Gly Pro Ala Val Leu Leu Thr Val Ala Leu Ala Thr Leu
Leu	
	1 5 10 15
	Ala Pro Gly <u>Ala**Lys Gln Gly</u> Ser Cys Arg Lys Lys Cys Phe Asp
Ala	
	20 25 30
	Ser Phe Arg Gly Leu Glu Asn Cys Arg Cys Asp Val Ala Cys Lys
Asp	
	35 40 45
	Arg Gly Asp Cys Cys Trp Asp Phe Glu Asp Thr Cys Val Glu Ser
Thr	
	50 55 60
	Arg Ile Trp Met Cys Asn Lys Phe Arg Cys Gly Glu Arg Leu Glu
Ala	
	65 70 75 80
	Ser Leu Cys Ser Cys Ser Asp Asp Cys Leu Gln Arg Lys Asp Cys
Cys	
	85 90 95
	Ala Asp Tyr Lys Ser Val Cys Gln Gly Glu Thr Ser Trp Leu Glu
Glu	
	100 105 110
	Asn Cys Asp Thr Ala Gln Gln Ser Gln Cys Pro Glu Gly Phe Asp
Leu	
	115 120 125
	Pro Pro Val Ile Leu Phe Ser Met Asp Gly Phe Arg Ala Glu Tyr
Leu	
	130 135 140
	Tyr Thr Trp Asp Thr Leu Met Pro Asn Ile Asn Lys Leu Lys Thr
Cys	
	145 150 155
160	
	Gly Ile His Ser Lys Tyr Met Arg Ala Met Tyr Pro Thr Lys Thr
Phe	
	165 170 175
	Pro Asn His Tyr Thr Ile Val Thr Gly Leu Tyr Pro Glu Ser His
Gly	
	180 185 190
	Ile Ile Asp Asn Asn Met Tyr Asp Val Asn Leu Asn Lys Asn Phe
Ser	

		195					200					205			
	Leu	Ser	Ser	Lys	Glu	Gln	Asn	Asn	Pro	Ala	Trp	Trp	His	Gly	Gln
Pro															
		210					215					220			
	Met	Trp	Leu	Thr	Ala	Met	Tyr	Gln	Gly	Leu	Lys	Ala	Ala	Thr	Tyr
Phe															
	225						230					235			
240															
	Trp	Pro	Gly	Ser	Glu	Val	Ala	Ile	Asn	Gly	Ser	Phe	Pro	Ser	Ile
Tyr															
				245						250					255
	Met	Pro	Tyr	Asn	Gly	Ser	Val	Pro	Phe	Glu	Glu	Arg	Ile	Ser	Thr
Leu															
			260						265					270	
	Leu	Lys	Trp	Leu	Asp	Leu	Pro	Lys	Ala	Glu	Arg	Pro	Arg	Phe	Tyr
Thr															
		275						280					285		
	Met	Tyr	Phe	Glu	Glu	Pro	Asp	Ser	Ser	Gly	His	Ala	Gly	Gly	Pro
Val															
		290					295					300			
	Ser	Ala	Arg	Val	Ile	Lys	Ala	Leu	Gln	Val	Val	Asp	His	Ala	Phe
Gly															
	305						310					315			
320															
	Met	Leu	Met	Glu	Gly	Leu	Lys	Gln	Arg	Asn	Leu	His	Asn	Cys	Val
Asn															
				325						330					335
	Ile	Ile	Leu	Leu	Ala	Asp	His	Gly	Met	Asp	Gln	Thr	Tyr	Cys	Asn
Lys															
			340						345				350		
	Met	Glu	Tyr	Met	Thr	Asp	Tyr	Phe	Pro	Arg	Ile	Asn	Phe	Phe	Tyr
Met															
		355						360					365		
	Tyr	Glu	Gly	Pro	Ala	Pro	Arg	Ile	Arg	Ala	His	Asn	Ile	Pro	His
Asp															
		370					375					380			
	Phe	Phe	Ser	Phe	Asn	Ser	Glu	Glu	Ile	Val	Arg	Asn	Leu	Ser	Cys
Arg															
	385						390					395			
400															
	Lys	Pro	Asp	Gln	His	Phe	Lys	Pro	Tyr	Leu	Thr	Pro	Asp	Leu	Pro
Lys															
				405						410					415
	Arg	Leu	His	Tyr	Ala	Lys	Asn	Val	Arg	Ile	Asp	Lys	Val	His	Leu
Phe															
			420						425					430	
	Val	Asp	Gln	Gln	Trp	Leu	Ala	Val	Arg	Ser	Lys	Ser	Asn	Thr	Asn
Cys															
		435						440					445		
	Gly	Gly	Gly	Asn	His	Gly	Tyr	Asn	Asn	Glu	Phe	Arg	Ser	Met	Glu
Ala															
		450					455					460			
	Ile	Phe	Leu	Ala	His	Gly	Pro	Ser	Phe	Lys	Glu	Lys	Thr	Glu	Val
Glu															
	465						470					475			
480															

	Pro	Phe	Glu	Asn	Ile	Glu	Val	Tyr	Asn	Leu	Met	Cys	Asp	Leu	Leu
Arg															
					485					490					495
	Ile	Gln	Pro	Ala	Pro	Asn	Asn	Gly	Thr	His	Gly	Ser	Leu	Asn	His
Leu															
				500					505					510	
	Leu	Lys	Val	Pro	Phe	Tyr	Glu	Pro	Ser	His	Ala	Glu	Glu	Val	Ser
Lys															
		515						520					525		
	Phe	Ser	Val	Cys	Gly	Phe	Ala	Asn	Pro	Leu	Pro	Thr	Glu	Ser	Leu
Asp															
		530					535					540			
	Cys	Phe	Cys	Pro	His	Leu	Gln	Asn	Ser	Thr	Gln	Leu	Glu	Gln	Val
Asn															
	545					550					555				
560															
	Gln	Met	Leu	Asn	Leu	Thr	Gln	Glu	Glu	Ile	Thr	Ala	Thr	Val	Lys
Val															
				565						570					575
	Asn	Leu	Pro	Phe	Gly	Arg	Pro	Arg	Val	Leu	Gln	Lys	Asn	Val	Asp
His															
				580					585					590	
	Cys	Leu	Leu	Tyr	His	Arg	Glu	Tyr	Val	Ser	Gly	Phe	Gly	Lys	Ala
Met															
		595						600					605		
	Arg	Met	Pro	Met	Trp	Ser	Ser	Tyr	Thr	Val	Pro	Gln	Leu	Gly	Asp
Thr															
		610					615					620			
	Ser	Pro	Leu	Pro	Pro	Thr	Val	Pro	Asp	Cys	Leu	Arg	Ala	Asp	Val
Arg															
	625					630					635				
640															
	Val	Pro	Pro	Ser	Glu	Ser	Gln	Lys	Cys	Ser	Phe	Tyr	Leu	Ala	Asp
Lys															
				645						650					655
	Asn	Ile	Thr	His	Gly	Phe	Leu	Tyr	Pro	Pro	Ala	Ser	Asn	Arg	Thr
Ser															
				660					665					670	
	Asp	Ser	Gln	Tyr	Asp	Ala	Leu	Ile	Thr	Ser	Asn	Leu	Val	Pro	Met
Tyr															
		675					680					685			
	Glu	Glu	Phe	Arg	Lys	Met	Trp	Asp	Tyr	Phe	His	Ser	Val	Leu	Leu
Ile															
		690					695					700			
	Lys	His	Ala	Thr	Glu	Arg	Asn	Gly	Val	Asn	Val	Val	Ser	Gly	Pro
Ile															
	705					710					715				
720															
	Phe	Asp	Tyr	Asn	Tyr	Asp	Gly	His	Phe	Asp	Ala	Pro	Asp	Glu	Ile
Thr															
				725						730					735
	Lys	His	Leu	Ala	Asn	Thr	Asp	Val	Pro	Ile	Pro	Thr	His	Tyr	Phe
Val															
				740					745					750	
	Val	Leu	Thr	Ser	Cys	Lys	Asn	Lys	Ser	His	Thr	Pro	Glu	Asn	Cys
Pro															

		755				760				765					
	Gly	Trp	Leu	Asp	Val	Leu	Pro	Phe	Ile	Ile	Pro	His	Arg	Pro	Thr
Asn															
		770				775				780					
	Val	Glu	Ser	Cys	Pro	Glu	Gly	Lys	Pro	Glu	Ala	Leu	Trp	Val	Glu
Glu															
	785					790				795					
800															
	Arg	Phe	Thr	Ala	His	Ile	Ala	Arg	Val	Arg	Asp	Val	Glu	Leu	Leu
Thr															
					805					810				815	
	Gly	Leu	Asp	Phe	Tyr	Gln	Asp	Lys	Val	Gln	Pro	Val	Ser	Glu	Ile
Leu															
				820					825				830		
	Gln	Leu	Lys	Thr	Tyr	Leu	<u>Pro</u>	<u>Thr</u>	<u>Phe</u>	<u>Glu</u>	<u>Thr</u>	<u>Thr</u>	<u>Ile</u>	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	Lys Glu	Lys Ala Leu Val	1060	Ser Ser Val Arg Gln	1065	Arg Met Lys Cys
1070	Ser Ser	Met Gln Lys Phe	1075	Gly Glu Arg Ala Phe	1080	Lys Ala Trp Ala
1085	Val Ala	Arg Leu Ser Gln	1090	Thr Phe Pro Asn Ala	1095	Asp Phe Ala Glu
1100	Ile Thr	Lys Leu Ala Thr	1105	Asp Leu Thr Lys Val	1110	Asn Lys Glu Cys
1115	Cys His	Gly Asp Leu Leu	1120	Glu Cys Ala Asp Asp	1125	Arg Ala Glu Leu
1130	Ala Lys	Tyr Met Cys Glu	1135	Asn Gln Ala Thr Ile	1140	Ser Ser Lys Leu
1145	Gln Thr	Cys Cys Asp Lys	1150	Pro Leu Leu Lys Lys	1155	Ala His Cys Leu
1160	Ser Glu	Val Glu His Asp	1165	Thr Met Pro Ala Asp	1170	Leu Pro Ala Ile
1175	Ala Ala	Asp Phe Val Glu	1180	Asp Gln Glu Val Cys	1185	Lys Asn Tyr Ala
1190	Glu Ala	Lys Asp Val Phe	1195	Leu Gly Thr Phe Leu	1200	Tyr Glu Tyr Ser
1205	Arg Arg	His Pro Asp Tyr	1210	Ser Val Ser Leu Leu	1215	Leu Arg Leu Ala
1220	Lys Lys	Tyr Glu Ala Thr	1225	Leu Glu Lys Cys Cys	1230	Ala Glu Ala Asn
1235	Pro Pro	Ala Cys Tyr Gly	1240	Thr Val Leu Ala Glu	1245	Phe Gln Pro Leu
1250	Val Glu	Glu Pro Lys Asn	1255	Leu Val Lys Thr Asn	1260	Cys Asp Leu Tyr
1265	Glu Lys	Leu Gly Glu Tyr	1270	Gly Phe Gln Asn Ala	1275	Ile Leu Val Arg
1280	Tyr Thr	Gln Lys Ala Pro	1285	Gln Val Ser Thr Pro	1290	Thr Leu Val Glu
1295	Ala Ala	Arg Asn Leu Gly	1300	Arg Val Gly Thr Lys	1305	Cys Cys Thr Leu
1310	Pro Glu	Asp Gln Arg Leu	1315	Pro Cys Val Glu Asp	1320	Tyr Leu Ser Ala
1325	Ile Leu	Asn Arg Val Cys	1330	Leu Leu His Glu Lys	1335	Thr Pro Val Ser
1340	Glu His	Val Thr Lys Cys	1345	Cys Ser Gly Ser Leu	1350	Val Glu Arg Arg
1355	Pro Cys	Phe Ser Ala Leu	1360	Thr Val Asp Glu Thr	1365	Tyr Val Pro Lys
1370	Glu Phe	Lys Ala Glu Thr	1375	Phe Thr Phe His Ser	1380	Asp Ile Cys Thr
1385	Leu Pro	Glu Lys Glu Lys	1390	Gln Ile Lys Lys Gln	1395	Thr Ala Leu Ala
1400	Glu Leu	Val Lys His Lys	1405	Pro Lys Ala Thr Ala	1410	Glu Gln Leu Lys
1415	Thr Val	Met Asp Asp Phe	1420	Ala Gln Phe Leu Asp	1425	Thr Cys Cys Lys
1430	Ala Ala	Asp Lys Asp Thr	1435	Cys Phe Ser Thr Glu	1440	Gly Pro Asn Leu
1445	Val Thr	Arg Cys Lys Asp	1450	Ala Leu Ala	1455	
1460			1465			

Singly underlined: signal peptide sequence; double-underlined: beginning and end of NPP3; ** = cleavage position at the signal peptide sequence; bold residues indicate albumin sequence

SEQ. ID NO: 25 - ENPP7-ENPP3-Albumin

	<u>Met Arg Gly Pro Ala Val Leu Leu Thr Val Ala Leu Ala Thr Leu</u>																			
<u>Leu</u>	1		5		10		15													
	<u>Ala Pro Gly Ala**<u>Lys Gln Gly</u> Ser Cys Arg Lys Lys Cys Phe Asp</u>																			
Ala			20		25		30													
	Ser Phe Arg Gly Leu Glu Asn Cys Arg Cys Asp Val Ala Cys Lys																			
Asp			35		40		45													
	Arg Gly Asp Cys Cys Trp Asp Phe Glu Asp Thr Cys Val Glu Ser																			
Thr			50		55		60													
	Arg Ile Trp Met Cys Asn Lys Phe Arg Cys Gly Glu Arg Leu Glu																			
Ala			65		70		75													80
	Ser Leu Cys Ser Cys Ser Asp Asp Cys Leu Gln Arg Lys Asp Cys																			
Cys			85		90		95													
	Ala Asp Tyr Lys Ser Val Cys Gln Gly Glu Thr Ser Trp Leu Glu																			
Glu			100		105		110													
	Asn Cys Asp Thr Ala Gln Gln Ser Gln Cys Pro Glu Gly Phe Asp																			
Leu			115		120		125													
	Pro Pro Val Ile Leu Phe Ser Met Asp Gly Phe Arg Ala Glu Tyr																			
Leu			130		135		140													
	Tyr Thr Trp Asp Thr Leu Met Pro Asn Ile Asn Lys Leu Lys Thr																			
Cys			145		150		155													
160																				
	Gly Ile His Ser Lys Tyr Met Arg Ala Met Tyr Pro Thr Lys Thr																			
Phe			165		170		175													
	Pro Asn His Tyr Thr Ile Val Thr Gly Leu Tyr Pro Glu Ser His																			
Gly			180		185		190													
	Ile Ile Asp Asn Asn Met Tyr Asp Val Asn Leu Asn Lys Asn Phe																			
Ser			195		200		205													
	Leu Ser Ser Lys Glu Gln Asn Asn Pro Ala Trp Trp His Gly Gln																			
Pro			210		215		220													
	Met Trp Leu Thr Ala Met Tyr Gln Gly Leu Lys Ala Ala Thr Tyr																			
Phe			225		230		235													
240																				

	Trp	Pro	Gly	Ser	Glu	Val	Ala	Ile	Asn	Gly	Ser	Phe	Pro	Ser	Ile
Tyr															
					245					250					255
	Met	Pro	Tyr	Asn	Gly	Ser	Val	Pro	Phe	Glu	Glu	Arg	Ile	Ser	Thr
Leu															
				260					265						270
	Leu	Lys	Trp	Leu	Asp	Leu	Pro	Lys	Ala	Glu	Arg	Pro	Arg	Phe	Tyr
Thr															
			275					280					285		
	Met	Tyr	Phe	Glu	Glu	Pro	Asp	Ser	Ser	Gly	His	Ala	Gly	Gly	Pro
Val															
		290					295					300			
	Ser	Ala	Arg	Val	Ile	Lys	Ala	Leu	Gln	Val	Val	Asp	His	Ala	Phe
Gly															
	305					310					315				
320															
	Met	Leu	Met	Glu	Gly	Leu	Lys	Gln	Arg	Asn	Leu	His	Asn	Cys	Val
Asn															
				325					330						335
	Ile	Ile	Leu	Leu	Ala	Asp	His	Gly	Met	Asp	Gln	Thr	Tyr	Cys	Asn
Lys															
			340					345					350		
	Met	Glu	Tyr	Met	Thr	Asp	Tyr	Phe	Pro	Arg	Ile	Asn	Phe	Phe	Tyr
Met															
		355					360					365			
	Tyr	Glu	Gly	Pro	Ala	Pro	Arg	Ile	Arg	Ala	His	Asn	Ile	Pro	His
Asp															
		370				375					380				
	Phe	Phe	Ser	Phe	Asn	Ser	Glu	Glu	Ile	Val	Arg	Asn	Leu	Ser	Cys
Arg															
	385					390					395				
400															
	Lys	Pro	Asp	Gln	His	Phe	Lys	Pro	Tyr	Leu	Thr	Pro	Asp	Leu	Pro
Lys															
				405					410						415
	Arg	Leu	His	Tyr	Ala	Lys	Asn	Val	Arg	Ile	Asp	Lys	Val	His	Leu
Phe															
			420					425					430		
	Val	Asp	Gln	Gln	Trp	Leu	Ala	Val	Arg	Ser	Lys	Ser	Asn	Thr	Asn
Cys															
		435					440				445				
	Gly	Gly	Gly	Asn	His	Gly	Tyr	Asn	Asn	Glu	Phe	Arg	Ser	Met	Glu
Ala															
		450				455				460					
	Ile	Phe	Leu	Ala	His	Gly	Pro	Ser	Phe	Lys	Glu	Lys	Thr	Glu	Val
Glu															
	465					470				475					
480															
	Pro	Phe	Glu	Asn	Ile	Glu	Val	Tyr	Asn	Leu	Met	Cys	Asp	Leu	Leu
Arg															
				485				490							495
	Ile	Gln	Pro	Ala	Pro	Asn	Asn	Gly	Thr	His	Gly	Ser	Leu	Asn	His
Leu															
			500				505						510		
	Leu	Lys	Val	Pro	Phe	Tyr	Glu	Pro	Ser	His	Ala	Glu	Glu	Val	Ser
Lys															

		515				520				525			
	Phe	Ser	Val	Cys	Gly	Phe	Ala	Asn	Pro	Leu	Pro	Thr	Glu Ser Leu
Asp													
		530				535				540			
	Cys	Phe	Cys	Pro	His	Leu	Gln	Asn	Ser	Thr	Gln	Leu	Glu Gln Val
Asn													
	545					550				555			
560													
	Gln	Met	Leu	Asn	Leu	Thr	Gln	Glu	Glu	Ile	Thr	Ala	Thr Val Lys
Val													
				565					570				575
	Asn	Leu	Pro	Phe	Gly	Arg	Pro	Arg	Val	Leu	Gln	Lys	Asn Val Asp
His													
			580					585				590	
	Cys	Leu	Leu	Tyr	His	Arg	Glu	Tyr	Val	Ser	Gly	Phe	Gly Lys Ala
Met													
		595					600				605		
	Arg	Met	Pro	Met	Trp	Ser	Ser	Tyr	Thr	Val	Pro	Gln	Leu Gly Asp
Thr													
		610				615				620			
	Ser	Pro	Leu	Pro	Pro	Thr	Val	Pro	Asp	Cys	Leu	Arg	Ala Asp Val
Arg													
	625					630				635			
640													
	Val	Pro	Pro	Ser	Glu	Ser	Gln	Lys	Cys	Ser	Phe	Tyr	Leu Ala Asp
Lys													
				645				650				655	
	Asn	Ile	Thr	His	Gly	Phe	Leu	Tyr	Pro	Pro	Ala	Ser	Asn Arg Thr
Ser													
			660				665				670		
	Asp	Ser	Gln	Tyr	Asp	Ala	Leu	Ile	Thr	Ser	Asn	Leu	Val Pro Met
Tyr													
		675				680				685			
	Glu	Glu	Phe	Arg	Lys	Met	Trp	Asp	Tyr	Phe	His	Ser	Val Leu Leu
Ile													
		690				695				700			
	Lys	His	Ala	Thr	Glu	Arg	Asn	Gly	Val	Asn	Val	Val	Ser Gly Pro
Ile													
	705					710				715			
720													
	Phe	Asp	Tyr	Asn	Tyr	Asp	Gly	His	Phe	Asp	Ala	Pro	Asp Glu Ile
Thr													
			725					730				735	
	Lys	His	Leu	Ala	Asn	Thr	Asp	Val	Pro	Ile	Pro	Thr	His Tyr Phe
Val													
			740				745			750			
	Val	Leu	Thr	Ser	Cys	Lys	Asn	Lys	Ser	His	Thr	Pro	Glu Asn Cys
Pro													
		755				760				765			
	Gly	Trp	Leu	Asp	Val	Leu	Pro	Phe	Ile	Ile	Pro	His	Arg Pro Thr
Asn													
		770				775				780			
	Val	Glu	Ser	Cys	Pro	Glu	Gly	Lys	Pro	Glu	Ala	Leu	Trp Val Glu
Glu													
	785					790				795			
800													

Arg Phe Thr Ala His Ile Ala Arg Val Arg Asp Val Glu Leu Leu
 Thr
 805 810 815
 Gly Leu Asp Phe Tyr Gln Asp Lys Val Gln Pro Val Ser Glu Ile
 Leu
 820 825 830
 Gln Leu Lys Thr Tyr Leu Pro Thr Phe Glu Thr Thr Ile Asp Lys
 Thr
 835 840 845
 His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
 Ser
 850 855 860
 Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
 Arg
 865 870 875
 880
 Thr Pro Glu Val Thr Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
 Gly
 885 890 895
 Gly Gly Ser Met Lys Trp Val Thr Phe Leu Leu Leu Leu Phe Val
 Ser
 900 905 910
 Gly Ser Ala Phe Ser Arg Gly Val Phe Arg Arg Glu Ala His Lys
 Ser
 915 920 925
 Glu Ile Ala His Arg Tyr Asn Asp Leu Gly Glu Gln His Phe Lys
 Gly
 930 935 940
 Leu Val Leu Ile Ala Phe Ser Gln Tyr Leu Gln Lys Cys Ser Tyr
 Asp
 945 950 955
 960
 Glu His Ala Lys Leu Val Gln Glu Val Thr Asp Phe Ala Lys Thr
 Cys
 965 970 975
 Val Ala Asp Glu Ser Ala Ala Asn Cys Asp Lys Ser Leu His Thr
 Leu
 980 985 990
 Phe Gly Asp Lys Leu Cys Ala Ile Pro Asn Leu Arg Glu Asn Tyr
 Gly
 995 1000 1005
 Glu Leu Ala Asp Cys Cys Thr Lys Gln Glu Pro Glu Arg Asn Glu
 1010 1015 1020
 Cys Phe Leu Gln His Lys Asp Asp Asn Pro Ser Leu Pro Pro Phe
 1025 1030 1035
 Glu Arg Pro Glu Ala Glu Ala Met Cys Thr Ser Phe Lys Glu Asn
 1040 1045 1050
 Pro Thr Thr Phe Met Gly His Tyr Leu His Glu Val Ala Arg Arg
 1055 1060 1065
 His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Tyr Tyr Ala Glu Gln
 1070 1075 1080
 Tyr Asn Glu Ile Leu Thr Gln Cys Cys Ala Glu Ala Asp Lys Glu
 1085 1090 1095
 Ser Cys Leu Thr Pro Lys Leu Asp Gly Val Lys Glu Lys Ala Leu
 1100 1105 1110
 Val Ser Ser Val Arg Gln Arg Met Lys Cys Ser Ser Met Gln Lys

1115	Phe Gly	Glu Arg Ala Phe	1120	Lys Ala Trp Ala Val	1125	Ala Arg Leu Ser
1130	Gln Thr	Phe Pro Asn Ala	1135	Asp Phe Ala Glu Ile	1140	Thr Lys Leu Ala
1145	Thr Asp	Leu Thr Lys Val	1150	Asn Lys Glu Cys Cys	1155	His Gly Asp Leu
1160	Leu Glu	Cys Ala Asp Asp	1165	Arg Ala Glu Leu Ala	1170	Lys Tyr Met Cys
1175	Glu Asn	Gln Ala Thr Ile	1180	Ser Ser Lys Leu Gln	1185	Thr Cys Cys Asp
1190	Lys Pro	Leu Leu Lys Lys	1195	Ala His Cys Leu Ser	1200	Glu Val Glu His
1205	Asp Thr	Met Pro Ala Asp	1210	Leu Pro Ala Ile Ala	1215	Ala Asp Phe Val
1220	Glu Asp	Gln Glu Val Cys	1225	Lys Asn Tyr Ala Glu	1230	Ala Lys Asp Val
1235	Phe Leu	Gly Thr Phe Leu	1240	Tyr Glu Tyr Ser Arg	1245	Arg His Pro Asp
1250	Tyr Ser	Val Ser Leu Leu	1255	Leu Arg Leu Ala Lys	1260	Lys Tyr Glu Ala
1265	Thr Leu	Glu Lys Cys Cys	1270	Ala Glu Ala Asn Pro	1275	Pro Ala Cys Tyr
1280	Gly Thr	Val Leu Ala Glu	1285	Phe Gln Pro Leu Val	1290	Glu Glu Pro Lys
1295	Asn Leu	Val Lys Thr Asn	1300	Cys Asp Leu Tyr Glu	1305	Lys Leu Gly Glu
1310	Tyr Gly	Phe Gln Asn Ala	1315	Ile Leu Val Arg Tyr	1320	Thr Gln Lys Ala
1325	Pro Gln	Val Ser Thr Pro	1330	Thr Leu Val Glu Ala	1335	Ala Arg Asn Leu
1340	Gly Arg	Val Gly Thr Lys	1345	Cys Cys Thr Leu Pro	1350	Glu Asp Gln Arg
1355	Leu Pro	Cys Val Glu Asp	1360	Tyr Leu Ser Ala Ile	1365	Leu Asn Arg Val
1370	Cys Leu	Leu His Glu Lys	1375	Thr Pro Val Ser Glu	1380	His Val Thr Lys
1385	Cys Cys	Ser Gly Ser Leu	1390	Val Glu Arg Arg Pro	1395	Cys Phe Ser Ala
1400	Leu Thr	Val Asp Glu Thr	1405	Tyr Val Pro Lys Glu	1410	Phe Lys Ala Glu
1415	Thr Phe	Thr Phe His Ser	1420	Asp Ile Cys Thr Leu	1425	Pro Glu Lys Glu
1430	Lys Gln	Ile Lys Lys Gln	1435	Thr Ala Leu Ala Glu	1440	Leu Val Lys His
1445	Lys Pro	Lys Ala Thr Ala	1450	Glu Gln Leu Lys Thr	1455	Val Met Asp Asp
1460	Phe Ala	Gln Phe Leu Asp	1465	Thr Cys Cys Lys Ala	1470	Ala Asp Lys Asp
1475	Thr Cys	Phe Ser Thr Glu	1480	Gly Pro Asn Leu Val	1485	Thr Arg Cys Lys
1490	Asp Ala	Leu Ala	1495		1500	
1505						

Singly underlined: signal peptide sequence; double-underlined: beginning and end of NPP3; ** = cleavage position at the signal peptide sequence; bold residues indicate albumin sequence

SEQ. ID NO: 26 - ENPP71 Amino Acid Sequence

	<u>Met</u>	<u>Arg</u>	<u>Gly</u>	<u>Pro</u>	<u>Ala</u>	<u>Val</u>	<u>Leu</u>	<u>Leu</u>	<u>Thr</u>	<u>Val</u>	<u>Ala</u>	<u>Leu</u>	<u>Ala</u>	<u>Thr</u>	<u>Leu</u>
<u>Leu</u>	1			5				10				15			
	<u>Ala</u>	<u>Pro</u>	<u>Gly</u>	<u>Ala</u>	<u>Gly</u>	<u>Ala</u>	<u>**Gly</u>	<u>Leu</u>	<u>Lys</u>	Pro	Ser	Cys	Ala	Lys	Glu
Val				20				25				30			
	Lys	Ser	Cys	Lys	Gly	Arg	Cys	Phe	Glu	Arg	Thr	Phe	Gly	Asn	Cys
Arg				35				40				45			
	Cys	Asp	Ala	Ala	Cys	Val	Glu	Leu	Gly	Asn	Cys	Cys	Leu	Asp	Tyr
Gln				50				55				60			
	Glu	Thr	Cys	Ile	Glu	Pro	Glu	His	Ile	Trp	Thr	Cys	Asn	Lys	Phe
Arg															
	65					70				75					80
	Cys	Gly	Glu	Lys	Arg	Leu	Thr	Arg	Ser	Leu	Cys	Ala	Cys	Ser	Asp
Asp															
				85					90				95		
	Cys	Lys	Asp	Lys	Gly	Asp	Cys	Cys	Ile	Asn	Tyr	Ser	Ser	Val	Cys
Gln															
				100				105				110			
	Gly	Glu	Lys	Ser	Trp	Val	Glu	Glu	Pro	Cys	Glu	Ser	Ile	Asn	Glu
Pro															
				115				120				125			
	Gln	Cys	Pro	Ala	Gly	Phe	Glu	Thr	Pro	Pro	Thr	Leu	Leu	Phe	Ser
Leu															
				130				135				140			
	Asp	Gly	Phe	Arg	Ala	Glu	Tyr	Leu	His	Thr	Trp	Gly	Gly	Leu	Leu
Pro															
	145					150					155				
160															
	Val	Ile	Ser	Lys	Leu	Lys	Lys	Cys	Gly	Thr	Tyr	Thr	Lys	Asn	Met
Arg															
				165					170				175		
	Pro	Val	Tyr	Pro	Thr	Lys	Thr	Phe	Pro	Asn	His	Tyr	Ser	Ile	Val
Thr															
				180				185				190			
	Gly	Leu	Tyr	Pro	Glu	Ser	His	Gly	Ile	Ile	Asp	Asn	Lys	Met	Tyr
Asp															
				195				200				205			
	Pro	Lys	Met	Asn	Ala	Ser	Phe	Ser	Leu	Lys	Ser	Lys	Glu	Lys	Phe
Asn															
				210				215				220			
	Pro	Glu	Trp	Tyr	Lys	Gly	Glu	Pro	Ile	Trp	Val	Thr	Ala	Lys	Tyr
Gln															
	225					230					235				
240															

	Gly	Leu	Lys	Ser	Gly	Thr	Phe	Phe	Trp	Pro	Gly	Ser	Asp	Val	Glu
Ile					245					250					255
	Asn	Gly	Ile	Phe	Pro	Asp	Ile	Tyr	Lys	Met	Tyr	Asn	Gly	Ser	Val
Pro				260						265					270
	Phe	Glu	Glu	Arg	Ile	Leu	Ala	Val	Leu	Gln	Trp	Leu	Gln	Leu	Pro
Lys			275					280					285		
	Asp	Glu	Arg	Pro	His	Phe	Tyr	Thr	Leu	Tyr	Leu	Glu	Glu	Pro	Asp
Ser		290						295				300			
	Ser	Gly	His	Ser	Tyr	Gly	Pro	Val	Ser	Ser	Glu	Val	Ile	Lys	Ala
Leu															
320	305					310					315				
	Gln	Arg	Val	Asp	Gly	Met	Val	Gly	Met	Leu	Met	Asp	Gly	Leu	Lys
Glu					325					330					335
	Leu	Asn	Leu	His	Arg	Cys	Leu	Asn	Leu	Ile	Leu	Ile	Ser	Asp	His
Gly															
	Met	Glu	Gln	Gly	Ser	Cys	Lys	Lys	Tyr	Ile	Tyr	Leu	Asn	Lys	Tyr
Leu															
	Gly	Asp	Val	Lys	Asn	Ile	Lys	Val	Ile	Tyr	Gly	Pro	Ala	Ala	Arg
Leu															
	Arg	Pro	Ser	Asp	Val	Pro	Asp	Lys	Tyr	Tyr	Ser	Phe	Asn	Tyr	Glu
Gly															
400	385					390						395			
	Ile	Ala	Arg	Asn	Leu	Ser	Cys	Arg	Glu	Pro	Asn	Gln	His	Phe	Lys
Pro															
	Tyr	Leu	Lys	His	Phe	Leu	Pro	Lys	Arg	Leu	His	Phe	Ala	Lys	Ser
Asp					405					410					415
	Arg	Ile	Glu	Pro	Leu	Thr	Phe	Tyr	Leu	Asp	Pro	Gln	Trp	Gln	Leu
Ala															
	Leu	Asn	Pro	Ser	Glu	Arg	Lys	Tyr	Cys	Gly	Ser	Gly	Phe	His	Gly
Ser															
	Asp	Asn	Val	Phe	Ser	Asn	Met	Gln	Ala	Leu	Phe	Val	Gly	Tyr	Gly
Pro															
480	465					470						475			
	Gly	Phe	Lys	His	Gly	Ile	Glu	Ala	Asp	Thr	Phe	Glu	Asn	Ile	Glu
Val															
	Tyr	Asn	Leu	Met	Cys	Asp	Leu	Leu	Asn	Leu	Thr	Pro	Ala	Pro	Asn
Asn															
	Gly	Thr	His	Gly	Ser	Leu	Asn	His	Leu	Leu	Lys	Asn	Pro	Val	Tyr
Thr															

	515		520		525		
Thr	Pro Lys His	Pro Lys Glu Val	His Pro Leu Val	Gln Cys	Pro Phe		
	530		535		540		
Leu	Arg Asn Pro	Arg Asp Asn Leu	Gly Cys Ser	Cys Asn Pro	Ser Ile		
560	545		550		555		
Glu	Pro Ile Glu	Asp Phe Gln Thr	Gln Phe Asn Leu	Thr Val Ala	Glu		
		565		570		575	
Leu	Lys Ile Ile	Lys His Glu Thr	Leu Pro Tyr	Gly Arg Pro	Arg Val		
		580		585		590	
Ser	Gln Lys Glu	Asn Thr Ile Cys	Leu Leu Ser	Gln His Gln	Phe Met		
	595		600		605		
Val	Gly Tyr Ser	Gln Asp Ile Leu	Met Pro Leu Trp	Thr Ser Tyr	Thr		
	610		615		620		
Tyr	Asp Arg Asn	Asp Ser Phe Ser	Thr Glu Asp	Phe Ser Asn	Cys Leu		
640	625		630		635		
Tyr	Gln Asp Phe	Arg Ile Pro Leu	Ser Pro Val	His Lys Cys	Ser Phe		
		645		650		655	
Leu	Lys Asn Asn	Thr Lys Val Ser	Tyr Gly Phe	Leu Ser Pro	Pro Gln		
		660		665		670	
Asn	Asn Lys Asn	Ser Ser Gly Ile	Tyr Ser Glu	Ala Leu Leu	Thr Thr		
	675		680		685		
His	Ile Val Pro	Met Tyr Gln Ser	Phe Gln Val	Ile Trp Arg	Tyr Phe		
	690		695		700		
Val	Asp Thr Leu	Leu Arg Lys Tyr	Ala Glu Glu	Arg Asn Gly	Val Asn		
720	705		710		715		
Ser	Val Ser Gly	Pro Val Phe Asp	Phe Asp Tyr	Asp Gly Arg	Cys Asp		
		725		730		735	
Ile	Leu Glu Asn	Leu Arg Gln Lys	Arg Arg Val	Ile Arg Asn	Gln Glu		
		740		745		750	
Thr	Leu Ile Pro	Thr His Phe Phe	Ile Val Leu	Thr Ser Cys	Lys Asp		
	755		760		765		
Ile	Ser Gln Thr	Pro Leu His Cys	Glu Asn Leu	Asp Thr Leu	Ala Phe		
	770		775		780		
His	Leu Pro His	Arg Thr Asp Asn	Ser Glu Ser	Cys Val His	Gly Lys		
800	785		790		795		

```

      Asp Ser Ser Trp Val Glu Glu Leu Leu Met Leu His Arg Ala Arg
Ile
      805                      810                      815
      Thr Asp Val Glu His Ile Thr Gly Leu Ser Phe Tyr Gln Gln Arg
Lys
      820                      825                      830
      Glu Pro Val Ser Asp Ile Leu Lys Leu Lys Thr His Leu Pro Thr
Phe
      835                      840                      845
      Ser Gln Glu Asp
      850

```

Singly underlined: signal peptide sequence; double-underlined: beginning and end of NPP1; ** = cleavage position at the signal peptide sequence

SEQ. ID NO: 27 - ENPP121 Amino Acid Sequence

```

Met Glu Arg Asp Gly Cys Ala Gly Gly Gly Ser Arg Gly Gly Glu
Gly
1           5           10           15
Gly Arg Ala Pro Arg Glu Gly Pro Ala Gly Asn Gly Arg Asp Arg
Gly
      20           25           30
Arg Ser His Ala Ala Glu Ala Pro Gly Asp Pro Gln Ala Ala Ala
Ser
      35           40           45
Leu Leu Ala Pro Met Asp Val Gly Glu Glu Pro Leu Glu Lys Ala
Ala
      50           55           60
Arg Ala Arg Thr Ala Lys Asp Pro Asn Thr Tyr Lys Ile Ile Ser
Leu
      65           70           75           80
Phe Thr Phe Ala Val Gly Val Asn Ile Cys Leu Gly**Phe Thr Ala
Gly
      85           90           95
Leu Lys Pro Ser Cys Ala Lys Glu Val Lys Ser Cys Lys Gly Arg
Cys
      100          105          110
Phe Glu Arg Thr Phe Gly Asn Cys Arg Cys Asp Ala Ala Cys Val
Glu
      115          120          125
Leu Gly Asn Cys Cys Leu Asp Tyr Gln Glu Thr Cys Ile Glu Pro
Glu
      130          135          140
His Ile Trp Thr Cys Asn Lys Phe Arg Cys Gly Glu Lys Arg Leu
Thr
      145          150          155
      Arg Ser Leu Cys Ala Cys Ser Asp Asp Cys Lys Asp Lys Gly Asp
Cys
      165          170          175

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	Cys	Ile	Asn	Tyr	Ser	Ser	Val	Cys	Gln	Gly	Glu	Lys	Ser	Trp	Val
Glu															
				180					185					190	
	Glu	Pro	Cys	Glu	Ser	Ile	Asn	Glu	Pro	Gln	Cys	Pro	Ala	Gly	Phe
Glu															
			195					200					205		
	Thr	Pro	Pro	Thr	Leu	Leu	Phe	Ser	Leu	Asp	Gly	Phe	Arg	Ala	Glu
Tyr															
		210					215					220			
	Leu	His	Thr	Trp	Gly	Gly	Leu	Leu	Pro	Val	Ile	Ser	Lys	Leu	Lys
Lys															
	225					230						235			
240															
	Cys	Gly	Thr	Tyr	Thr	Lys	Asn	Met	Arg	Pro	Val	Tyr	Pro	Thr	Lys
Thr															
				245						250				255	
	Phe	Pro	Asn	His	Tyr	Ser	Ile	Val	Thr	Gly	Leu	Tyr	Pro	Glu	Ser
His															
			260						265					270	
	Gly	Ile	Ile	Asp	Asn	Lys	Met	Tyr	Asp	Pro	Lys	Met	Asn	Ala	Ser
Phe															
		275						280					285		
	Ser	Leu	Lys	Ser	Lys	Glu	Lys	Phe	Asn	Pro	Glu	Trp	Tyr	Lys	Gly
Glu															
		290					295					300			
	Pro	Ile	Trp	Val	Thr	Ala	Lys	Tyr	Gln	Gly	Leu	Lys	Ser	Gly	Thr
Phe															
	305					310						315			
320															
	Phe	Trp	Pro	Gly	Ser	Asp	Val	Glu	Ile	Asn	Gly	Ile	Phe	Pro	Asp
Ile															
				325						330				335	
	Tyr	Lys	Met	Tyr	Asn	Gly	Ser	Val	Pro	Phe	Glu	Glu	Arg	Ile	Leu
Ala															
			340						345					350	
	Val	Leu	Gln	Trp	Leu	Gln	Leu	Pro	Lys	Asp	Glu	Arg	Pro	His	Phe
Tyr															
		355						360					365		
	Thr	Leu	Tyr	Leu	Glu	Glu	Pro	Asp	Ser	Ser	Gly	His	Ser	Tyr	Gly
Pro															
		370					375					380			
	Val	Ser	Ser	Glu	Val	Ile	Lys	Ala	Leu	Gln	Arg	Val	Asp	Gly	Met
Val															
	385					390						395			
400															
	Gly	Met	Leu	Met	Asp	Gly	Leu	Lys	Glu	Leu	Asn	Leu	His	Arg	Cys
Leu															
				405					410					415	
	Asn	Leu	Ile	Leu	Ile	Ser	Asp	His	Gly	Met	Glu	Gln	Gly	Ser	Cys
Lys															
			420						425				430		
	Lys	Tyr	Ile	Tyr	Leu	Asn	Lys	Tyr	Leu	Gly	Asp	Val	Lys	Asn	Ile
Lys															
		435						440				445			
	Val	Ile	Tyr	Gly	Pro	Ala	Ala	Arg	Leu	Arg	Pro	Ser	Asp	Val	Pro
Asp															

	450		455		460										
	Lys	Tyr	Tyr	Ser	Phe	Asn	Tyr	Glu	Gly	Ile	Ala	Arg	Asn	Leu	Ser
Cys															
	465					470					475				
480															
	Arg	Glu	Pro	Asn	Gln	His	Phe	Tys	Pro	Tyr	Leu	Lys	His	Phe	Leu
Pro															
					485					490					495
	Lys	Arg	Leu	His	Phe	Ala	Lys	Ser	Asp	Arg	Ile	Glu	Pro	Leu	Thr
Phe															
				500					505					510	
	Tyr	Leu	Asp	Pro	Gln	Trp	Gln	Leu	Ala	Leu	Asn	Pro	Ser	Glu	Arg
Lys															
			515				520					525			
	Tyr	Cys	Gly	Ser	Gly	Phe	His	Gly	Ser	Asp	Asn	Val	Phe	Ser	Asn
Met															
		530				535					540				
	Gln	Ala	Leu	Phe	Val	Gly	Tyr	Gly	Pro	Gly	Phe	Lys	His	Gly	Ile
Glu															
	545				550					555					
560															
	Ala	Asp	Thr	Phe	Glu	Asn	Ile	Glu	Val	Tyr	Asn	Leu	Met	Cys	Asp
Leu															
				565					570					575	
	Leu	Asn	Leu	Thr	Pro	Ala	Pro	Asn	Asn	Gly	Thr	His	Gly	Ser	Leu
Asn															
			580					585					590		
	His	Leu	Leu	Lys	Asn	Pro	Val	Tyr	Thr	Pro	Lys	His	Pro	Lys	Glu
Val															
		595					600				605				
	His	Pro	Leu	Val	Gln	Cys	Pro	Phe	Thr	Arg	Asn	Pro	Arg	Asp	Asn
Leu															
		610				615				620					
	Gly	Cys	Ser	Cys	Asn	Pro	Ser	Ile	Leu	Pro	Ile	Glu	Asp	Phe	Gln
Thr															
	625				630					635					
640															
	Gln	Phe	Asn	Leu	Thr	Val	Ala	Glu	Glu	Lys	Ile	Ile	Lys	His	Glu
Thr															
				645				650					655		
	Leu	Pro	Tyr	Gly	Arg	Pro	Arg	Val	Leu	Gln	Lys	Glu	Asn	Thr	Ile
Cys															
			660				665					670			
	Leu	Leu	Ser	Gln	His	Gln	Phe	Met	Ser	Gly	Tyr	Ser	Gln	Asp	Ile
Leu															
		675				680				685					
	Met	Pro	Leu	Trp	Thr	Ser	Tyr	Thr	Val	Asp	Arg	Asn	Asp	Ser	Phe
Ser															
		690			695				700						
	Thr	Glu	Asp	Phe	Ser	Asn	Cys	Leu	Tyr	Gln	Asp	Phe	Arg	Ile	Pro
Leu															
	705				710				715						
720															
	Ser	Pro	Val	His	Lys	Cys	Ser	Phe	Tyr	Lys	Asn	Asn	Thr	Lys	Val
Ser															
				725					730				735		

Ile	Tyr	Gly	Phe	Leu	Ser	Pro	Pro	Gln	Leu	Asn	Lys	Asn	Ser	Ser	Gly
				740					745					750	
Ser	Tyr	Ser	Glu	Ala	Leu	Leu	Thr	Thr	Asn	Ile	Val	Pro	Met	Tyr	Gln
			755					760					765		
Tyr	Phe	Gln	Val	Ile	Trp	Arg	Tyr	Phe	His	Asp	Thr	Leu	Leu	Arg	Lys
		770					775					780			
Asp	Ala	Glu	Glu	Arg	Asn	Gly	Val	Asn	Val	Val	Ser	Gly	Pro	Val	Phe
	785					790						795			
800															
Lys	Phe	Asp	Tyr	Asp	Gly	Arg	Cys	Asp	Ser	Leu	Glu	Asn	Leu	Arg	Gln
					805					810				815	
Phe	Arg	Arg	Val	Ile	Arg	Asn	Gln	Glu	Ile	Leu	Ile	Pro	Thr	His	Phe
				820					825					830	
Cys	Ile	Val	Leu	Thr	Ser	Cys	Lys	Asp	Thr	Ser	Gln	Thr	Pro	Leu	His
			835					840					845		
Asn	Glu	Asn	Leu	Asp	Thr	Leu	Ala	Phe	Ile	Leu	Pro	His	Arg	Thr	Asp
		850					855				860				
Glu	Ser	Glu	Ser	Cys	Val	His	Gly	Lys	His	Asp	Ser	Ser	Trp	Val	Glu
	865					870					875				
880															
Thr	Leu	Leu	Met	Leu	His	Arg	Ala	Arg	Ile	Thr	Asp	Val	Glu	His	Ile
				885					890					895	
Leu	Gly	Leu	Ser	Phe	Tyr	Gln	Gln	Arg	Lys	Glu	Pro	Val	Ser	Asp	Ile
			900					905					910		
	Lys	Leu	Lys	Thr	His	Leu	Pro	Thr	Phe	Ser	<u>Gln</u>	<u>Glu</u>	<u>Asp</u>		
		915					920					925			

Singly underlined: signal peptide sequence; double-underlined: beginning and end of NPP1; ** = cleavage position at the signal peptide sequence

SEQ. ID. NO: 28 - ENPP121-Fc Amino Acid Sequence

Gly	Met	Glu	Arg	Asp	Gly	Cys	Ala	Gly	Gly	Gly	Ser	Arg	Gly	Gly	Glu
	1				5					10				15	
Gly	Gly	Arg	Ala	Pro	Arg	Glu	Gly	Pro	Ala	Gly	Asn	Gly	Arg	Asp	Arg
				20					25				30		
Ser	Arg	Ser	His	Ala	Ala	Glu	Ala	Pro	Gly	Asp	Pro	Gln	Ala	Ala	Ala
			35					40					45		

Leu Leu Ala Pro Met Asp Val Gly Glu Glu Pro Leu Glu Lys Ala
 Ala 50 55 60
 Arg Ala Arg Thr Ala Lys Asp Pro Asn Thr Tyr Lys Ile Ile Ser
 Leu 65 70 75 80
Phe Thr Phe Ala Val Gly Val Asn Ile Cys Leu Gly**Phe Thr Ala
Gly
 85 90 95
Leu Lys Pro Ser Cys Ala Lys Glu Val Lys Ser Cys Lys Gly Arg
 Cys 100 105 110
 Phe Glu Arg Thr Phe Gly Asn Cys Arg Cys Asp Ala Ala Cys Val
 Glu 115 120 125
 Leu Gly Asn Cys Cys Leu Asp Tyr Gln Glu Thr Cys Ile Glu Pro
 Glu 130 135 140
 His Ile Trp Thr Cys Asn Lys Phe Arg Cys Gly Glu Lys Arg Leu
 Thr 145 150 155
 160
 Arg Ser Leu Cys Ala Cys Ser Asp Asp Cys Lys Asp Lys Gly Asp
 Cys 165 170 175
 Cys Ile Asn Tyr Ser Ser Val Cys Gln Gly Glu Lys Ser Trp Val
 Glu 180 185 190
 Glu Pro Cys Glu Ser Ile Asn Glu Pro Gln Cys Pro Ala Gly Phe
 Glu 195 200 205
 Thr Pro Pro Thr Leu Leu Phe Ser Leu Asp Gly Phe Arg Ala Glu
 Tyr 210 215 220
 Leu His Thr Trp Gly Gly Leu Leu Pro Val Ile Ser Lys Leu Lys
 Lys 225 230 235
 240
 Cys Gly Thr Tyr Thr Lys Asn Met Arg Pro Val Tyr Pro Thr Lys
 Thr 245 250 255
 Phe Pro Asn His Tyr Ser Ile Val Thr Gly Leu Tyr Pro Glu Ser
 His 260 265 270
 Gly Ile Ile Asp Asn Lys Met Tyr Asp Pro Lys Met Asn Ala Ser
 Phe 275 280 285
 Ser Leu Lys Ser Lys Glu Lys Phe Asn Pro Glu Trp Tyr Lys Gly
 Glu 290 295 300
 Pro Ile Trp Val Thr Ala Lys Tyr Gln Gly Leu Lys Ser Gly Thr
 Phe 305 310 315
 320
 Phe Trp Pro Gly Ser Asp Val Glu Ile Asn Gly Ile Phe Pro Asp
 Ile

				325					330				335		
	Tyr	Lys	Met	Tyr	Asn	Gly	Ser	Val	Pro	Phe	Glu	Glu	Arg	Ile	Leu
Ala															
				340					345				350		
	Val	Leu	Gln	Trp	Leu	Gln	Leu	Pro	Lys	Asp	Glu	Arg	Pro	His	Phe
Tyr															
			355					360					365		
	Thr	Leu	Tyr	Leu	Glu	Glu	Pro	Asp	Ser	Ser	Gly	His	Ser	Tyr	Gly
Pro															
		370					375				380				
	Val	Ser	Ser	Glu	Val	Ile	Lys	Ala	Leu	Gln	Arg	Val	Asp	Gly	Met
Val															
400	385					390					395				
	Gly	Met	Leu	Met	Asp	Gly	Leu	Lys	Glu	Leu	Asn	Leu	His	Arg	Cys
Leu															
				405					410					415	
	Asn	Leu	Ile	Leu	Ile	Ser	Asp	His	Gly	Met	Glu	Gln	Gly	Ser	Cys
Lys															
			420						425				430		
	Lys	Tyr	Ile	Tyr	Leu	Asn	Lys	Tyr	Leu	Gly	Asp	Val	Lys	Asn	Ile
Lys															
		435						440				445			
	Val	Ile	Tyr	Gly	Pro	Ala	Ala	Arg	Leu	Arg	Pro	Ser	Asp	Val	Pro
Asp															
		450					455				460				
	Lys	Tyr	Tyr	Ser	Phe	Asn	Tyr	Glu	Gly	Ile	Ala	Arg	Asn	Leu	Ser
Cys															
480	465					470					475				
	Arg	Glu	Pro	Asn	Gln	His	Phe	Lys	Pro	Tyr	Leu	Lys	His	Phe	Leu
Pro															
				485					490				495		
	Lys	Arg	Leu	His	Phe	Ala	Lys	Ser	Asp	Arg	Ile	Glu	Pro	Leu	Thr
Phe															
			500						505				510		
	Tyr	Leu	Asp	Pro	Gln	Trp	Gln	Leu	Ala	Leu	Asn	Pro	Ser	Glu	Arg
Lys															
		515						520				525			
	Tyr	Cys	Gly	Ser	Gly	Phe	His	Gly	Ser	Asp	Asn	Val	Phe	Ser	Asn
Met															
		530					535				540				
	Gln	Ala	Leu	Phe	Val	Gly	Tyr	Gly	Pro	Gly	Phe	Lys	His	Gly	Ile
Glu															
560	545					550					555				
	Ala	Asp	Thr	Phe	Glu	Asn	Ile	Glu	Val	Tyr	Asn	Leu	Met	Cys	Asp
Leu															
				565					570				575		
	Leu	Asn	Leu	Thr	Pro	Ala	Pro	Asn	Asn	Gly	Thr	His	Gly	Ser	Leu
Asn															
			580						585			590			
	His	Leu	Leu	Lys	Asn	Pro	Val	Tyr	Thr	Pro	Lys	His	Pro	Lys	Glu
Val															
			595					600				605			

	His	Pro	Leu	Val	Gln	Cys	Pro	Phe	Thr	Arg	Asn	Pro	Arg	Asp	Asn
Leu															
		610					615				620				
	Gly	Cys	Ser	Cys	Asn	Pro	Ser	Ile	Leu	Pro	Ile	Glu	Asp	Phe	Gln
Thr															
		625				630					635				
640															
	Gln	Phe	Asn	Leu	Thr	Val	Ala	Glu	Glu	Lys	Ile	Ile	Lys	His	Glu
Thr															
				645					650					655	
	Leu	Pro	Tyr	Gly	Arg	Pro	Arg	Val	Leu	Gln	Lys	Glu	Asn	Thr	Ile
Cys															
			660					665					670		
	Leu	Leu	Ser	Gln	His	Gln	Phe	Met	Ser	Gly	Tyr	Ser	Gln	Asp	Ile
Leu															
		675					680					685			
	Met	Pro	Leu	Trp	Thr	Ser	Tyr	Thr	Val	Asp	Arg	Asn	Asp	Ser	Phe
Ser															
		690				695					700				
	Thr	Glu	Asp	Phe	Ser	Asn	Cys	Leu	Tyr	Gln	Asp	Phe	Arg	Ile	Pro
Leu															
		705				710					715				
720															
	Ser	Pro	Val	His	Lys	Cys	Ser	Phe	Tyr	Lys	Asn	Asn	Thr	Lys	Val
Ser															
				725					730					735	
	Tyr	Gly	Phe	Leu	Ser	Pro	Pro	Gln	Leu	Asn	Lys	Asn	Ser	Ser	Gly
Ile															
			740					745					750		
	Tyr	Ser	Glu	Ala	Leu	Leu	Thr	Thr	Asn	Ile	Val	Pro	Met	Tyr	Gln
Ser															
		755					760					765			
	Phe	Gln	Val	Ile	Trp	Arg	Tyr	Phe	His	Asp	Thr	Leu	Leu	Arg	Lys
Tyr															
		770				775					780				
	Ala	Glu	Glu	Arg	Asn	Gly	Val	Asn	Val	Val	Ser	Gly	Pro	Val	Phe
Asp															
		785				790					795				
800															
	Phe	Asp	Tyr	Asp	Gly	Arg	Cys	Asp	Ser	Leu	Glu	Asn	Leu	Arg	Gln
Lys															
				805					810					815	
	Arg	Arg	Val	Ile	Arg	Asn	Gln	Glu	Ile	Leu	Ile	Pro	Thr	His	Phe
Phe															
			820					825					830		
	Ile	Val	Leu	Thr	Ser	Cys	Lys	Asp	Thr	Ser	Gln	Thr	Pro	Leu	His
Cys															
		835					840					845			
	Glu	Asn	Leu	Asp	Thr	Leu	Ala	Phe	Ile	Leu	Pro	His	Arg	Thr	Asp
Asn															
		850				855					860				
	Ser	Glu	Ser	Cys	Val	His	Gly	Lys	His	Asp	Ser	Ser	Trp	Val	Glu
Glu															
		865				870					875				
880															

Thr Leu Leu Met Leu His Arg Ala Arg Ile Thr Asp Val Glu His Ile
 885 890 895
 Gly Leu Ser Phe Tyr Gln Gln Arg Lys Glu Pro Val Ser Asp Ile
 Leu 900 905 910
 Lys Leu Lys Thr His Leu Pro Thr Phe Ser Gln Glu Asp Leu Ile
 Asn 915 920 925
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
 Gly 930 935 940
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
 Met 945 950 955
 960
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
 His 965 970 975
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
 Val 980 985 990
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
 Tyr 995 1000 1005
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
 1010 1015 1020
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
 1025 1030 1035
Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
 1040 1045 1050
Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys
 1055 1060 1065
Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
 1070 1075 1080
Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
 1085 1090 1095
Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
 1100 1105 1110
Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
 1115 1120 1125
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
 1130 1135 1140
Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 1145 1150 1155

Singly underlined: signal peptide sequence; double-underlined:
 beginning and end of NPP1; ** = cleavage position at the signal
 peptide sequence; bold residues indicate Fc sequence

SEQ. ID NO: 29 - ENPP121-ALB Amino Acid Sequence:

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

	Gly	Arg	Ala	Pro	Arg	Glu	Gly	Pro	Ala	Gly	Asn	Gly	Arg	Asp	Arg
Gly															
			20					25					30		
	Arg	Ser	His	Ala	Ala	Glu	Ala	Pro	Gly	Asp	Pro	Gln	Ala	Ala	Ala
Ser															
			35					40					45		
	Leu	Leu	Ala	Pro	Met	Asp	Val	Gly	Glu	Glu	Pro	Leu	Glu	Lys	Ala
Ala															
		50					55					60			
	Arg	Ala	Arg	Thr	Ala	Lys	Asp	Pro	Asn	Thr	Tyr	Lys	Ile	Ile	Ser
Leu															
	65					70					75				80
	<u>Phe</u>	<u>Thr</u>	<u>Phe</u>	<u>Ala</u>	<u>Val</u>	<u>Gly</u>	<u>Val</u>	<u>Asn</u>	<u>Ile</u>	<u>Cys</u>	<u>Leu</u>	<u>Gly</u>	<u>**Phe</u>	<u>Thr</u>	<u>Ala</u>
<u>Gly</u>															
					85				90					95	
	<u>Leu</u>	<u>Lys</u>		Pro	Ser	Cys	Ala	Lys	Glu	Val	Lys	Ser	Cys	Lys	Gly
Cys															
			100						105					110	
	Phe	Glu	Arg	Thr	Phe	Gly	Asn	Cys	Arg	Cys	Asp	Ala	Ala	Cys	Val
Glu															
			115					120					125		
	Leu	Gly	Asn	Cys	Cys	Leu	Asp	Tyr	Gln	Glu	Thr	Cys	Ile	Glu	Pro
Glu															
		130					135					140			
	His	Ile	Trp	Thr	Cys	Asn	Lys	Phe	Arg	Cys	Gly	Glu	Lys	Arg	Leu
Thr															
	145					150					155				
160															
	Arg	Ser	Leu	Cys	Ala	Cys	Ser	Asp	Asp	Cys	Lys	Asp	Lys	Gly	Asp
Cys															
			165						170					175	
	Cys	Ile	Asn	Tyr	Ser	Ser	Val	Cys	Gln	Gly	Glu	Lys	Ser	Trp	Val
Glu															
			180					185					190		
	Glu	Pro	Cys	Glu	Ser	Ile	Asn	Glu	Pro	Gln	Cys	Pro	Ala	Gly	Phe
Glu															
		195						200					205		
	Thr	Pro	Pro	Thr	Leu	Leu	Phe	Ser	Leu	Asp	Gly	Phe	Arg	Ala	Glu
Tyr															
		210					215					220			
	Leu	His	Thr	Trp	Gly	Gly	Leu	Leu	Pro	Val	Ile	Ser	Lys	Leu	Lys
Lys															
	225					230					235				
240															
	Cys	Gly	Thr	Tyr	Thr	Lys	Asn	Met	Arg	Pro	Val	Tyr	Pro	Thr	Lys
Thr															
			245					250						255	
	Phe	Pro	Asn	His	Tyr	Ser	Ile	Val	Thr	Gly	Leu	Tyr	Pro	Glu	Ser
His															
			260					265					270		
	Gly	Ile	Ile	Asp	Asn	Lys	Met	Tyr	Asp	Pro	Lys	Met	Asn	Ala	Ser
Phe															
		275					280				285				
	Ser	Leu	Lys	Ser	Lys	Glu	Lys	Phe	Asn	Pro	Glu	Trp	Tyr	Lys	Gly
Glu															
		290				295					300				

Phe	Pro	Ile	Trp	Val	Thr	Ala	Lys	Tyr	Gln	Gly	Leu	Lys	Ser	Gly	Thr
	305					310					315				
320	Phe	Trp	Pro	Gly	Ser	Asp	Val	Glu	Ile	Asn	Gly	Ile	Phe	Pro	Asp
Tle					325					330					335
	Tyr	Lys	Met	Tyr	Asn	Gly	Ser	Val	Pro	Phe	Glu	Glu	Arg	Ile	Leu
Ala				340					345					350	
	Val	Leu	Gln	Trp	Leu	Gln	Leu	Pro	Lys	Asp	Glu	Arg	Pro	His	Phe
Tyr			355					360					365		
	Thr	Leu	Tyr	Leu	Glu	Glu	Pro	Asp	Ser	Ser	Gly	His	Ser	Tyr	Gly
Pro		370					375				380				
	Val	Ser	Ser	Glu	Val	Ile	Lys	Ala	Leu	Gln	Arg	Val	Asp	Gly	Met
Val															
400		385				390					395				
	Gly	Met	Leu	Met	Asp	Gly	Leu	Lys	Glu	Leu	Asn	Leu	His	Arg	Cys
Leu					405					410				415	
	Asn	Leu	Ile	Leu	Ile	Ser	Asp	His	Gly	Met	Glu	Gln	Gly	Ser	Cys
Lys															
		420						425					430		
	Lys	Tyr	Ile	Tyr	Leu	Asn	Lys	Tyr	Leu	Gly	Asp	Val	Lys	Asn	Ile
Lys															
		435					440					445			
	Val	Ile	Tyr	Gly	Pro	Ala	Ala	Arg	Leu	Arg	Pro	Ser	Asp	Val	Pro
Asp															
		450				455					460				
	Lys	Tyr	Tyr	Ser	Phe	Asn	Tyr	Glu	Gly	Ile	Ala	Arg	Asn	Leu	Ser
Cys															
480		465				470					475				
	Arg	Glu	Pro	Asn	Gln	His	Phe	Lys	Pro	Tyr	Leu	Lys	His	Phe	Leu
Pro															
				485					490					495	
	Lys	Arg	Leu	His	Phe	Ala	Lys	Ser	Asp	Arg	Ile	Glu	Pro	Leu	Thr
Phe															
			500					505					510		
	Tyr	Leu	Asp	Pro	Gln	Trp	Gln	Leu	Ala	Leu	Asn	Pro	Ser	Glu	Arg
Lys															
		515					520				525				
	Tyr	Cys	Gly	Ser	Gly	Phe	His	Gly	Ser	Asp	Asn	Val	Phe	Ser	Asn
Met															
		530				535					540				
	Gln	Ala	Leu	Phe	Val	Gly	Tyr	Gly	Pro	Gly	Phe	Lys	His	Gly	Ile
Glu															
560		545				550					555				
	Ala	Asp	Thr	Phe	Glu	Asn	Ile	Glu	Val	Tyr	Asn	Leu	Met	Cys	Asp
Leu															
					565					570				575	

	Leu	Asn	Leu	Thr	Pro	Ala	Pro	Asn	Asn	Gly	Thr	His	Gly	Ser	Leu
Asn															
				580					585					590	
	His	Leu	Leu	Lys	Asn	Pro	Val	Tyr	Thr	Pro	Lys	His	Pro	Lys	Glu
Val															
			595					600					605		
	His	Pro	Leu	Val	Gln	Cys	Pro	Phe	Thr	Arg	Asn	Pro	Arg	Asp	Asn
Leu															
	610					615				620					
	Gly	Cys	Ser	Cys	Asn	Pro	Ser	Ile	Leu	Pro	Ile	Glu	Asp	Phe	Gln
Thr															
	625					630					635				
640															
	Gln	Phe	Asn	Leu	Thr	Val	Ala	Glu	Glu	Lys	Ile	Ile	Lys	His	Glu
Thr															
				645					650					655	
	Leu	Pro	Tyr	Gly	Arg	Pro	Arg	Val	Leu	Gln	Lys	Glu	Asn	Thr	Ile
Cys															
			660					665					670		
	Leu	Leu	Ser	Gln	His	Gln	Phe	Met	Ser	Gly	Tyr	Ser	Gln	Asp	Ile
Leu															
		675					680					685			
	Met	Pro	Leu	Trp	Thr	Ser	Tyr	Thr	Val	Asp	Arg	Asn	Asp	Ser	Phe
Ser															
		690				695				700					
	Thr	Glu	Asp	Phe	Ser	Asn	Cys	Leu	Tyr	Gln	Asp	Phe	Arg	Ile	Pro
Leu															
	705					710				715					
720															
	Ser	Pro	Val	His	Lys	Cys	Ser	Phe	Tyr	Lys	Asn	Asn	Thr	Lys	Val
Ser															
				725					730					735	
	Tyr	Gly	Phe	Leu	Ser	Pro	Pro	Gln	Leu	Asn	Lys	Asn	Ser	Ser	Gly
Ile															
			740					745					750		
	Tyr	Ser	Glu	Ala	Leu	Leu	Thr	Thr	Asn	Ile	Val	Pro	Met	Tyr	Gln
Ser															
		755					760				765				
	Phe	Gln	Val	Ile	Trp	Arg	Tyr	Phe	His	Asp	Thr	Leu	Leu	Arg	Lys
Tyr															
	770					775				780					
	Ala	Glu	Glu	Arg	Asn	Gly	Val	Asn	Val	Val	Ser	Gly	Pro	Val	Phe
Asp															
	785					790				795					
800															
	Phe	Asp	Tyr	Asp	Gly	Arg	Cys	Asp	Ser	Leu	Glu	Asn	Leu	Arg	Gln
Lys															
				805					810					815	
	Arg	Arg	Val	Ile	Arg	Asn	Gln	Glu	Ile	Leu	Ile	Pro	Thr	His	Phe
Phe															
			820					825					830		
	Ile	Val	Leu	Thr	Ser	Cys	Lys	Asp	Thr	Ser	Gln	Thr	Pro	Leu	His
Cys															
		835					840				845				
	Glu	Asn	Leu	Asp	Thr	Leu	Ala	Phe	Ile	Leu	Pro	His	Arg	Thr	Asp
Asn															

		850					855				860				
Glu	Ser	Glu	Ser	Cys	Val	His	Gly	Lys	His	Asp	Ser	Ser	Trp	Val	Glu
	865						870				875				
880	Leu	Leu	Met	Leu	His	Arg	Ala	Arg	Ile	Thr	Asp	Val	Glu	His	Ile
Thr					885					890				895	
	Gly	Leu	Ser	Phe	Tyr	Gln	Gln	Arg	Lys	Glu	Pro	Val	Ser	Asp	Ile
Leu				900					905					910	
	Lys	Leu	Lys	Thr	His	Leu	Pro	Thr	Phe	Ser	<u>Gln</u>	<u>Glu</u>	<u>Asp</u>	Arg	Ser
Gly				915				920				925			
Val	Ser	Gly	Gly	Ser	Met	Lys	Trp	Val	Thr	Phe	Leu	Leu	Leu	Leu	Phe
		930					935				940				
	Ser	Gly	Ser	Ala	Phe	Ser	Arg	Gly	Val	Phe	Arg	Arg	Glu	Ala	His
Lys															
960	945					950					955				
	Ser	Glu	Ile	Ala	His	Arg	Tyr	Asn	Asp	Leu	Gly	Glu	Gln	His	Phe
Lys															
				965					970					975	
Tyr	Gly	Leu	Val	Leu	Ile	Ala	Phe	Ser	Gln	Tyr	Leu	Gln	Lys	Cys	Ser
		980						985					990		
Thr	Asp	Glu	His	Ala	Lys	Leu	Val	Gln	Glu	Val	Thr	Asp	Phe	Ala	Lys
		995					1000					1005			
	Cys	Val	Ala	Asp	Glu	Ser	Ala	Ala	Asn	Cys	Asp	Lys	Ser	Leu	His
	1010						1015					1020			
	Thr	Leu	Phe	Gly	Asp	Lys	Leu	Cys	Ala	Ile	Pro	Asn	Leu	Arg	Glu
	1025						1030					1035			
	Asn	Tyr	Gly	Glu	Leu	Ala	Asp	Cys	Cys	Thr	Lys	Gln	Glu	Pro	Glu
	1040						1045					1050			
	Arg	Asn	Glu	Cys	Phe	Leu	Gln	His	Lys	Asp	Asp	Asn	Pro	Ser	Leu
	1055						1060					1065			
	Pro	Pro	Phe	Glu	Arg	Pro	Glu	Ala	Glu	Ala	Met	Cys	Thr	Ser	Phe
	1070						1075					1080			
	Lys	Glu	Asn	Pro	Thr	Thr	Phe	Met	Gly	His	Tyr	Leu	His	Glu	Val
	1085						1090					1095			
	Ala	Arg	Arg	His	Pro	Tyr	Phe	Tyr	Ala	Pro	Glu	Leu	Leu	Tyr	Tyr
	1100						1105					1110			
	Ala	Glu	Gln	Tyr	Asn	Glu	Ile	Leu	Thr	Gln	Cys	Cys	Ala	Glu	Ala
	1115						1120					1125			
	Asp	Lys	Glu	Ser	Cys	Leu	Thr	Pro	Lys	Leu	Asp	Gly	Val	Lys	Glu
	1130						1135					1140			
	Lys	Ala	Leu	Val	Ser	Ser	Val	Arg	Gln	Arg	Met	Lys	Cys	Ser	Ser
	1145						1150					1155			
	Met	Gln	Lys	Phe	Gly	Glu	Arg	Ala	Phe	Lys	Ala	Trp	Ala	Val	Ala
	1160						1165					11			

Gly	Asp	Leu	Leu	Glu	Cys	Ala	Asp	Asp	Arg	Ala	Glu	Leu	Ala	Lys
	1205					1210					1215			
Tyr	Met	Cys	Glu	Asn	Gln	Ala	Thr	Ile	Ser	Ser	Lys	Leu	Gln	Thr
	1220					1225					1230			
Cys	Cys	Asp	Lys	Pro	Leu	Leu	Lys	Lys	Ala	His	Cys	Leu	Ser	Glu
	1235					1240					1245			
Val	Glu	His	Asp	Thr	Met	Pro	Ala	Asp	Leu	Pro	Ala	Ile	Ala	Ala
	1250					1255					1260			
Asp	Phe	Val	Glu	Asp	Gln	Glu	Val	Cys	Lys	Asn	Tyr	Ala	Glu	Ala
	1265					1270					1275			
Lys	Asp	Val	Phe	Leu	Gly	Thr	Phe	Leu	Tyr	Glu	Tyr	Ser	Arg	Arg
	1280					1285					1290			
His	Pro	Asp	Tyr	Ser	Val	Ser	Leu	Leu	Leu	Arg	Leu	Ala	Lys	Lys
	1295					1300					1305			
Tyr	Glu	Ala	Thr	Leu	Glu	Lys	Cys	Cys	Ala	Glu	Ala	Asn	Pro	Pro
	1310					1315					1320			
Ala	Cys	Tyr	Gly	Thr	Val	Leu	Ala	Glu	Phe	Gln	Pro	Leu	Val	Glu
	1325					1330					1335			
Glu	Pro	Lys	Asn	Leu	Val	Lys	Thr	Asn	Cys	Asp	Leu	Tyr	Glu	Lys
	1340					1345					1350			
Leu	Gly	Glu	Tyr	Gly	Phe	Gln	Asn	Ala	Ile	Leu	Val	Arg	Tyr	Thr
	1355					1360					1365			
Gln	Lys	Ala	Pro	Gln	Val	Ser	Thr	Pro	Thr	Leu	Val	Glu	Ala	Ala
	1370					1375					1380			
Arg	Asn	Leu	Gly	Arg	Val	Gly	Thr	Lys	Cys	Cys	Thr	Leu	Pro	Glu
	1385					1390					1395			
Asp	Gln	Arg	Leu	Pro	Cys	Val	Glu	Asp	Tyr	Leu	Ser	Ala	Ile	Leu
	1400					1405					1410			
Asn	Arg	Val	Cys	Leu	Leu	His	Glu	Lys	Thr	Pro	Val	Ser	Glu	His
	1415					1420					1425			
Val	Thr	Lys	Cys	Cys	Ser	Gly	Ser	Leu	Val	Glu	Arg	Arg	Pro	Cys
	1430					1435					1440			
Phe	Ser	Ala	Leu	Thr	Val	Asp	Glu	Thr	Tyr	Val	Pro	Lys	Glu	Phe
	1445					1450					1455			
Lys	Ala	Glu	Thr	Phe	Thr	Phe	His	Ser	Asp	Ile	Cys	Thr	Leu	Pro
	1460					1465					1470			
Glu	Lys	Glu	Lys	Gln	Ile	Lys	Lys	Gln	Thr	Ala	Leu	Ala	Glu	Leu
	1475					1480					1485			
Val	Lys	His	Lys	Pro	Lys	Ala	Thr	Ala	Glu	Gln	Leu	Lys	Thr	Val
	1490					1495					1500			
Met	Asp	Asp	Phe	Ala	Gln	Phe	Leu	Asp	Thr	Cys	Cys	Lys	Ala	Ala
	1505					1510					1515			
Asp	Lys	Asp	Thr	Cys	Phe	Ser	Thr	Glu	Gly	Pro	Asn	Leu	Val	Thr
	1520					1525					1530			
Arg	Cys	Lys	Asp	Ala	Leu	Ala	Arg	Ser	Trp	Ser	His	Pro	Gln	Phe
	1535					1540					1545			
Glu	Lys													
	1550													

Singly underlined: signal peptide sequence; double-underlined: beginning and end of NPP1; ** = cleavage position at the signal peptide sequence; bold residues indicate albumin sequence

SEQ. ID NO: 30 - ENPP121-NPP3-Fc sequence

Met Glu Arg Asp Gly Cys Ala Gly Gly Gly Ser Arg Gly Gly Glu
 Gly 1 5 10 15
 Gly Arg Ala Pro Arg Glu Gly Pro Ala Gly Asn Gly Arg Asp Arg
 Gly 20 25 30
 Arg Ser His Ala Ala Glu Ala Pro Gly Asp Pro Gln Ala Ala Ala
 Ser 35 40 45
 Leu Leu Ala Pro Met Asp Val Gly Glu Glu Pro Leu Glu Lys Ala
 Ala 50 55 60
 Arg Ala Arg Thr Ala Lys Asp Pro Asn Thr Tyr Lys Ile Ile Ser
 Leu 65 70 75 80
Ala**Lys Phe Thr Phe Ala Val Gly Val Asn Ile Cys Leu Gly Phe Thr
 85 90 95
 Gln Gly Ser Cys Arg Lys Lys Cys Phe Asp Ala Ser Phe Arg Gly
 Leu 100 105 110
 Glu Asn Cys Arg Cys Asp Val Ala Cys Lys Asp Arg Gly Asp Cys
 Cys 115 120 125
 Trp Asp Phe Glu Asp Thr Cys Val Glu Ser Thr Arg Ile Trp Met
 Cys 130 135 140
 Asn Lys Phe Arg Cys Gly Glu Arg Leu Glu Ala Ser Leu Cys Ser
 Cys 145 150 155
 160 Ser Asp Asp Cys Leu Gln Arg Lys Asp Cys Cys Ala Asp Tyr Lys
 Ser 165 170 175
 Val Cys Gln Gly Glu Thr Ser Trp Leu Glu Glu Asn Cys Asp Thr
 Ala 180 185 190
 Gln Gln Ser Gln Cys Pro Glu Gly Phe Asp Leu Pro Pro Val Ile
 Leu 195 200 205
 Phe Ser Met Asp Gly Phe Arg Ala Glu Tyr Leu Tyr Thr Trp Asp
 Thr 210 215 220
 Leu Met Pro Asn Ile Asn Lys Leu Lys Thr Cys Gly Ile His Ser
 Lys 225 230 235
 240 Tyr Met Arg Ala Met Tyr Pro Thr Lys Thr Phe Pro Asn His Tyr
 Thr 245 250 255
 Ile Val Thr Gly Leu Tyr Pro Glu Ser His Gly Ile Ile Asp Asn
 Asn 260 265 270

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

	545		550		555										
560	Glu	Val	Tyr	Asn	Leu	Met	Cys	Asp	Leu	Leu	Arg	Ile	Gln	Pro	Ala
Pro															
				565					570					575	
	Asn	Asn	Gly	Thr	His	Gly	Ser	Leu	Asn	His	Leu	Leu	Lys	Val	Pro
Phe															
			580						585					590	
	Tyr	Glu	Pro	Ser	His	Ala	Glu	Glu	Val	Ser	Lys	Phe	Ser	Val	Cys
Gly															
		595						600					605		
	Phe	Ala	Asn	Pro	Leu	Pro	Thr	Glu	Ser	Leu	Asp	Cys	Phe	Cys	Pro
His															
		610					615					620			
	Leu	Gln	Asn	Ser	Thr	Gln	Leu	Glu	Gln	Val	Asn	Gln	Met	Leu	Asn
Leu															
	625					630					635				
640															
	Thr	Gln	Glu	Glu	Ile	Thr	Ala	Thr	Val	Lys	Val	Asn	Leu	Pro	Phe
Gly															
				645					650					655	
	Arg	Pro	Arg	Val	Leu	Gln	Lys	Asn	Val	Asp	His	Cys	Leu	Leu	Tyr
His															
			660						665				670		
	Arg	Glu	Tyr	Val	Ser	Gly	Phe	Gly	Lys	Ala	Met	Arg	Met	Pro	Met
Trp															
		675						680				685			
	Ser	Ser	Tyr	Thr	Val	Pro	Gln	Leu	Gly	Asp	Thr	Ser	Pro	Leu	Pro
Pro															
		690					695				700				
	Thr	Val	Pro	Asp	Cys	Leu	Arg	Ala	Asp	Val	Arg	Val	Pro	Pro	Ser
Glu															
	705					710					715				
720															
	Ser	Gln	Lys	Cys	Ser	Phe	Tyr	Leu	Ala	Asp	Lys	Asn	Ile	Thr	His
Gly															
			725						730				735		
	Phe	Leu	Tyr	Pro	Pro	Ala	Ser	Asn	Arg	Thr	Ser	Asp	Ser	Gln	Tyr
Asp															
			740						745				750		
	Ala	Leu	Ile	Thr	Ser	Asn	Leu	Val	Pro	Met	Tyr	Glu	Glu	Phe	Arg
Lys															
		755						760				765			
	Met	Trp	Asp	Tyr	Phe	His	Ser	Val	Leu	Leu	Ile	Lys	His	Ala	Thr
Glu															
		770					775				780				
	Arg	Asn	Gly	Val	Asn	Val	Val	Ser	Gly	Pro	Ile	Phe	Asp	Tyr	Asn
Tyr															
	785					790					795				
800															
	Asp	Gly	His	Phe	Asp	Ala	Pro	Asp	Glu	Ile	Thr	Lys	His	Leu	Ala
Asn															
			805						810				815		
	Thr	Asp	Val	Pro	Ile	Pro	Thr	His	Tyr	Phe	Val	Val	Leu	Thr	Ser
Cys															
			820						825				830		

Lys Asn Lys Ser His Thr Pro Glu Asn Cys Pro Gly Trp Leu Asp
 Val
 835 840 845
 Leu Pro Phe Ile Ile Pro His Arg Pro Thr Asn Val Glu Ser Cys
 Pro
 850 855 860
 Glu Gly Lys Pro Glu Ala Leu Trp Val Glu Glu Arg Phe Thr Ala
 His
 865 870 875
 880
 Ile Ala Arg Val Arg Asp Val Glu Leu Leu Thr Gly Leu Asp Phe
 Tyr
 885 890 895
 Gln Asp Lys Val Gln Pro Val Ser Glu Ile Leu Gln Leu Lys Thr
 Tyr
 900 905 910
Leu Pro Thr Phe Glu Thr Thr Ile Asp Lys Thr His Thr Cys Pro
 Pro
 915 920 925
 Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
 Pro
 930 935 940
 Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
 Thr
 945 950 955
 960
 Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
 Asn
 965 970 975
 Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
 Arg
 980 985 990
 Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
 Val
 995 1000 1005
 Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
 1010 1015 1020
 Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
 1025 1030 1035
 Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
 1040 1045 1050
 Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
 1055 1060 1065
 Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
 1070 1075 1080
 Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
 1085 1090 1095
 Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
 1100 1105 1110
 Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
 1115 1120 1125
 His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
 1130 1135 1140
 Ser Pro Gly Lys
 1145

Singly underlined: signal peptide sequence; double-underlined: beginning and end of NPP1; ** = cleavage position at the signal peptide sequence; bold residues indicate Fc sequence

SEQ. ID NO: 31 - ENPP121-NPP3-Albumin sequence

	Met	Glu	Arg	Asp	Gly	Cys	Ala	Gly	Gly	Gly	Ser	Arg	Gly	Gly	Glu
Gly	1				5					10					15
	Gly	Arg	Ala	Pro	Arg	Glu	Gly	Pro	Ala	Gly	Asn	Gly	Arg	Asp	Arg
Gly					20					25					30
	Arg	Ser	His	Ala	Ala	Glu	Ala	Pro	Gly	Asp	Pro	Gln	Ala	Ala	Ala
Ser					35					40					45
	Leu	Leu	Ala	Pro	Met	Asp	Val	Gly	Glu	Glu	Pro	Leu	Glu	Lys	Ala
Ala					50					55					60
	Arg	Ala	Arg	Thr	Ala	Lys	Asp	Pro	Asn	Thr	Tyr	Lys	Ile	Ile	Ser
Leu					65					70					75
	<u>Phe</u>	<u>Thr</u>	<u>Phe</u>	<u>Ala</u>	<u>Val</u>	<u>Gly</u>	<u>Val</u>	<u>Asn</u>	<u>Ile</u>	<u>Cys</u>	<u>Leu</u>	<u>Gly</u>	<u>Phe</u>	<u>Thr</u>	80
Ala**	<u>Lys</u>														
					85					90					95
	<u>Gln</u>	<u>Gly</u>	Ser	Cys	Arg	Lys	Lys	Cys	Phe	Asp	Ala	Ser	Phe	Arg	Gly
Leu					100					105					110
	Glu	Asn	Cys	Arg	Cys	Asp	Val	Ala	Cys	Lys	Asp	Arg	Gly	Asp	Cys
Cys					115					120					125
	Trp	Asp	Phe	Glu	Asp	Thr	Cys	Val	Glu	Ser	Thr	Arg	Ile	Trp	Met
Cys					130					135					140
	Asn	Lys	Phe	Arg	Cys	Gly	Glu	Arg	Leu	Glu	Ala	Ser	Leu	Cys	Ser
Cys					145					150					155
160															
	Ser	Asp	Asp	Cys	Leu	Gln	Arg	Lys	Asp	Cys	Cys	Ala	Asp	Tyr	Lys
Ser					165					170					175
	Val	Cys	Gln	Gly	Glu	Thr	Ser	Trp	Leu	Glu	Glu	Asn	Cys	Asp	Thr
Ala					180					185					190
	Gln	Gln	Ser	Gln	Cys	Pro	Glu	Gly	Phe	Asp	Leu	Pro	Pro	Val	Ile
Leu					195					200					205
	Phe	Ser	Met	Asp	Gly	Phe	Arg	Ala	Glu	Tyr	Leu	Tyr	Thr	Trp	Asp
Thr					210					215					220
	Leu	Met	Pro	Asn	Ile	Asn	Lys	Leu	Lys	Thr	Cys	Gly	Ile	His	Ser
Lys					225					230					235
240															

	Tyr	Met	Arg	Ala	Met	Tyr	Pro	Thr	Lys	Thr	Phe	Pro	Asn	His	Tyr
Thr															
					245					250					255
	Ile	Val	Thr	Gly	Leu	Tyr	Pro	Glu	Ser	His	Gly	Ile	Ile	Asp	Asn
Asn															
				260					265					270	
	Met	Tyr	Asp	Val	Asn	Leu	Asn	Lys	Asn	Phe	Ser	Leu	Ser	Ser	Lys
Glu															
			275					280					285		
	Gln	Asn	Asn	Pro	Ala	Trp	Trp	His	Gly	Gln	Pro	Met	Trp	Leu	Thr
Ala															
		290					295					300			
	Met	Tyr	Gln	Gly	Leu	Lys	Ala	Ala	Thr	Tyr	Phe	Trp	Pro	Gly	Ser
Glu															
	305					310					315				
320															
	Val	Ala	Ile	Asn	Gly	Ser	Phe	Pro	Ser	Ile	Tyr	Met	Pro	Tyr	Asn
Gly															
				325						330					335
	Ser	Val	Pro	Phe	Glu	Glu	Arg	Ile	Ser	Thr	Leu	Leu	Lys	Trp	Leu
Asp															
				340					345					350	
	Leu	Pro	Lys	Ala	Glu	Arg	Pro	Arg	Phe	Tyr	Thr	Met	Tyr	Phe	Glu
Glu															
			355					360					365		
	Pro	Asp	Ser	Ser	Gly	His	Ala	Gly	Gly	Pro	Val	Ser	Ala	Arg	Val
Ile															
		370					375					380			
	Lys	Ala	Leu	Gln	Val	Val	Asp	His	Ala	Phe	Gly	Met	Leu	Met	Glu
Gly															
	385					390					395				
400															
	Leu	Lys	Gln	Arg	Asn	Leu	His	Asn	Cys	Val	Asn	Ile	Ile	Leu	Leu
Ala															
				405						410					415
	Asp	His	Gly	Met	Asp	Gln	Thr	Tyr	Cys	Asn	Lys	Met	Glu	Tyr	Met
Thr															
				420					425					430	
	Asp	Tyr	Phe	Pro	Arg	Ile	Asn	Phe	Phe	Tyr	Met	Tyr	Glu	Gly	Pro
Ala															
			435					440					445		
	Pro	Arg	Ile	Arg	Ala	His	Asn	Ile	Pro	His	Asp	Phe	Phe	Ser	Phe
Asn															
		450					455					460			
	Ser	Glu	Glu	Ile	Val	Arg	Asn	Leu	Ser	Cys	Arg	Lys	Pro	Asp	Gln
His															
	465					470					475				
480															
	Phe	Lys	Pro	Tyr	Leu	Thr	Pro	Asp	Leu	Pro	Lys	Arg	Leu	His	Tyr
Ala															
				485					490						495
	Lys	Asn	Val	Arg	Ile	Asp	Lys	Val	His	Leu	Phe	Val	Asp	Gln	Gln
Trp															
				500					505					510	
	Leu	Ala	Val	Arg	Ser	Lys	Ser	Asn	Thr	Asn	Cys	Gly	Gly	Gly	Asn
His															

		515				520			525						
	Gly	Tyr	Asn	Asn	Glu	Phe	Arg	Ser	Met	Glu	Ala	Ile	Phe	Leu	Ala
His															
		530				535			540						
	Gly	Pro	Ser	Phe	Lys	Glu	Lys	Thr	Glu	Val	Glu	Pro	Phe	Glu	Asn
Tle															
	545					550					555				
560															
	Glu	Val	Tyr	Asn	Leu	Met	Cys	Asp	Leu	Leu	Arg	Ile	Gln	Pro	Ala
Pro															
				565				570						575	
	Asn	Asn	Gly	Thr	His	Gly	Ser	Leu	Asn	His	Leu	Leu	Lys	Val	Pro
Phe															
			580					585					590		
	Tyr	Glu	Pro	Ser	His	Ala	Glu	Glu	Val	Ser	Lys	Phe	Ser	Val	Cys
Gly															
		595				600						605			
	Phe	Ala	Asn	Pro	Leu	Pro	Thr	Glu	Ser	Leu	Asp	Cys	Phe	Cys	Pro
His															
		610				615						620			
	Leu	Gln	Asn	Ser	Thr	Gln	Leu	Glu	Gln	Val	Asn	Gln	Met	Leu	Asn
Leu															
	625					630					635				
640															
	Thr	Gln	Glu	Glu	Ile	Thr	Ala	Thr	Val	Lys	Val	Asn	Leu	Pro	Phe
Gly															
				645				650						655	
	Arg	Pro	Arg	Val	Leu	Gln	Lys	Asn	Val	Asp	His	Cys	Leu	Leu	Tyr
His															
			660					665					670		
	Arg	Glu	Tyr	Val	Ser	Gly	Phe	Gly	Lys	Ala	Met	Arg	Met	Pro	Met
Trp															
		675				680						685			
	Ser	Ser	Tyr	Thr	Val	Pro	Gln	Leu	Gly	Asp	Thr	Ser	Pro	Leu	Pro
Pro															
		690				695						700			
	Thr	Val	Pro	Asp	Cys	Leu	Arg	Ala	Asp	Val	Arg	Val	Pro	Pro	Ser
Glu															
	705					710					715				
720															
	Ser	Gln	Lys	Cys	Ser	Phe	Tyr	Leu	Ala	Asp	Lys	Asn	Ile	Thr	His
Gly															
			725					730						735	
	Phe	Leu	Tyr	Pro	Pro	Ala	Ser	Asn	Arg	Thr	Ser	Asp	Ser	Gln	Tyr
Asp															
			740					745					750		
	Ala	Leu	Ile	Thr	Ser	Asn	Leu	Val	Pro	Met	Tyr	Glu	Glu	Phe	Arg
Lys															
		755				760						765			
	Met	Trp	Asp	Tyr	Phe	His	Ser	Val	Leu	Leu	Ile	Lys	His	Ala	Thr
Glu															
		770				775						780			
	Arg	Asn	Gly	Val	Asn	Val	Val	Ser	Gly	Pro	Ile	Phe	Asp	Tyr	Asn
Tyr															
	785					790						795			
800															

Asp Gly His Phe Asp Ala Pro Asp Glu Ile Thr Lys His Leu Ala
 Asn
 805 810 815
 Thr Asp Val Pro Ile Pro Thr His Tyr Phe Val Val Leu Thr Ser
 Cys
 820 825 830
 Lys Asn Lys Ser His Thr Pro Glu Asn Cys Pro Gly Trp Leu Asp
 Val
 835 840 845
 Leu Pro Phe Ile Ile Pro His Arg Pro Thr Asn Val Glu Ser Cys
 Pro
 850 855 860
 Glu Gly Lys Pro Glu Ala Leu Trp Val Glu Glu Arg Phe Thr Ala
 His
 865 870 875
 880
 Ile Ala Arg Val Arg Asp Val Glu Leu Leu Thr Gly Leu Asp Phe
 Tyr
 885 890 895
 Gln Asp Lys Val Gln Pro Val Ser Glu Ile Leu Gln Leu Lys Thr
 Tyr
 900 905 910
 Leu Pro Thr Phe Glu Thr Thr Ile Gly Gly Gly Ser Gly Gly Gly
 Gly
 915 920 925
 Ser Gly Gly Gly Gly Ser Met Lys Trp Val Thr Phe Leu Leu Leu
 Leu
 930 935 940
 Phe Val Ser Gly Ser Ala Phe Ser Arg Gly Val Phe Arg Arg Glu
 Ala
 945 950 955
 960
 His Lys Ser Glu Ile Ala His Arg Tyr Asn Asp Leu Gly Glu Gln
 His
 965 970 975
 Phe Lys Gly Leu Val Leu Ile Ala Phe Ser Gln Tyr Leu Gln Lys
 Cys
 980 985 990
 Ser Tyr Asp Glu His Ala Lys Leu Val Gln Glu Val Thr Asp Phe
 Ala
 995 1000 1005
 Lys Thr Cys Val Ala Asp Glu Ser Ala Ala Asn Cys Asp Lys Ser
 1010 1015 1020
 Leu His Thr Leu Phe Gly Asp Lys Leu Cys Ala Ile Pro Asn Leu
 1025 1030 1035
 Arg Glu Asn Tyr Gly Glu Leu Ala Asp Cys Cys Thr Lys Gln Glu
 1040 1045 1050
 Pro Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro
 1055 1060 1065
 Ser Leu Pro Pro Phe Glu Arg Pro Glu Ala Glu Ala Met Cys Thr
 1070 1075 1080
 Ser Phe Lys Glu Asn Pro Thr Thr Phe Met Gly His Tyr Leu His
 1085 1090 1095
 Glu Val Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu
 1100 1105 1110
 Tyr Tyr Ala Glu Gln Tyr Asn Glu Ile Leu Thr Gln Cys Cys Ala

1115	Glu Ala Asp Lys Glu Ser	1120	Cys Leu Thr Pro Lys	1125	Leu Asp Gly Val
1130	Lys Glu Lys Ala Leu Val	1135	Ser Ser Val Arg Gln	1140	Arg Met Lys Cys
1145	Ser Ser Met Gln Lys Phe	1150	Gly Glu Arg Ala Phe	1155	Lys Ala Trp Ala
1160	Val Ala Arg Leu Ser Gln	1165	Thr Phe Pro Asn Ala	1170	Asp Phe Ala Glu
1175	Ile Thr Lys Leu Ala Thr	1180	Asp Leu Thr Lys Val	1185	Asn Lys Glu Cys
1190	Cys His Gly Asp Leu Leu	1195	Glu Cys Ala Asp Asp	1200	Arg Ala Glu Leu
1205	Ala Lys Tyr Met Cys Glu	1210	Asn Gln Ala Thr Ile	1215	Ser Ser Lys Leu
1220	Gln Thr Cys Cys Asp Lys	1225	Pro Leu Leu Lys Lys	1230	Ala His Cys Leu
1235	Ser Glu Val Glu His Asp	1240	Thr Met Pro Ala Asp	1245	Leu Pro Ala Ile
1250	Ala Ala Asp Phe Val Glu	1255	Asp Gln Glu Val Cys	1260	Lys Asn Tyr Ala
1265	Glu Ala Lys Asp Val Phe	1270	Leu Gly Thr Phe Leu	1275	Tyr Glu Tyr Ser
1280	Arg Arg His Pro Asp Tyr	1285	Ser Val Ser Leu Leu	1290	Leu Arg Leu Ala
1295	Lys Lys Tyr Glu Ala Thr	1300	Leu Glu Lys Cys Cys	1305	Ala Glu Ala Asn
1310	Pro Pro Ala Cys Tyr Gly	1315	Thr Val Leu Ala Glu	1320	Phe Gln Pro Leu
1325	Val Glu Glu Pro Lys Asn	1330	Leu Val Lys Thr Asn	1335	Cys Asp Leu Tyr
1340	Glu Lys Leu Gly Glu Tyr	1345	Gly Phe Gln Asn Ala	1350	Ile Leu Val Arg
1355	Tyr Thr Gln Lys Ala Pro	1360	Gln Val Ser Thr Pro	1365	Thr Leu Val Glu
1370	Ala Ala Arg Asn Leu Gly	1375	Arg Val Gly Thr Lys	1380	Cys Cys Thr Leu
1385	Pro Glu Asp Gln Arg Leu	1390	Pro Cys Val Glu Asp	1395	Tyr Leu Ser Ala
1400	Ile Leu Asn Arg Val Cys	1405	Leu Leu His Glu Lys	1410	Thr Pro Val Ser
1415	Glu His Val Thr Lys Cys	1420	Cys Ser Gly Ser Leu	1425	Val Glu Arg Arg
1430	Pro Cys Phe Ser Ala Leu	1435	Thr Val Asp Glu Thr	1440	Tyr Val Pro Lys
1445	Glu Phe Lys Ala Glu Thr	1450	Phe Thr Phe His Ser	1455	Asp Ile Cys Thr
1460	Leu Pro Glu Lys Glu Lys	1465	Gln Ile Lys Lys Gln	1470	Thr Ala Leu Ala
1475	Glu Leu Val Lys His Lys	1480	Pro Lys Ala Thr Ala	1485	Glu Gln Leu Lys
1490	Thr Val Met Asp Asp Phe	1495	Ala Gln Phe Leu Asp	1500	Thr Cys Cys Lys
1505	Ala Ala Asp Lys Asp Thr	1510	Cys Phe Ser Thr Glu	1515	Gly Pro Asn Leu
1520	Val Thr Arg Cys Lys Asp	1525	Ala Leu Ala	1530	

1535

1540

Singly underlined: signal peptide sequence; double-underlined: beginning and end of NPP3; ** = cleavage position at the signal peptide sequence; bold residues indicate albumin sequence

SEQ. ID NO: 32 - ENPP121GLK Protein Export Signal Sequence

Met Glu Arg Asp Gly Cys Ala Gly Gly Gly Ser Arg Gly Gly Glu
 Gly 1 5 10 15
 Gly Arg Ala Pro Arg Glu Gly Pro Ala Gly Asn Gly Arg Asp Arg
 Gly 20 25 30
 Arg Ser His Ala Ala Glu Ala Pro Gly Asp Pro Gln Ala Ala Ala
 Ser 35 40 45
 Leu Leu Ala Pro Met Asp Val Gly Glu Glu Pro Leu Glu Lys Ala
 Ala 50 55 60
 Arg Ala Arg Thr Ala Lys Asp Pro Asn Thr Tyr Lys Ile Ile Ser
 Leu 65 70 75 80
Phe Thr Phe Ala Val Gly Val Asn Ile Cys Leu Gly Phe Thr Ala
Gly 85 90 95
Leu Lys

SEQ. ID NO: 33 - Albumin Sequence

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
 Met 1 5 10 15
 Lys Trp Val Thr Phe Leu Leu Leu Leu Phe Val Ser Gly Ser Ala
 Phe 20 25 30
 Ser Arg Gly Val Phe Arg Arg Glu Ala His Lys Ser Glu Ile Ala
 His 35 40 45
 Arg Tyr Asn Asp Leu Gly Glu Gln His Phe Lys Gly Leu Val Leu
 Ile 50 55 60
 Ala Phe Ser Gln Tyr Leu Gln Lys Cys Ser Tyr Asp Glu His Ala
 Lys 65 70 75 80
 Leu Val Gln Glu Val Thr Asp Phe Ala Lys Thr Cys Val Ala Asp
 Glu 85 90 95
 Ser Ala Ala Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
 Lys

				100					105				110		
	Leu	Cys	Ala	Ile	Pro	Asn	Leu	Arg	Glu	Asn	Tyr	Gly	Glu	Leu	Ala
Asp															
			115					120					125		
	Cys	Cys	Thr	Lys	Gln	Glu	Pro	Glu	Arg	Asn	Glu	Cys	Phe	Leu	Gln
His															
		130					135					140			
	Lys	Asp	Asp	Asn	Pro	Ser	Leu	Pro	Pro	Phe	Glu	Arg	Pro	Glu	Ala
Glu															
	145						150					155			
160															
	Ala	Met	Cys	Thr	Ser	Phe	Lys	Glu	Asn	Pro	Thr	Thr	Phe	Met	Gly
His															
				165					170					175	
	Tyr	Leu	His	Glu	Val	Ala	Arg	Arg	His	Pro	Tyr	Phe	Tyr	Ala	Pro
Glu															
			180					185					190		
	Leu	Leu	Tyr	Tyr	Ala	Glu	Gln	Tyr	Asn	Glu	Ile	Leu	Thr	Gln	Cys
Cys															
		195					200					205			
	Ala	Glu	Ala	Asp	Lys	Glu	Ser	Cys	Leu	Thr	Pro	Lys	Leu	Asp	Gly
Val															
		210					215				220				
	Lys	Glu	Lys	Ala	Leu	Val	Ser	Ser	Val	Arg	Gln	Arg	Met	Lys	Cys
Ser															
	225						230				235				
240															
	Ser	Met	Gln	Lys	Phe	Gly	Glu	Arg	Ala	Phe	Lys	Ala	Trp	Ala	Val
Ala															
				245				250					255		
	Arg	Leu	Ser	Gln	Thr	Phe	Pro	Asn	Ala	Asp	Phe	Ala	Glu	Ile	Thr
Lys															
			260				265					270			
	Leu	Ala	Thr	Asp	Leu	Thr	Lys	Val	Asn	Lys	Glu	Cys	Cys	His	Gly
Asp															
		275					280					285			
	Leu	Leu	Glu	Cys	Ala	Asp	Asp	Arg	Ala	Glu	Leu	Ala	Lys	Tyr	Met
Cys															
		290					295				300				
	Glu	Asn	Gln	Ala	Thr	Ile	Ser	Ser	Lys	Leu	Gln	Thr	Cys	Cys	Asp
Lys															
	305						310				315				
320															
	Pro	Leu	Leu	Lys	Lys	Ala	His	Cys	Leu	Ser	Glu	Val	Glu	His	Asp
Thr															
				325				330					335		
	Met	Pro	Ala	Asp	Leu	Pro	Ala	Ile	Ala	Ala	Asp	Phe	Val	Glu	Asp
Gln															
			340				345				350				
	Glu	Val	Cys	Lys	Asn	Tyr	Ala	Glu	Ala	Lys	Asp	Val	Phe	Leu	Gly
Thr															
		355					360				365				
	Phe	Leu	Tyr	Glu	Tyr	Ser	Arg	Arg	His	Pro	Asp	Tyr	Ser	Val	Ser
Leu															
		370					375				380				

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

SEQ. ID NO: 34 - Human IgG Fc domain, Fc

Gly Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
 1 5 10 15
 Met Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu

				20					25					30		
His	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Val	Asp	Val	Ser
			35					40					45			
Val	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	
		50					55					60				
Tyr	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	
	65				70				75						80	
Gly	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	
				85					90					95		
Ile	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	
				100					105					110		
Val	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	
		115						120					125			
Ser	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	
		130					135					140				
Glu	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	
	145					150					155					
160																
Pro	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	
				165					170					175		
Val	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	
				180					185					190		
Met	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	
		195						200					205			
Ser	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	
		210					215					220				
	Pro	Gly	Lys													
	225															

SEQ. ID NO: 35 - Albumin Sequence

Ala	Met	Lys	Trp	Val	Thr	Phe	Leu	Leu	Leu	Leu	Phe	Val	Ser	Gly	Ser
	1			5					10					15	
Ala	Phe	Ser	Arg	Gly	Val	Phe	Arg	Arg	Glu	Ala	His	Lys	Ser	Glu	Ile
				20					25					30	
Leu	His	Arg	Tyr	Asn	Asp	Leu	Gly	Glu	Gln	His	Phe	Lys	Gly	Leu	Val
			35					40					45		
Ala	Ile	Ala	Phe	Ser	Gln	Tyr	Leu	Gln	Lys	Cys	Ser	Tyr	Asp	Glu	His
		50					55					60			

	Lys	Leu	Val	Gln	Glu	Val	Thr	Asp	Phe	Ala	Lys	Thr	Cys	Val	Ala
Asp	65					70					75				80
	Glu	Ser	Ala	Ala	Asn	Cys	Asp	Lys	Ser	Leu	His	Thr	Leu	Phe	Gly
Asp					85					90					95
	Lys	Leu	Cys	Ala	Ile	Pro	Asn	Leu	Arg	Glu	Asn	Tyr	Gly	Glu	Leu
Ala				100					105					110	
	Asp	Cys	Cys	Thr	Lys	Gln	Glu	Pro	Glu	Arg	Asn	Glu	Cys	Phe	Leu
Gln				115				120					125		
	His	Lys	Asp	Asp	Asn	Pro	Ser	Leu	Pro	Pro	Phe	Glu	Arg	Pro	Glu
Ala				130				135					140		
	Glu	Ala	Met	Cys	Thr	Ser	Phe	Lys	Glu	Asn	Pro	Thr	Thr	Phe	Met
Gly															
145						150					155				
160															
	His	Tyr	Leu	His	Glu	Val	Ala	Arg	Arg	His	Pro	Tyr	Phe	Tyr	Ala
Pro															
				165					170					175	
	Glu	Leu	Leu	Tyr	Tyr	Ala	Glu	Gln	Tyr	Asn	Glu	Ile	Leu	Thr	Gln
Cys															
				180					185					190	
	Cys	Ala	Glu	Ala	Asp	Lys	Glu	Ser	Cys	Leu	Thr	Pro	Lys	Leu	Asp
Gly															
				195				200					205		
	Val	Lys	Glu	Lys	Ala	Leu	Val	Ser	Ser	Val	Arg	Gln	Arg	Met	Lys
Cys															
				210				215					220		
	Ser	Ser	Met	Gln	Lys	Phe	Gly	Glu	Arg	Ala	Phe	Lys	Ala	Trp	Ala
Val															
225						230					235				
240															
	Ala	Arg	Leu	Ser	Gln	Thr	Phe	Pro	Asn	Ala	Asp	Phe	Ala	Glu	Ile
Thr															
				245					250					255	
	Lys	Leu	Ala	Thr	Asp	Leu	Thr	Lys	Val	Asn	Lys	Glu	Cys	Cys	His
Gly															
				260				265					270		
	Asp	Leu	Leu	Glu	Cys	Ala	Asp	Asp	Arg	Ala	Glu	Leu	Ala	Lys	Tyr
Met															
				275				280					285		
	Cys	Glu	Asn	Gln	Ala	Thr	Ile	Ser	Ser	Lys	Leu	Gln	Thr	Cys	Cys
Asp															
				290				295					300		
	Lys	Pro	Leu	Leu	Lys	Lys	Ala	His	Cys	Leu	Ser	Glu	Val	Glu	His
Asp															
305						310					315				
320															
	Thr	Met	Pro	Ala	Asp	Leu	Pro	Ala	Ile	Ala	Ala	Asp	Phe	Val	Glu
Asp															
				325				330					335		
	Gln	Glu	Val	Cys	Lys	Asn	Tyr	Ala	Glu	Ala	Lys	Asp	Val	Phe	Leu
Gly															

				340					345				350		
	Thr	Phe	Leu	Tyr	Glu	Tyr	Ser	Arg	Arg	His	Pro	Asp	Tyr	Ser	Val
Ser															
			355					360				365			
	Leu	Leu	Leu	Arg	Leu	Ala	Lys	Lys	Tyr	Glu	Ala	Thr	Leu	Glu	Lys
Cys															
		370					375				380				
	Cys	Ala	Glu	Ala	Asn	Pro	Pro	Ala	Cys	Tyr	Gly	Thr	Val	Leu	Ala
Glu															
	385					390					395				
400															
	Phe	Gln	Pro	Leu	Val	Glu	Glu	Pro	Lys	Asn	Leu	Val	Lys	Thr	Asn
Cys															
				405						410				415	
	Asp	Leu	Tyr	Glu	Lys	Leu	Gly	Glu	Tyr	Gly	Phe	Gln	Asn	Ala	Ile
Leu															
			420					425					430		
	Val	Arg	Tyr	Thr	Gln	Lys	Ala	Pro	Gln	Val	Ser	Thr	Pro	Thr	Leu
Val															
		435					440				445				
	Glu	Ala	Ala	Arg	Asn	Leu	Gly	Arg	Val	Gly	Thr	Lys	Cys	Cys	Thr
Leu															
		450				455			460						
	Pro	Glu	Asp	Gln	Arg	Leu	Pro	Cys	Val	Glu	Asp	Tyr	Leu	Ser	Ala
Ile															
	465				470				475						
480															
	Leu	Asn	Arg	Val	Cys	Leu	Leu	His	Glu	Lys	Thr	Pro	Val	Ser	Glu
His															
			485					490					495		
	Val	Thr	Lys	Cys	Cys	Ser	Gly	Ser	Leu	Val	Glu	Arg	Arg	Pro	Cys
Phe															
			500				505				510				
	Ser	Ala	Leu	Thr	Val	Asp	Glu	Thr	Tyr	Val	Pro	Lys	Glu	Phe	Lys
Ala															
		515				520			525						
	Glu	Thr	Phe	Thr	Phe	His	Ser	Asp	Ile	Cys	Thr	Leu	Pro	Glu	Lys
Glu															
		530				535			540						
	Lys	Gln	Ile	Lys	Lys	Gln	Thr	Ala	Leu	Ala	Glu	Leu	Val	Lys	His
Lys															
	545				550				555						
560															
	Pro	Lys	Ala	Thr	Ala	Glu	Gln	Leu	Lys	Thr	Val	Met	Asp	Asp	Phe
Ala															
			565				570					575			
	Gln	Phe	Leu	Asp	Thr	Cys	Cys	Lys	Ala	Ala	Asp	Lys	Asp	Thr	Cys
Phe															
			580				585				590				
	Ser	Thr	Glu	Gly	Pro	Asn	Leu	Val	Thr	Arg	Cys	Lys	Asp	Ala	Leu
Ala															
		595				600			605						
	Arg	Ser	Trp	Ser	His	Pro	Gln	Phe	Glu	Lys					
		610				615									

SEQ. ID NO: 36 - ENPP2 Signal Peptide

Leu Phe Thr Phe Ala Val Gly Val Asn Ile Cys Leu Gly
 1 5 10 15
 Phe Thr Ala

SEQ. ID NO: 37 - Signal Sequence ENPP7

Met Arg Gly Pro Ala Val Leu Leu Thr Val Ala Leu Ala Thr Leu
 Leu 1 5 10 15
 Ala Pro Gly Ala
 20

SEQ. ID NO: 38 - Signal sequence ENPP7

Met Arg Gly Pro Ala Val Leu Leu Thr Val Ala Leu Ala Thr Leu
 Leu 1 5 10 15
 Ala Pro Gly Ala Gly Ala
 20

SEQ. ID NO: 39 - Signal Sequence ENPP1-2-1

Met Glu Arg Asp Gly Cys Ala Gly Gly Gly Ser Arg Gly Gly Glu
 Gly 1 5 10 15
 Gly Arg Ala Pro Arg Glu Gly Pro Ala Gly Asn Gly Arg Asp Arg
 Gly 20 25 30
 Arg Ser His Ala Ala Glu Ala Pro Gly Asp Pro Gln Ala Ala Ala
 Ser 35 40 45
 Leu Leu Ala Pro Met Asp Val Gly Glu Glu Pro Leu Glu Lys Ala
 Ala 50 55 60
 Arg Ala Arg Thr Ala Lys Asp Pro Asn Thr Tyr Lys Ile Ile Ser
 Leu 65 70 75 80
Phe Thr Phe Ala Val Gly Val Asn Ile Cys Leu Gly Phe Thr Ala
 85 90 95

SEQ. ID NO: 40 - exENPP3

Leu Leu Val Ile Met Ser Leu Gly Leu Gly Leu Gly Leu Gly Leu
 Arg 1 5 10 15
 Lys

SEQ. ID NO: 41 - Signal Sequence ENPP5:

Met Thr Ser Lys Phe Leu Leu Val Ser Phe Ile Leu Ala Ala Leu
 Ser
 1 5 10 15
 Leu Ser Thr Thr Phe Ser
 20

SEQ. ID NO: 42 - Signal Sequence - Azurocidin

Met Thr Arg Leu Thr Val Leu Ala Leu Leu Ala Gly Leu Leu Ala
 Ser

Ser Arg Ala

SEQ. ID NO: 43 - Linker

Asp Ser Ser

SEQ. ID NO: 44 - Linker

Glu Ser Ser

SEQ. ID NO: 45 - Linker

Arg Gln Gln

SEQ. ID NO: 46 - Linker

Lys Arg

SEQ. ID NO: 47 - Linker

(Arg)_m ; m=0-15

SEQ. ID NO: 48 - Linker

Asp Ser Ser Ser Glu Glu Lys Phe Leu Arg Arg Ile Gly Arg Phe
 Gly

SEQ. ID NO: 49 - Linker

Glu Glu Glu Glu Glu Glu Glu Pro Arg Gly Asp Thr

1 5 10

SEQ. ID NO: 50 - Linker

Ala Pro Trp His Leu Ser Ser Gln Tyr Ser Arg Thr

1 5 10

SEQ. ID NO: 51 - Linker

Ser Thr Leu Pro Ile Pro His Glu Phe Ser Arg Glu

1 5 10

SEQ. ID NO: 52 - Linker

Val Thr Lys His Leu Asn Gln Ile Ser Gln Ser Tyr

1 5 10

SEQ. ID NO: 53 - Linker

(Glu)_m; m=1-15

SEQ. ID NO: 54 - Linker

Leu Ile Asn

SEQ. ID NO: 55 - Linker

Gly Gly Ser Gly Gly Ser

1 5

SEQ. ID NO: 56 - Linker

Arg Ser Gly Ser Gly Gly Ser

1 5

SEQ. ID NO: 57 - Linker

(Asp)_m; m=1-15

1

SEQ. ID NO: 58 - Linker

Lys Leu Val Ile Met Ser Leu Gly Leu Gly Leu Gly Leu Gly Leu Arg

1 5 10 15

SEQ. ID NO: 59 - Linker

Val Ile Met Ser Leu Gly Leu Gly Leu Gly Leu Gly Leu Arg Lys

1 5 10 15

SEQ. ID NO: 60 - Linker

Ile Met Ser Leu Gly Leu Gly Leu Gly Leu Gly Leu Arg Lys

1 5 10

SEQ. ID NO: 61 - Linker

Met Ser Leu Gly Leu Gly Leu Gly Leu Gly Leu Arg Lys

1 5 10

SEQ. ID NO: 62 - Linker

Ser Leu Gly Leu Gly Leu Gly Leu Gly Leu Arg Lys

1 5 10

SEQ. ID NO: 63 - Linker

Leu Gly Leu Gly Leu Gly Leu Gly Leu Arg Lys

1 5 10

SEQ. ID NO: 64 - Linker

Gly Leu Gly Leu Gly Leu Gly Leu Arg Lys

1 5 10

SEQ. ID NO: 65 - Linker

Leu Gly Leu Gly Leu Gly Leu Arg Lys

1 5

SEQ. ID NO: 66 - Linker

Gly Leu Gly Leu Gly Leu Arg Lys

1 5

SEQ. ID NO: 67 - Linker

Leu Gly Leu Gly Leu Arg Lys

1 5

SEQ. ID NO: 68 - Linker

Gly Leu Gly Leu Arg Lys

1 5

SEQ. ID NO: 69 - Linker

Leu Gly Leu Arg Lys

1 5

SEQ. ID NO: 70 - Linker

Gly Leu Arg Lys

1

SEQ. ID NO: 71 - Linker

Leu Arg Lys

1

SEQ. ID NO: 72 - Linker

Arg Lys

1

SEQ. ID NO: 73 - Linker

(Lys)_m; m=0-15

1

SEQ. ID NO: 74 -Linker

D_m; m=1-15

SEQ. ID NO: 75 -Linker

(GGGGS)_n; n=1-10

SEQ. ID NO: 76 - ENPP3 Nucleotide sequence

atggaatcta cgttgacttt agcaacggaa caacctgtta agaagaacac tcttaagaaa	60
tataaaatag cttgcattgt tcttcttgct ttgctggtga tcatgtcact tggattaggc	120
ctggggccttg gactcaggaa actggaaaag caaggcagct gcaggaagaa gtgctttgat	180
gcatcattta gaggactgga gaactgccgg tgtgatgtgg catgtaaaga ccgagggtgat	240
tgctgctggg attttgaaga cacctgtgtg gaatcaactc gaatatggat gtgcaataaa	300
tttcgttgty gagagaccag attagaggcc agcctttgct cttgttcaga tgactgtttg	360
cagaggaaag attgctgtgc tgactataag agtggttgcc aaggagaaac ctcatggctg	420
gaagaaaact gtgacacagc ccagcagtct cagtgccagc aagggtttga cctgccacca	480
gttatcttgt tttctatgga tggatttaga gctgaatatt tatacacatg ggatacttta	540
atgccaaata tcaataaact gaaaacatgt ggaattcatt caaaatacat gagagctatg	600
tatcctacca aaaccttccc aaatcattac accattgtca cgggcttgta tccagagtca	660
catggcatca ttgacaataa tatgtatgat gtaaatctca acaagaatth ttcactttct	720
tcaaaggaac aaaataatcc agcctggtgg catgggcaac caatgtggct gacagcaatg	780
tatcaagggt taaaagccgc tacctacttt tggcccgat cagaagtggc tataaatggc	840
tcctttcctt ccatatacat gccttacaac ggaagtgtcc catttgaaga gaggatttct	900
acactgttaa aatggctgga cctgcccata gctgaaagac ccagggtttta taccatgtat	960

tttgaagaac	ctgattcctc	tggacatgca	ggtggaccag	tcagtgccag	agtaattaaa	1020
gccttacagg	tagtagatca	tgtttttggg	atgttgatgg	aaggcctgaa	gcagcggaat	1080
ttgcacaact	gtgtcaatat	catccttctg	gctgaccatg	gaatggacca	gacttattgt	1140
aacaagatgg	aatacatgac	tgattatfff	cccagaataa	acttcttcta	catgtacgaa	1200
gggcctgccc	ccgcacccg	agctcataat	atacctcatg	acttttttag	ttttaattct	1260
gaggdaallg	llagadaacc	cagllgcca	aaacctgac	agcattlcaa	gccccallly	1320
actcctgatt	tgccaaagcg	actgcactat	gccaaagacg	tcagaatcga	caaagtccat	1380
ctctttgtgg	atcaacagtg	gctggctggt	aggagtaa	caaatacaaa	ttgtggagga	1440
ggcaaccatg	gttataacaa	tgagtttagg	agcatggagg	ctatctttct	ggcacatgga	1500
cccagtttta	aagagaagac	tgaagttgaa	ccatttgaaa	atattgaagt	ctataaccta	1560
atgtgtgac	ttctacgc	tcaaccagca	ccaaacaatg	gaaccatgg	tagtttaaac	1620
catcttctga	aggtgccttt	ttatgagcca	tcccatgcag	aggagggtgc	aaagttttct	1680
gtttgtggct	ttgctaatac	attgccacaa	gagtcctctg	actgtttctg	ccctcaccta	1740
caaaatagta	ctcagctgga	acaagtgaat	cagatgctaa	atctcaccca	agaagaaata	1800
acagcaacag	tgaaagtaaa	tttgccatft	gggaggccta	gggtactgca	gaagaacgtg	1860
gaccactgtc	tcctttacca	cagggaatat	gtcagtggat	ttggaaaagc	tatgaggatg	1920
cccagtggtg	gttcatacac	agtcctccag	ttgggagaca	catgcctct	gcctccact	1980
gtcccagact	gtctgcgggc	tgatgtcagg	gttctctctt	ctgagagcca	aaaatgttcc	2040
ttctatttag	cagacaagaa	tatcacccac	ggtctctct	atcctctctg	cagcaataga	2100
acatcagata	gccaatatga	tgttttaatt	actagcaatt	tggtacctat	gtatgaagaa	2160
ttcagaaaaa	tgtgggacta	cttcacacgt	gttcttctta	taaaacatgc	cacagaaaga	2220
aatggagtaa	atgtgggttag	tggaccaata	tttgattata	attatgatgg	ccatftttgat	2280
gtccagatg	aaattaccaa	acatttagcc	aacactgatg	ttccatccc	aacacactac	2340
tttgtggtgc	tgaccagtty	taaaaacaag	agccacacac	cggaaaactg	ccctgggtgg	2400
ctggatgtcc	taccotftat	catccctcac	cgacctacca	acgtggagag	ctgtcctgaa	2460
ggtaaaccag	aagctctttg	ggttgaagaa	agattttacag	ctcacattgc	ccgggtccgt	2520
gatgtagaac	ttctcactgg	gcttgacttc	tatcaggata	aagtgcagcc	tgtctctgaa	2580
atftttgcaac	taaagacata	tttaccaaca	tttgaaacca	ctatt		2625

SEQ. ID NO: 77 - ENFP1 Nucleotide sequence:

atggaacggg	acggctgtgc	cggcggagga	tcaagaggcg	gagaaggcgg	cagagccct	60
agagaaggac	ctgcgggcaa	cggcagagac	agaggcagat	ctcatgccgc	cgaagccct	120
ggcgatcctc	aggetgctgc	ttctctgctg	gccccatgg	atgtgggcga	ggaacctctg	180
gaaaaggccg	ccagagccag	aacgcgcaag	gaccccaaca	cctacaaggt	gctgagcctg	240

gtgctgtccg	tgtgogtgct	gaccaccatc	ctgggctgca	tcttcggcct	gaagcccagc	300
tgcgccaaag	aagtgaagtc	ctgcaagggc	cggtgcttcg	agcggacctt	cggaactgc	360
agatgcgacg	ccgcctgtgt	ggaactgggc	aactgctgcc	tggactacca	ggaaacctgc	420
atcgagcccc	agcacatctg	gacctgcaac	aagttcagat	gcggcgagaa	gcggctgacc	480
agatccctgt	gtgcctgcag	cgacgactgc	aaggacaagg	gcgactgctg	catcaactac	540
agcagcgtgt	gccagggcga	gaagtccctg	gtggaagaac	cctgcgagag	catcaacgag	600
ccccagtgcc	ctgcgggctt	cgagacacct	cctaccctgc	tgttcagcct	ggacggcttt	660
cgggccgagt	acctgcacac	atggggaggc	ctgctgcccg	tgatcagcaa	gctgaagaag	720
tgcggcacct	acaccaagaa	catgcgggcc	gtgtacccca	ccaagacctt	ccccaacccac	780
tactccatcg	tgaccggcct	gtaccccag	agccacggca	tcatcgacaa	caagatgtac	840
gaccccaaga	tgaacgccag	cttcagcctg	aagtccaaag	agaagttcaa	ccccgagtgg	900
tataagggcg	agcccatctg	ggtcaccgcc	aagtaccagg	gcctgaaaag	cggcacattc	960
ttttggcccc	gcagcgacgt	ggaaatcaac	ggcatcttcc	ccgacatcta	taagatgtac	1020
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agctacggcc	ctgtgtccag	cgaagtgate	aaggccctgc	agcgggtgga	cggcattggtg	1200
ggaatgctga	tggacggcct	gaaagagctg	aacctgcaca	gatgcctgaa	cctgatcctg	1260
atcagcgacc	acggcatgga	acagggatcc	tgcaagaagt	acatctacct	gaacaagtac	1320
ctgggcgacg	tgaagaacat	caaagtgate	tacggcccag	ccgccagact	gaggcctagc	1380
gacgtgcccc	acaagtacta	cagcttcaac	tacgagggaa	tcgcccgaa	cctgagctgc	1440
agagagccca	accagcactt	caagccctac	ctgaagcact	tcccgcccaa	gcggctgcac	1500
ttcgccaaga	gcgacagaat	cgagcccctg	accttctacc	tggaccccca	gtggcagctg	1560
gccctgaatc	ccagcgagag	aaagtactgc	ggcagcggct	tccacggctc	cgacaacgtg	1620
ttcagcaaca	tgcaggccct	gttcgtgggc	tacggaccgc	gctttaagca	cggcattcgag	1680
gccgacacct	tcgagaacat	cgagggtgtac	aatctgatgt	gcgacctgct	gaatctgacc	1740
cctgccccca	acaatggcac	ccacggcagc	ctgaaccatc	tgetgaagaa	ccccgtgtac	1800
accctaagc	accccaaaga	ggtgcacccc	ctgggtgcagt	gccccttcac	cagaaacccc	1860
agagacaacc	tgggctgtag	ctgcaacccc	agcatcctgc	ccatcgagga	cttcagacc	1920
cagttcaacc	tgaccgtggc	cgaggaaaag	atcatcaagc	acgagacact	gccctacggc	1980
agaccccggg	tgetgcagaa	agagaacacc	atctgcctgc	tgagccagca	ccagttcatg	2040
agcggctact	cccaggacat	cctgatgccc	ctgtggacca	gctacaccgt	ggaccggaac	2100
gacagcttct	ccaccgagga	tttcagcaac	tgctgtacc	aggatttccg	gatccccctg	2160
agccccgtgc	acaagtgcag	cttctacaag	aacaacacca	aggtgtccta	cggttctctg	2220
agccctcccc	agctgaacaa	gaacagctcc	ggcatctaca	gcgaggccct	gctgactacc	2280

aacatcgtgc ccatgtacca gagcttccaa gtgatctggc ggtacttcca cgacaccctg	2340
ctgcggaagt acgccgaaga acggaacggc gtgaacgtgg tgtccggccc agtgttcgac	2400
ttcgactacg acggcagatg tgacagcctg gaaaatctgc ggcagaaaag aagagtgatc	2460
cggaaccagg aaattctgat ccctaccac ttctttatcg tgetgacaag ctgcaaggat	2520
accagccaga ccccccctgca ctgcgagaac ctggataccc tggccttcat cctgcctcac	2580
cggaccgaca acagcgagag ctgtgtgcac ggcaagcacg acagctcttg ggtggaagaa	2640
ctgctgatgc tgcaccgggc cagaatcacc gatgtggaac acatcaccgg cctgagcttt	2700
taccagcagc ggaaagaacc cgtgtccgat atcctgaagc tgaaaaccca tctgccacc	2760
ttcagccagg aagat	2775

SEQ ID NO: 78 - Azurocidin-ENPP1-FC Nucleotide sequence

ggtaccgccaccatgacaagactgacagtgtctggctctgctggccggactgttgccctcttctagagc
tgctccttctctgcgccaaagaagtgaagtcctgcaagggcagatgcttcgagcggaccttcggcaact
 gtatgtgtgacgccgcttgctggaactgggcaactgctgcctggactaccaagagacatgcatcgag
 cccgagcacatctggacctgcaacaagttcagatgcggcgagaagcggctgaccagatctctgtgcgc
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 gaggaacgcattctggctgttctgcagtggtgcagctgcctaaggatgagaggcctcacttctacac
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 gaacaagtacctgggcgacgtgaagaacatcaaagtgatctacggcccagccgcccagactgaggcctt
 ctgatgtgcctgacaagtactactccttcaactacgagggaatcgcccggaaacctgtcctgcagagag
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 tacggccctggctttaaacacggcatcgaggccgacaccttcgagaacatcgaggtgtacaatctgat
 gtgtgacctgctgaatctgaccttgccttaacaacggcacccacggatctctgaacctctgtgta
 agaatcccgtgtacacccctaagcaccoccaaagaggttcacctctggtccagtgctcctttcaccaga
 aatcctcgggacaacctgggctgctcttgcaaccttctatcctgcctatcgaggactttcagaccca
 gttcaacctgacctggccgaggaaaagatcatcaagcacgagacactgccttacggcagacctagag
 tgctgcagaaagagaacaccatctgcctgtgtgccagcaccagttcatgtccggctactccaggac
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 caagagatcctgattcctacacacttctttatcgtgctgaccagctgcaaggatacctctcagacccc
 tctgcaactgcgagaatctggacacctggccttcatctgcctcacagaaccgacaactccgagtcct
 gtgtgcacggcaagcacgactcctctgggtcgaagaactgctgatgtgtgcaccggggccagaatcacc
 gatgtggaacacatcaccggcctgagcttctaccagcagcggaaagaacctgtgtccgatatcctgaa
 gctgaaaacccatctgccaaccttcagccaagaggacctgatcaacgacaagaccacacctgtcctc

catgtcctgctccagaactgctcggaggccccctctgtgttccctgtttccacctaagccaaaggacaca
 ctgatgatctctcggacccccctgaagtgaactgacctgcgtggtggtgatgtgtctcacgaagatcccgaagt
 caagttcaattggtacgtggacggcgtggaagtgcacaacgccaaagacctagagaggaacagt
 acaactccacctacagagtgggtgtccgtgctgactgtgctgcaccaggattggctgaacggcgaagag
 tacaagtgc aaagtgtccaacaaggctctgcccgtcctatcgaaaagaccatctccaaggctaaggg
 ccagcctcgggaacctcaggtttacacccctgacctccatctcgggaagagatgaccaagaaccaggtgt
 ccctgacctgacctggtcaagggtcttacccttccgatatcgccgtggaatgggagtccaatggccag
 cctgagaacaactacaagacaacccccctctgtgctggacagcgacggctcattcttctgtactctaa
 gctgacagtggacaagtcccgggtggcagcaaggcaatgtgttttctgtctgtgatgcacgaggccc
 tccacaatcactacaccagaagtccctgtctctgtccctggcaaat**tgatag**ctcgag

Legend - **bold** = start/stop codon; underlined = nucleotide sequence of signal peptide.

SEQ ID NO: 79 - Azurocidin-ENPP3-FC Nucleotide sequence

atgaccagactgacctgtgctggccctgctggccggcctgctggccagcagcagagccgccaa
 gcagggcagctgcagaaagaagtgtcttcgacgccagcttcagaggcctggagaactgcagatgcgacg
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 ctgcctgcagagaaaggactgctgcgccgactacaagagcgtgtgccaggggcgagaccagctggctgg
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 acgacttcttcagcttcaacagcgaggagatcgtgagaaacctgagctgcagaaagcccgaccagcac
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 ggtgcacctgttcgtggaccagcagtggtggccgtgagaagcaagagcaaccaactgcggcgggcg
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 aatccagccccgcccccaaacggcaccacggcagcctgaaccacctgctgaaggtgccttctacg
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 agcctggactgcttctgtccccacacctgcagaacagcaccagctggagcaggtgaaccagatgctgaa
 cctgaccagaggagatcacccgccacctgaagggtgaacctgtcccttcggcagaccagagtgctgc
 agaagaacctggaccactgctgctgtaccacagagagtacgtgagcggttcggcaaggccatgaga
 atgcccctgtggagcagctacacctgtccccagctgggagacaccagccccctgtccccccacctgtcc
 cgactgcctgagagccgacgtgagagtgtccccccagcgagagccagaagtgcagcttctacctggccg
 acaagaacatcacccacggcttctgtacccccccgcccagcaacagaaccagcgacagccagtagcag
 gccctgatcaccagcaacctgggtgcccattgtacgaggagttcagaaagatgtgggactacttccacag
 cgtgctgctgatcaagcagcgcaccgagagaaacggcgtgaacctgtgtgagcggtcccatcttcgact
 acaactacgacggccacttcgacgcccccgacgagatcaccaagcacctggccaacaccgacgtgtcc
 atccccaccactacttctgtggtgctgaccagctgcaagaacaagagccacacccccgagaactgtcc
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 ctgctgaccggcctggacttctaccaggacaaggtgcagcccgtgagcgagatcctgcagctgaagac
 ctacctgtccaccttcgagaccaccatcgacaagaccacacctgtccccccctgtccccgcccccgagc

tgctgggcgccccagcgtgttctctgttcccccccaagcccaaggacaccctgatgatcagcagaacc
 cccgaggtgacctgcgtggtggtggacgtgagccacgaggaccccgaggtgaagttcaactggtacgt
 ggacggcgtggaggtgcacaacgcccaagaccaagcccagagaggagcagtacaacagcacctacagag
 tggtagcgtgctgacctgctgcaccaggactggctgaacggcaaggagtacaagtgcgaaggtgagc
 aacaaggccctgccccccccatcgagaagaccatcagcaaggccaagggccagcccagagagcccca
 ggtgtacacctgccccccagcagagaggagatgaccaagaaccaggtgagcctgacctgacctggtga
 agggcttctaccccagcgacatcgccgtggagtgaggagagcaacggccagcccagagaacaactacaag
 accaccccccccgctgctggacagcgacggcagcttcttctctgtacagcaagctgacctgggacaagag
 cagatggcagcagggcaacgtgttcagctgcagcgtgatgcacgaggccctgcacaaccactacacc
 agaagagcctgagcctgagccccggcaag

Cloning and Expression of ENPP1 and ENPP3 fusion polypeptides

ENPP1, or an ENPP1 polypeptide, is prepared as described in US 2015/0359858 A1, which is incorporated herein in its entirety by reference. ENPP1 is a transmembrane protein localized to the cell surface with distinct intramembrane domains. In order to express ENPP1 as a soluble extracellular protein, the transmembrane domain of ENPP1 may be swapped for the transmembrane domain of ENPP2 or a signal peptide sequence such as Azurocidin, which results in the accumulation of soluble, recombinant ENPP1 in the extracellular fluid of the baculovirus cultures. Signal sequences of any other known proteins may be used to target the extracellular domain of ENPP1 for secretion as well, such as but not limited to the signal sequence of the immunoglobulin kappa and lambda light chain proteins. Further, the disclosure should not be construed to be limited to the polypeptides described herein, but also includes polypeptides comprising any enzymatically active truncation of the ENPP1 extracellular domain.

ENPP1 is made soluble by omitting the transmembrane domain. Human ENPP1 (SEQ ID NO: 1) was modified to express a soluble, recombinant protein by replacing its transmembrane region (e.g., residues 77-98) with the corresponding subdomain of human ENPP2 (NCBI accession NP 00112433 5, e.g., residues 12-30) or Azurocidin signal sequence (SEQ ID NO: 42).

The modified ENPP1 sequence was cloned into a modified pFastbac FIT vector possessing a TEV protease cleavage site followed by a C-terminus 9-His tag, and cloned and expressed in insect cells, and both proteins were expressed in a baculovirus system as described previously (*Albright, et al., 2012, Blood 120:4432-4440; Saunders, et al., 2011, J. Biol. Chem. 18:994-1004; Saunders, et al., 2008, Mol. Cancer Ther. 7:3352-3362*), resulting in the accumulation of soluble, recombinant protein in the extracellular fluid.

ENPP3 is poorly exported to the cell surface. Soluble ENPP3 polypeptide is constructed by replacing the signal sequence of ENPP3 with the native signal sequence of other ENPPs or Azurocidin or suitable signal sequences. Several examples of ENPP3 fusion

constructs are disclosed in WO 2017/087936. 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. Soluble ENPP3 constructs are prepared using a signal sequence comprised of a combination of the signal sequences of ENPP1 and ENPP2 ("ENPP1-2-1" or "ENPP121" hereinafter). 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 disclosure 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.

In certain embodiments, the ENPP3 polypeptide is soluble. In some embodiments, the polypeptide of the disclosure includes an ENPP3 polypeptide that lacks the ENPP3 transmembrane domain. In another embodiment, the polypeptide of the disclosure includes an ENPP3 polypeptide wherein the ENPP3 transmembrane domain has been removed and replaced with the transmembrane domain of another polypeptide, such as, by way of non-limiting example, ENPP2, ENPP5 or ENPP7 or Azurocidin signal sequence.

In some embodiments, the polypeptide of the disclosure comprises an IgG Fc domain. In certain embodiments, the polypeptide of the disclosure comprises an albumin domain. In other embodiments, the albumin domain is located at the C terminal region of the ENPP3 polypeptide. In yet other embodiments, the IgG Fc domain is located at the C terminal region of the ENPP3 polypeptide. In yet other embodiments, the presence of IgG Fc domain or albumin domain improves half-life, solubility, reduces immunogenicity and increases the activity of the ENPP3 polypeptide.

In certain embodiments, the polypeptide of the disclosure comprises a signal peptide resulting in the secretion of a precursor of the ENPP3 polypeptide, which undergoes proteolytic processing to yield the ENPP3 polypeptide. In other embodiments, the signal peptide is selected from the group consisting of signal peptides of ENPP2, ENPP5 and ENPP7. In yet other embodiments, the signal peptide is selected from the group consisting of SEQ ID NOs:36-42.

In certain embodiments, the IgG Fc domain or the albumin domain is connected to the C terminal region of the ENPP3 polypeptide by a linker region. In other embodiments, the linker is selected from SEQ ID NOs:43-75, where n is an integer ranging from 1-20.

Production and Purification of ENPP1 and ENPP3 fusion polypeptides

To produce soluble, recombinant ENPP1 polypeptide for in vitro use, polynucleotide encoding ENPP1 (Human NPP1 (NCBI accession NP_006199)) was fused to the Fc domain of IgG (referred to as "ENPP1-Fc") and was expressed in stable CHO cell lines. In some embodiments, ENPP1 polynucleotide encoding residues 96 to 925 of NCBI accession NP_006199 were fused to Fc domain to generate ENPP1 polypeptide.

Alternately the ENPP1 polypeptide can also be expressed from HEK293 cells, Baculovirus insect cell system or CHO cells or Yeast *Pichia* expression system using suitable vectors. The ENPP1 polypeptide can be produced in either adherent or suspension cells. Preferably the ENPP1 polypeptide is expressed in CHO cells. To establish stable cell lines the nucleic acid sequence encoding ENPP1 constructs are cloned into an appropriate vector for large scale protein production.

ENPP3 is produced by establishing stable transfections in either CHO or HEK293 mammalian cells. ENPP3 polynucleotide encoding ENPP3 (Human NPP3 (UniProtKB/Swiss-Prot: O14638.2) was fused to the Fc domain of IgG (referred to as "ENPP3-Fc") and was expressed in stable CHO cell lines. In some embodiments, ENPP3 polynucleotide encoding residues 49-875 of UniProtKB/Swiss-Prot: O14638.2 was fused to Fc domain to generate ENPP3 polypeptide. The ENPP3 polypeptide can be produced in either adherent or suspension cells. To establish stable cell lines the nucleic acid sequence encoding NPP3 fusion polypeptides of the disclosure 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. ENPP3 polypeptides are produced following the protocols established in WO 2017/087936, the contents of which are hereby incorporated by reference in their entirety. ENPP1 polypeptides are produced following the protocols established in Albright, et al, 2015, *Nat Commun.* 6:10006, the contents of which are hereby incorporated by reference in their entirety.

A suitable plasmid containing the desired polypeptide constructs of ENPP1 or ENPP3 can be stably transfected into expression plasmid using established techniques such as electroporation or lipofectamine, and the cells can be grown under antibiotic selection to enhance for stably transfected cells. Clones of single, stably transfected cells are then established and screened for high expressing clones of the desired fusion protein. Screening of the single cell clones for ENPP1 or ENPP3 polypeptide expression can be accomplished in a high-throughput manner in 96 well plates using the synthetic enzymatic substrate pNP-TMP as previously described (*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 for ENPP3 or ENPP1 polypeptides through screening, protein production can be accomplished in shaking flasks or bio-reactors previously described for ENPP1 (*Albright, et al, 2015, Nat Commun. 6:10006*). Purification of ENPP3 or ENPP1 polypeptides can be accomplished using a combination of standard purification techniques known in the art. These techniques are well known in the art and are selected from techniques such as column chromatography, ultracentrifugation, filtration, and precipitation. Column chromatographic purification is accomplished using affinity chromatography such as protein-A and protein-G resins, metal affinity resins such as nickel or copper, hydrophobic exchange chromatography, and reverse-phase high-pressure chromatography (HPLC) using C8-C14 resins. Ion exchange may also be employed, such as anion and cation exchange chromatography using commercially available resins such as Q-sepharose (anion exchange) and SP-sepharose (cation exchange), blue sepharose resin and blue-sephadex resin, and hydroxyapatite resins. Size exclusion chromatography using commercially available S-75 and S200 Superdex resins can also be employed, as known in the art. Buffers used to solubilize the protein and provide the selection media for the above described chromatographic steps, are standard biological buffers known to practitioners of the art and science of protein chemistry.

Some examples of buffers that are used in preparation include citrate, phosphate, acetate, tris(hydroxymethyl)aminomethane, saline buffers, glycine-HCL buffers, Cacodylate buffers, and sodium barbital buffers, which are well known in art. Using a single technique, or a series of techniques in combination, and the appropriate buffer systems purified ENPP3 and the crude starting material side by side on a Coomassie stained polyacrylamide gel after a single purification step. The ENPP3 protein can then be additionally purified using additional techniques and/or chromatographic steps as described above, to reach substantially higher purity such as ~99% purity adjusted to the appropriate pH, one can purify the ENPP1 or ENPP3 polypeptides described to greater than 99% purity from crude material.

Following purification, ENPP1-Fc or ENPP3-Fc was dialyzed into PBS supplemented with Zn²⁺ and Mg²⁺ (PBSplus) concentrated to between 5 and 7 mg/ml, and frozen at -80 °C in aliquots of 200-500 µl. Aliquots were thawed immediately prior to use and the specific activity of the solution was adjusted to 31.25 au/ml (or about 0.7 mg/ml depending on the preparation) by dilution in PBSplus.

Dosage & Mode of Administration

In another embodiment, the hsNPP1 or hsNPP3 is administered in one or more doses containing about 1.0 mg/kg to about 5.0 mg/kg NPP1 or about 1.0 mg/kg to about 5.0 mg/kg NPP3 respectively. In another embodiment, the hsNPP1 or hsNPP3 is administered in one or more doses containing about 1.0 mg/kg to about 10.0 mg/kg NPP1 or about 1.0 mg/kg to about 10.0 mg/kg NPP3.

The time period between doses of the hsNPP1 or hsNPP3 is at least 2 days and can be longer, for example at least 3 days, at least 1 week, 2 weeks or 1 month. In one embodiment, the administration is weekly, bi-weekly, or monthly.

The recombinant hsNPP1 or hsNPP3 can be administered in any suitable way, such as intravenously, subcutaneously, or intraperitoneally.

The recombinant hsNPP1 or hsNPP3 can be administered in combination with one or more additional therapeutic agents. Exemplary therapeutic agents include, but are not limited to Bisphosphonate, Statins, Fibrates, Niacin, Aspirin, Clopidogrel, and warfarin.

In some embodiments, the recombinant hsNPP1 or hsNPP3 and additional therapeutic agents are administered separately and are administered concurrently or sequentially. In some embodiments, the recombinant hsNPP1 or hsNPP3 is administered prior to the administration of the additional therapeutic agent. In some embodiments, the recombinant hsNPP1 or hsNPP3 is administered after the administration of the additional therapeutic agent. In other embodiments, the recombinant hsNPP1 or hsNPP3 and additional therapeutic agents are administered together.

Nucleic Acid Administration and Therapy

Viral Vectors for *in vivo* expression of ENPP1 and ENPP3

The nucleic acids encoding the polypeptide(s) useful within the disclosure may be used in gene therapy protocols for the treatment of the diseases or disorders contemplated herein. The improved construct encoding the polypeptide(s) can be inserted into the appropriate gene therapy vector and administered to a patient to treat or prevent the diseases or disorder of interest.

Vectors, such as viral vectors, have been used in the prior art to introduce genes into a wide variety of different target cells. Typically, the vectors are exposed to the target cells so that transformation can take place in a sufficient proportion of the cells to provide a useful therapeutic or prophylactic effect from the expression of the desired polypeptide (e.g., a

receptor). The transfected nucleic acid may be permanently incorporated into the genome of each of the targeted cells, providing long lasting effect, or alternatively, the treatment may have to be repeated periodically. In certain embodiments, the (viral) vector transfects liver cells *in vivo* with genetic material encoding the polypeptide(s) of the disclosure.

A variety of vectors, both viral vectors and plasmid vectors are known in the art (see for example U.S. Patent No. 5,252,479 and WO 93/07282). In particular, a number of viruses have been used as gene transfer vectors, including papovaviruses, such as SV40, vaccinia virus, herpes viruses including HSV and EBV, and retroviruses. Many gene therapy protocols in the prior art have employed disabled murine retroviruses. Several recently issued patents are directed to methods and compositions for performing gene therapy (see for example U.S. Patent Nos. 6,168,916; 6,135,976; 5,965,541 and 6,129,705). Each of the foregoing patents is incorporated by reference in its entirety herein. Hence, genetic material such as a polynucleotide comprising an NPP1 or an NPP3 sequence can be introduced to a mammal in order to treat VSMC proliferation.

Certain modified viruses are often used as vectors to carry a coding sequence because after administration to a mammal, a virus infects a cell and expresses the encoded protein. Modified viruses useful according to the disclosure are derived from viruses which include, for example: parvovirus, picornavirus, pseudorabies virus, hepatitis virus A, B or C, papillomavirus, papovavirus (such as polyoma and SV40) or herpes virus (such as Epstein-Barr Virus, Varicella Zoster Virus, Cytomegalovirus, Herpes Zoster and Herpes Simplex Virus types 1 and 2), an RNA virus or a retrovirus, such as the Moloney murine leukemia virus or a lentivirus (i.e. derived from Human Immunodeficiency Virus, Feline Immunodeficiency Virus, equine infectious anemia virus, etc.). Among DNA viruses useful according to the disclosure are: Adeno-associated viruses adenoviruses, Alphaviruses, and Lentiviruses.

A viral vector is generally administered by injection, most often intravenously (by IV) directly into the body, or directly into a specific tissue, where it is taken up by individual cells. Alternately, a viral vector may be administered by contacting the viral vector *ex vivo* with a sample of the patient's cells, thereby allowing the viral vector to infect the cells, and cells containing the vector are then returned to the patient. Once the viral vector is delivered, the coding sequence expressed and results in a functioning protein. Generally, the infection and transduction of cells by viral vectors occur by a series of sequential events as follows: interaction of the viral capsid with receptors on the surface of the target cell, internalization by endocytosis, intracellular trafficking through the endocytic/ proteasomal compartment,

endosomal escape, nuclear import, virion uncoating, and viral DNA double-strand conversion that leads to the transcription and expression of the recombinant coding sequence interest. (Colella *et al.*, *Mol Ther Methods Clin Dev.* 2017 Dec 1;8:87-104.).

Adeno-Associated Viral Vectors according to the disclosure

AAV refers to viruses belonging to the genus Dependovirus of the Parvoviridae family. The AAV genome is approximately 4.7 kilobases long and is composed of linear single-stranded deoxyribonucleic acid (ssDNA) which may be either positive- or negative-sensed. The genome comprises inverted terminal repeats (ITRs) at both ends of the DNA strand, and two open reading frames (ORFs): rep and cap. The rep frame is made of four overlapping genes encoding non-structural replication (Rep) proteins required for the AAV life cycle. The cap frame contains overlapping nucleotide sequences of structural VP capsid proteins: VP1, VP2 and VP3, which interact together to form a capsid of icosahedral symmetry.

The terminal 145 nucleotides are self-complementary and are organized so that an energetically stable intramolecular duplex forming a T-shaped hairpin may be formed. These hairpin structures function as an origin for viral DNA replication, serving as primers for the cellular DNA polymerase complex. Following wild type AAV infection in mammalian cells, the rep genes (i.e. Rep78 and Rep52) are expressed from the P5 promoter and the P19 promoter, respectively, and both Rep proteins have a function in the replication of the viral genome. A splicing event in the rep ORF results in the expression of actually four Rep proteins (i.e. Rep78, Rep68, Rep52 and Rep40). However, it has been shown that the unspliced mRNA, encoding Rep78 and Rep52 proteins, in mammalian cells are sufficient for AAV vector production. Also in insect cells the Rep78 and Rep52 proteins suffice for AAV vector production.

AAV is a helper-dependent virus, that is, it requires co-infection with a helper virus (e.g., adenovirus, herpesvirus, or vaccinia virus) in order to form functionally complete AAV virions. In the absence of co-infection with a helper virus, AAV establishes a latent state in which the viral genome inserts into a host cell chromosome or exists in an episomal form, but infectious virions are not produced. Subsequent infection by a helper virus "rescues" the integrated genome, allowing it to be replicated and packaged into viral capsids, thereby reconstituting the infectious virion. While AAV can infect cells from different species, the

helper virus must be of the same species as the host cell. Thus, for example, human AAV replicates in canine cells that have been co-infected with a canine adenovirus.

To produce infectious recombinant AAV (rAAV) containing a heterologous nucleic acid sequence, a suitable host cell line can be transfected with an AAV vector containing the heterologous nucleic acid sequence, but lacking the AAV helper function genes, rep and cap. The AAV-helper function genes can then be provided on a separate vector. Also, only the helper virus genes necessary for AAV production (i.e., the accessory function genes) can be provided on a vector, rather than providing a replication-competent helper virus (such as adenovirus, herpesvirus, or vaccinia).

Collectively, the AAV helper function genes (i.e., rep and cap) and accessory function genes can be provided on one or more vectors. Helper and accessory function gene products can then be expressed in the host cell where they will act in trans on rAAV vectors containing the heterologous nucleic acid sequence. The rAAV vector containing the heterologous nucleic acid sequence will then be replicated and packaged as though it were a wild-type (wt) AAV genome, forming a recombinant virion. When a patient's cells are infected with the resulting rAAV virions, the heterologous nucleic acid sequence enters and is expressed in the patient's cells.

Because the patient's cells lack the rep and cap genes, as well as the accessory function genes, the rAAV cannot further replicate and package their genomes. Moreover, without a source of 5 rep and cap genes, wtAAV cannot be formed in the patient's cells.

The AAV vector typically lacks rep and cap frames. Such AAV vectors can be replicated and packaged into infectious viral particles when present in a host cell that has been transfected with a vector encoding and expressing rep and cap gene products (i.e. AAV Rep and Cap proteins), and wherein the host cell has been transfected with a vector which encodes and expresses a protein from the adenovirus open reading frame E4orf6.

Delivery of a protein of interest to the cells of a mammal is accomplished by first generating an AAV vector comprising DNA encoding the protein of interest and then administering the vector to the mammal. Thus, the disclosure should be construed to include AAV vectors comprising DNA encoding the polypeptide(s) of interest. Once armed with the present disclosure, the generation of AAV vectors comprising DNA encoding this/these polypeptide(s) will be apparent to the skilled artisan.

In one embodiment, the disclosure relates to an adeno-associated viral (AAV) expression vector comprising a sequence encoding mammal ENPP1 or mammal ENPP3, and upon administration to a mammal the vector expresses an ENPP1 or ENPP3 precursor in a

cell, the precursor including an Azurocidin signal peptide fused at its carboxy terminus to the amino terminus of ENPP1 or ENPP3. The ENPP1 or ENPP3 precursor may include a stabilizing domain, such as an IgG Fc region or human albumin. Upon secretion of the precursor from the cell, the signal peptide is cleaved off and enzymatically active soluble mammal ENPP1 or ENPP3 is provided extracellularly.

An AAV expression vector may include an expression cassette comprising a transcriptional regulatory region operatively linked to a nucleotide sequence comprising a transcriptional regulatory region operatively linked to a recombinant nucleic acid sequence encoding a polypeptide comprising a Azurocidin signal peptide sequence and an ectonucleotide pyrophosphatase/phosphodiesterase (ENPP1) polypeptide sequence.

In some embodiments, the expression cassette comprises a promoter and enhancer, the Kozak sequence GCCACCATGG, a nucleotide sequence encoding mammal NPP1 protein or a nucleotide sequence encoding mammal NPP3 protein, other suitable regulatory elements and a polyadenylation signal.

In some embodiments, the AAV recombinant genome of the AAV vector according to the disclosure lacks the rep open reading frame and/or the cap open reading frame.

The AAV vector according to the disclosure comprises a capsid from any serotype. In general, the AAV serotypes have genomic sequences of significant homology at the amino acid and the nucleic acid levels, provide an identical set of genetic functions, and replicate and assemble through practically identical mechanisms. In particular, the AAV of the present disclosure may belong to the serotype 1 of AAV (AAV1), AAV2, AAV3 (including types 3A and 3B), AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAVrh10, AAV11, avian AAV, bovine AAV, canine AAV, equine AAV, or ovine AAV.

Examples of the sequences of the genome of the different AAV serotypes may be found in the literature or in public databases such as GenBank. For example, GenBank accession numbers NC_001401.2 (AAV2), NC_001829.1 (AAV4), NC_006152.1 (AAV5), AF028704.1 (AAV6), NC_006260.1 (AAV7), NC_006261.1 (AAV8), AX753250.1 (AAV9) and AX753362.1 (AAV10).

In some embodiments, the adeno-associated viral vector according to the disclosure comprises a capsid derived from a serotype selected from the group consisting of the AAV2, AAV5, AAV7, AAV8, AAV9, AAV10 and AAVrh10 serotypes. In another embodiment, the serotype of the AAV is AAV8. If the viral vector comprises sequences encoding the capsid proteins, these may be modified so as to comprise an exogenous sequence to direct the AAV to a particular cell type or types, or to increase the efficiency of the delivery of the targeted

vector to a cell, or to facilitate purification or detection of the AAV, or to reduce the host response.

In certain embodiments, the rAAV vector of the disclosure comprises several essential DNA elements. In certain embodiments, these DNA elements include at least two copies of an AAV ITR sequence, a promoter/enhancer element, a transcription termination signal, any necessary 5' or 3' untranslated regions which flank DNA encoding the protein of interest or a biologically active fragment thereof. The rAAV vector of the disclosure may also include a portion of an intron of the protein of interest. Also, optionally, the rAAV vector of the disclosure comprises DNA encoding a mutated polypeptide of interest.

In certain embodiments, the vector comprises a promoter/regulatory sequence that comprises a promiscuous promoter which is capable of driving the expression of a heterologous gene to high levels in many different cell types. Such promoters include but are not limited to the cytomegalovirus (CMV) immediate early promoter/enhancer sequences, the Rous sarcoma virus promoter/enhancer sequences and the like. In certain embodiments, the promoter/regulatory sequence in the rAAV vector of the disclosure is the CMV immediate early promoter/enhancer. However, the promoter sequence used to drive expression of the heterologous gene may also be an inducible promoter, for example, but not limited to, a steroid inducible promoter, or maybe a tissue specific promoter, such as, but not limited to, the skeletal α -actin promoter which is muscle tissue specific and the muscle creatine kinase promoter/enhancer, and the like.

In certain embodiments, the rAAV vector of the disclosure comprises a transcription termination signal. While any transcription termination signal may be included in the vector of the disclosure, in certain embodiments, the transcription termination signal is the SV40 transcription termination signal.

In certain embodiments, the rAAV vector of the disclosure comprises isolated DNA encoding the polypeptide of interest, or a biologically active fragment of the polypeptide of interest. The disclosure should be construed to include any mammalian sequence of the polypeptide of interest, which is either known or unknown. Thus, the disclosure should be construed to include genes from mammals other than humans, which polypeptide functions in a substantially similar manner to the human polypeptide. Preferably, the nucleotide sequence comprising the gene encoding the polypeptide of interest is about 50% homologous, more preferably about 70% homologous, even more preferably about 80% homologous and most preferably about 90% homologous to the gene encoding the polypeptide of interest.

Further, the disclosure should be construed to include naturally occurring variants or recombinantly derived mutants of wild type protein sequences, which variants or mutants render the polypeptide encoded thereby either as therapeutically effective as full-length polypeptide, or even more therapeutically effective than full-length polypeptide in the gene therapy methods of the disclosure.

The disclosure should also be construed to include DNA encoding variants which retain the polypeptide's biological activity. Such variants include proteins or polypeptides which have been or may be modified using recombinant DNA technology, such that the protein or polypeptide possesses additional properties which enhance its suitability for use in the methods described herein, for example, but not limited to, variants conferring enhanced stability on the protein in plasma and enhanced specific activity of the protein. Analogs can differ from naturally occurring proteins or peptides by conservative amino acid sequence differences or by modifications which do not affect sequence, or by both. For example, conservative amino acid changes may be made, which although they alter the primary sequence of the protein or peptide, do not normally alter its function.

The disclosure is not limited to the specific rAAV vector exemplified in the experimental examples; rather, the disclosure should be construed to include any suitable AAV vector, including, but not limited to, vectors based on AAV-1, AAV-3, AAV-4 and AAV-6, and the like. Also included in the disclosure is a method of treating a mammal having a disease or disorder in an amount effective to provide a therapeutic effect.

The method comprises administering to the mammal an rAAV vector encoding the polypeptide of interest. Preferably, the mammal is a human. Typically, the number of viral vector genomes/mammal which are administered in a single injection ranges from about 1×10^8 to about 5×10^{16} . Preferably, the number of viral vector genomes/mammal which are administered in a single injection is from about 1×10^{10} to about 1×10^{15} ; more preferably, the number of viral vector genomes/mammal which are administered in a single injection is from about 5×10^{10} to about 5×10^{15} ; and, most preferably, the number of viral vector genomes which are administered to the mammal in a single injection is from about 5×10^{10} to about 5×10^{14} .

When the method of the disclosure comprises multiple site simultaneous injections, or several multiple site injections comprising injections into different sites over a period of several hours (for example, from about less than one hour to about two or three hours) the total number of viral vector genomes administered may be identical, or a fraction thereof or a multiple thereof, 15 to that recited in the single site injection method.

For administration of the rAAV vector of the disclosure in a single site injection, in certain embodiments a composition comprising the virus is injected directly into an organ of the subject (such as, but not limited to, the liver of the subject).

For administration to the mammal, the rAAV vector may be suspended in a pharmaceutically acceptable carrier, for example, HEPES buffered saline at a pH of about 7.8. Other useful pharmaceutically acceptable carriers include, but are not limited to, glycerol, water, saline, ethanol and other pharmaceutically acceptable salt solutions such as phosphates and salts of organic acids. Examples of these and other pharmaceutically acceptable carriers are described in Remington's Pharmaceutical Sciences (1991, Mack Publication Co., New Jersey). The rAAV vector of the disclosure may also be provided in the form of a kit, the kit comprising, for example, a freeze-dried preparation of vector in a dried salts formulation, sterile water for suspension of the vector/salts composition and instructions for suspension of the vector and administration of the same to the mammal.

The published application, US 2017/0290926 –Smith et al., the contents of which are incorporated by reference in their entirety herein, describe in detail the process by which AAV vectors are generated, delivered and administered.

RNA based in vivo expression of ENPP1 and ENPP3 polypeptides

The present disclosure provides compositions and methods for the production and delivery of recombinant double-stranded RNA molecules (dsRNA that encode ENPP1 or ENPP3 polypeptides described herein. The double stranded RNA particle (dsRP) can contain a dsRNA molecule enclosed in a capsid or coat protein. The dsRNA molecule can be a viral genome or portion of a genome, which can be derived from a wild-type viral genome. The RNA molecule can encode an RNA-dependent RNA polymerase (RDRP) and a polyprotein that forms at least part of a capsid or coat protein. The RNA molecule can also contain an RNA sub-sequence that encodes an ENPP1 or ENPP3 polypeptides that are translated by the cellular components of a host cell. When the dsRP is transfected into a host cell the sub-sequence can be translated by the cellular machinery of the host cell to produce the ENPP1 or ENPP3 polypeptides.

In another aspect the disclosure provides a method of producing a protein product in a host cell. The method includes transfecting a host cell with a dsRP having a recombinant double-stranded RNA molecule (dsRNA) and a capsid or coat protein. The RNA molecule can encode an RNA-dependent RNA polymerase and a polyprotein that forms at least part of

the capsid or coat protein, and the dsRP can be able to replicate in the host cell. The RNA molecule has at least one RNA sub-sequence that encodes ENPP1 or ENPP3 polypeptides that is translated by cellular components of the host cell.

In another aspect the disclosure provides an RNA molecule translatable by a host cell. The RNA molecule can be any RNA molecule that encodes the ENPP1 or ENPP3 polypeptides described herein. In one embodiment the RNA molecule encodes an RNA-dependent RNA polymerase and a polyprotein that forms at least part of a capsid or coat protein of a dsRP and, optionally, can have at least one sub-sequence of RNA that encodes an additional protein product.

Production of dsRP

A dsRP of the disclosure can also be produced by presenting to a host cell a plasmid or other DNA molecule encoding a dsRP of the disclosure or encoding the genes of the dsRP. The plasmid or DNA molecule containing nucleotide sequences encoding desired protein such as ENPP1 or ENPP3 polypeptide is then transfected into the host cell and the host cell begins producing the dsRP of the disclosure. The dsRP can also be produced in the host cell by presenting to the host cell an RNA molecule encoding the genes of the dsRP. The RNA molecule can be (+)-strand RNA.

Once the dsRP of the disclosure has been presented to the host cell (or a plasmid encoding the genes of the dsRP of the disclosure, or an RNA molecule encoding the genes of the dsRP), the dsRP will be produced within the host cell using the cellular components of the host cell. The dsRP of the disclosure is therefore self-sustaining within the host cell and is propagated within the host cell. The host cell can be any suitable host cell such as, for example, a eukaryotic cell, a mammalian cell, a fungal cell, a bacterial cell, an insect cell, or a yeast cell. The host cell can propagate a recombinant dsRP after a recombinant dsRNA molecule of the disclosure or a DNA molecule encoding a dsRP of the disclosure is presented to and taken up by the host cell.

Methods of Producing a dsRNA Virus or dsRP

The disclosure also provides methods of producing a dsRP of the disclosure. A double-stranded or single-stranded RNA or DNA molecule can be presented to a host cell. The amplification of the dsRNA molecules in the host cell utilizes the natural production and

assembly processes already present in many types of host cells (e.g., yeast). The disclosure can thus be applied by presenting to a host cell a single-stranded or double-stranded RNA or DNA molecule of the disclosure, which is taken up by the host cell and is utilized to produce the recombinant dsRP and protein or peptide encoded by the RNA sub-sequence using the host cell's cellular components. The disclosure can also be applied by providing to the host cell a linear or circular DNA molecule (e.g., a plasmid or vector) containing one or more sequences coding for an RNA-dependent RNA polymerase, a polyprotein that forms at least part of the capsid or coat protein of the dsRP, and a sub-sequence encoding the protein of interest such as ENPP1 or ENPP3 polypeptides as disclosed herein.

The presentation of a dsRNA or ssRNA molecule of the disclosure can be performed in any suitable way such as, for example, by presenting an RNA molecule of the disclosure directly to the host cell as “naked” or unmodified single-stranded or double-stranded RNA. The RNA molecule can be transfected (or transformed) into a yeast, bacterial, or mammalian host cell by any suitable method, for example by electroporation, exposure of the host cell to calcium phosphate, or by the production of liposomes that fuse with the cell membrane and deposit the viral sequence inside. It can also be performed by a specific mechanism of direct introduction of dsRNA from killer viruses or heterologous dsRNA into the host cell. This step can be optimized using a reporter system, such as red fluorescent protein (RFP), or by targeting a specific constitutive gene transcript within the host cell genome. This can be done by using a target with an obvious phenotype or by monitoring by quantitative reverse transcriptase PCR (RT-PCR).

In some embodiments a DNA molecule (e.g., a plasmid or other vector) that encodes an RNA molecule of the disclosure is introduced into the host cell. The DNA molecule can contain a sequence coding for the RNA molecule of a dsRP of the disclosure. The DNA molecule can code for an entire genome of the dsRP, or a portion thereof. The DNA molecule can further code for the at least one sub-sequence of RNA that produces the additional (heterologous) protein product. The DNA sequence can also code for gag protein or gag-pol protein, and as well as any necessary or desirable promoters or other sequences supporting the expression and purpose of the molecule. The DNA molecule can be a linear DNA, a circular DNA, a plasmid, a yeast artificial chromosome, or may take another form convenient for the specific application.

In one embodiment the DNA molecule can further comprise T7 ends for producing concatamers and hairpin structures, thus allowing for propagation of the virus or dsRP sequence in the host cell. The DNA molecule can be transfected or transformed into the host

cell and then, using the host cellular machinery, transcribed and thus provide the dsRNA molecule having the at least one sub-sequence of RNA to the host cell. The host cell can then produce the encoded desired ENPP1 or ENPP3 polypeptide. The dsRNA can be packaged in the same manner that a wild-type virus would be, using the host cell's metabolic processes and machinery. The ENPP1 or ENPP3 polypeptide is also produced using the host cell's metabolic processes and cellular components.

The patent, US 10266834 by Brown et al., the contents of which are incorporated by reference in their entirety herein, describes in detail the process by which dsRNA particles that encode polypeptides are generated, delivered and administered.

ENPP1 Coated Stents and ENPP3 Coated stents

Stents are typically elongated structures used to keep open lumens (e.g., openings in the body) found in various parts of the body so that the parts of the body containing those lumens may function properly. Stents are often used in the treatment of atherosclerosis, a disease of the vascular system in which arteries become partially, and sometimes completely, occluded with substances that may include lipids, cholesterol, calcium, and various types of cells, such as smooth muscle cells and platelets.

Stents located within any lumen in the body may not always prevent partial or complete restenosis. In particular, stents do not always prevent the re-narrowing of an artery following Percutaneous transluminal angioplasty (PTA). In some cases, the introduction and presence of the stent itself in the artery or vein can create regions of trauma or tissue injury such as, e.g., tears in the inner lining of the artery, called the endothelium requiring further surgeries post stent placement.

It is believed that such trauma or tissue injury can trigger migration of vascular smooth muscle cells, which are usually separated from the arterial lumen by the endothelium, into the arterial lumen, where they proliferate to create a mass of cells that may, in a matter of days or weeks, occlude the artery. Such re-occlusion, which is sometimes seen after PTA, is an example of restenosis. Coating a stent with therapeutic agent such as ENPP1 agent or ENPP3 agent is expected to prevent and/or reduce vascular smooth muscle cell proliferation which in return reduces the occurrence of or treats restenosis.

In some embodiments, the patient is need of surgery and/or has tissue injury due to the presence of a prior implanted non-eluting stent.

In some embodiments, the patient is in need of surgery and/or has tissue injury due to the presence of a prior implanted eluting stent that elutes therapeutic agents other than ENPP1 agent or ENPP3 agent.

In some embodiments, the prior stent that had caused the tissue injury is removed and replaced with ENPP1 agent coated stent.

In some embodiments, the prior stent that had caused the tissue injury is removed and replaced with ENPP3 agent coated stent.

In some embodiments, the prior stent that had caused the tissue injury is not removed and the ENPP1 agent coated stent is implanted adjacent to the prior stent.

In some embodiments, the prior stent that had caused the tissue injury is not removed and the ENPP3 agent coated stent is implanted adjacent to the prior stent.

ENPP1 or ENPP3 coated stents are typically hollow, cylindrical structures made from struts or interconnected filaments. Stents are usually implanted at their site of use in the body by attaching them in a compressed state to a catheter that is directed through the body to the site of stent use. Vascular stents are frequently used in blood vessels to open the vessel and provide improved blood flow. The stent can be expanded to a size which enables it to keep the lumen open by supporting the walls of the lumen once it is positioned at the desired site. Vascular stents can be collapsed to reduce their diameter so that the stent can be guided through a patient's arteries or veins to reach the site of deployment. Stents are typically either coupled to the outside of the balloon for expansion by the expanding balloon or are self-expanding upon removal of a restraint such as a wire or sleeve maintaining the stent in its collapsed state.

Vascular stents are often made of metal to provide the strength necessary to support the occluded arterial walls. Two of the preferred metals are Nitinol alloys of nickel and titanium, and stainless steel. Other materials that can be used in fabricating stents are ceramics, polymers, and plastics. The polymer may be a polymer having no functional groups. Alternatively, the polymer may be one having functional groups, but none that are reactive with the ENPP1 agent or ENPP3 agent. The polymer may include a biodegradable polymer. For example, the polymer may include a polymer selected from the group consisting of polyhydroxy acids, polyanhydrides, polyphosphazenes, polyalkylene oxalates, biodegradable polyamides, polyorthoesters, polyphosphoesters, polyorthocarbonates, and blends or copolymers thereof. The polymer may also include a biostable polymer, alone or in combination with a biodegradable polymer. For example, the polymer may include a polymer selected from the group consisting of polyurethanes, silicones, polyacrylates, polyesters,

polyalkylene oxides, polyalcohols, polyolefins, polyvinyl chlorides, cellulose and its derivatives, fluorinated polymers, biostable polyamides, and blends or copolymers thereof.

The effect of different stent designs on the drug distribution pattern has been scrutinized in experimental studies and also tested in clinical trials (*Hwang CW, Wu D, Edelman ER. 2001. Physiological transport forces govern drug distribution for stent-based delivery. Circulation, 104: 600–5; & Takebayashi H, Mintz GS, Carlier SG, et al. 2004. Nonuniform strut distribution correlates with more neointimal hyperplasia after Sirolimus-eluting stent implantation. Circulation, 110:3430–4*). Although a large number of stent designs have been developed to date, only the multicellular design is currently most commonly used; they can be categorized into “closed cell” and “open cell” configurations (*Rogers CDK. 2002. Drug-eluting stents: role of stent design, delivery vehicle, and drug selection. Rev Cardiovasc Med, 3(Suppl 5): S10–15.*). A closed cell stent has a uniform cell expansion and constant cell spacing when deployed in a curved vascular segment, which gives more uniform drug distribution (Rogers 2002). An open cell stent has a greater variation in the surface coverage between the inner and outer curvatures in the curved segment but gives better conformability to curved surface at the expense of less uniform drug distribution (Rogers 2002). The majority of current stents use a closed cell design. The optimal stent design for drug delivery would have a large stent surface area, a small cell gap, and minimal strut deformation after deployment while maintaining conformability, radial support, and flexibility to reach the complex coronary lesions. Several examples of the different geometrical stent structures are described in Paisal et al. (*Muhammad Sufyan Amir Paisal et al 2017 IOP Conf. Ser.: Mater. Sci. Eng. 165 012003*)

ENPP1 coated stents or ENPP3 coated stents are prepared by applying a coating composition comprising an effective amount of ENPP1 agent or ENPP3 agent respectively. The coating composition preferably includes an amount of the ENPP1 agent or ENPP3 agent that is sufficient to be therapeutically effective for inhibiting regrowth of plaque or inhibiting restenosis or preventing vascular smooth cell proliferation.

In one embodiment, the coating composition comprises from about 1 wt % to about 50 wt % ENPP1 polypeptide, based on the total weight of the coating composition. In another embodiment, the coating composition comprises from about 5 wt % to about 30 wt % ENPP1 polypeptide. In yet another embodiment, the coating composition comprises from about 10 wt % to about 20 wt % ENPP1 polypeptide.

In one embodiment, the coating composition comprises from about 1 wt % to about 50 wt % ENPP3 polypeptide, based on the total weight of the coating composition. In another

embodiment, the coating composition comprises from about 5 wt % to about 30 wt % ENPP3 polypeptide. In yet another embodiment, the coating composition comprises from about 10 wt % to about 20 wt % ENPP3 polypeptide.

In one embodiment, the coating composition comprises from about 1 µg/ml to about 10 mg/ml of ENPP1 polypeptide. In another embodiment, the coating composition comprises from about 100 µg/ml to 5mg/ml ENPP1 polypeptide. In yet another embodiment, the coating composition comprises from about 500 µg/ml to about 2 mg/ml ENPP1 polypeptide.

In a related embodiment, the ENPP1 polypeptide of the coating composition is ENPP1-Fc.

In a related embodiment, the ENPP1 polypeptide of the coating composition is ENPP1-Albumin.

In one embodiment, the coating composition comprises from about 1 µg/ml to about 10 mg/ml of ENPP3 polypeptide. In another embodiment, the coating composition comprises from about 100 µg/ml to 5mg/ml ENPP3 polypeptide. In yet another embodiment, the coating composition comprises from about 500 µg/ml to about 2 mg/ml ENPP3 polypeptide.

In a related embodiment, the ENPP3 polypeptide of the coating composition is ENPP3-Fc.

In a related embodiment, the ENPP3 polypeptide of the coating composition is ENPP3-Albumin.

In one embodiment, the coating composition comprises from about 1ng/µl to about 1000 µg/µl of ENPP1 mRNA. In another embodiment, the coating composition comprises from about 100 ng/µl to 10µg/µl ENPP1 mRNA. In yet another embodiment, the coating composition comprises from about 50 ng/µl to about 5 µg/µl ENPP1 mRNA.

In one embodiment, the coating composition comprises from about 1ng/µl to about 1000 µg/µl of ENPP1-Fc mRNA. In another embodiment, the coating composition comprises from about 100 ng/µl to 10µg/µl ENPP1 -Fc mRNA. In yet another embodiment, the coating composition comprises from about 50 ng/µl to about 5 µg/µl ENPP1-Fc mRNA.

In one embodiment, the coating composition comprises from about 1ng/µl to about 1000 µg/µl of ENPP1-Albumin mRNA. In another embodiment, the coating composition comprises from about 100 ng/µl to 10µg/µl ENPP1-Albumin mRNA. In yet another embodiment, the coating composition comprises from about 50 ng/µl to about 5 µg/µl ENPP1-Albumin mRNA.

In one embodiment, the coating composition comprises from about 1ng/µl to about 1000 µg/µl of ENPP3 mRNA. In another embodiment, the coating composition comprises

from about 100 ng/ μ l to 5 μ g/ μ l ENPP3 mRNA. In yet another embodiment, the coating composition comprises from about 500 ng/ μ l to about 2 μ g/ μ l ENPP3 mRNA.

In one embodiment, the coating composition comprises from about 1 ng/ μ l to about 1000 μ g/ μ l of ENPP3-Fc mRNA. In another embodiment, the coating composition comprises from about 100 ng/ μ l to 5 μ g/ μ l ENPP3-Fc mRNA. In yet another embodiment, the coating composition comprises from about 500 ng/ μ l to about 2 μ g/ μ l ENPP3-Fc mRNA.

In one embodiment, the coating composition comprises from about 1 ng/ μ l to about 1000 μ g/ μ l of ENPP3-Albumin mRNA. In another embodiment, the coating composition comprises from about 100 ng/ μ l to 5 μ g/ μ l ENPP3-Albumin mRNA. In yet another embodiment, the coating composition comprises from about 500 ng/ μ l to about 2 μ g/ μ l ENPP3-Albumin mRNA.

Stents may be coated with a substance, such as a biodegradable or biostable polymer, to improve the biocompatibility of the stent, making it less likely to cause an allergic or other immunological response in a patient. A coating substance may also add to the strength of the stent. Some known coating substances include organic acids, their derivatives, and synthetic polymers that are either biodegradable or biostable. Biostable coating substances do not degrade in the body, biodegradable coating substances can degrade in the body.

The coating composition comprises an effective amount of carrier which helps in the coating process to ensure that the therapeutic molecules such as ENPP1 agent or ENPP3 agent adhere to the stent surface and also facilitate in eluting the therapeutic agent into the body at the site of stent placement. The carrier could be a liquid carrier or a solid carrier. The coating composition may alternatively comprise more than one solid compound in a solid carrier. The coating composition may further comprise both a liquid carrier and a solid carrier. In a still further aspect, the coating composition may also comprise more than one type of nonpolymeric or polymeric compound in the carrier and may further comprise both a polymeric material and a nonpolymeric material in a solid or liquid carrier.

In another embodiment, two or more types of biodegradable compounds (polymers or non-polymers) may be blended together to obtain a liquid carrier for use in the coating composition. The biodegradable compounds can be liquids before they are mixed together, e.g., forming a homogeneous solution, mixture, or suspension. Alternatively, some of the biodegradable compounds may be solids before they are mixed with other liquid biodegradable compounds. The solid biodegradable compounds preferably dissolve when they are mixed with the liquid biodegradable compounds, resulting in a liquid carrier composition containing the different biodegradable compounds.

In another embodiment, the biodegradable carrier component of the coating composition is a solid, which dissolves when mixed with the biologically active component and any other components included in the coating composition.

The carrier could be a polymeric carrier. Some polymeric carriers are synthetic polymers. Examples of synthetic polymers that serve as reservoir matrices include but not limited to poly-n-butyl methacrylate, polyethylene-vinyl acetate, poly (lactide-co- Σ -caprolactone) copolymer, Fibrin, cellulose, Phosphorylcholine. Some eluting stent comprise porous 300 μ m ceramic layer containing therapeutic molecule -loaded nanocavities. Examples of drug eluting stents, stent structures and stent designs can be found in *Drug-Eluting Stent: A Review and Update, Vasc Health Risk Manag. 2005 Dec; 1(4): 263–276* and *Modern Stents: Where Are We Going?, Rambam Maimonides Med J. 2020 Apr; 11(2): e0017*.

The carriers in the coating composition may be either biodegradable or biostable. Biodegradable polymers are often used in synthetic biodegradable sutures. These polymers include polyhydroxy acids. Polyhydroxy acids suitable for use in the present invention include poly-L-lactic acids, poly- DL-lactic acids, polyglycolic acids, polylactides including homopolymers and copolymers of lactide (including lactides made from all stereo isomers of lactic acids, such as D-,L-lactic acid and meso lactic acid), polylactones, polycaprolactones, polyglycolides, polyparadioxanone, poly 1,4-dioxepan- 2-one, poly 1,5-dioxepan-2-one, poly 6,6-dimethyl-1, 4-dioxan-2-one, polyhydroxyvalerate, polyhydroxybuterate, polytrimethylene carbonate polymers, and blends of the foregoing.

Polylactones suitable for use in the present invention include polycaprolactones such as poly(ϵ -caprolactone), polyvalerolactones such as poly(d-valerolactone), and polybutyrolactones such as poly(butyrolactone). Other biodegradable polymers that can be used are polyanhydrides, polyphosphazenes, biodegradable polyamides such as synthetic polypeptides such as polylysine and polyaspartic acid, polyalkylene oxalates, polyorthoesters, polyphosphoesters, and polyorthocarbonates. Copolymers and blends of any of the listed polymers may be used. Polymer names that are identical except for the presence or absence of brackets represent the same polymers.

Biostable polymers suitable for use in the present invention include, but are not limited to polyurethanes, silicones such as polyalkyl siloxanes such as polydimethyl siloxane and polybutyl methacrylate, polyesters such as poly(ethylene terephthalate), polyalkylene oxides such as polyethylene oxide or polyethylene glycol, polyalcohols such as polyvinyl alcohols and polyethylene glycols, polyolefins such as poly- 5 ethylene, polypropylene,

poly(ethylene-propylene) rubber and natural rubber, polyvinyl chloride, cellulose and modified cellulose derivatives such as rayon, rayon-triacetate, cellulose acetate, cellulose acetate butyrate, cellophane, cellulose nitrate, cellulose propionate, cellulose ethers such as carboxymethyl cellulose and hydroxyalkyl celluloses, fluorinated polymers such as polytetrafluoroethylene (Teflon), and bio stable polyamides such as Nylon 66 and polycaprolactam. Fixed animal tissues such as glutaraldehyde fixed bovine pericardium can also be used. Polyesters and polyamides can be either biodegradable or biostable. Ester and amide bonds are susceptible to hydrolysis, which can contribute to biodegradation.

In some cases, the coating composition further comprises an effective amount of a non-polymeric carrier. The non-polymeric carrier can include one or more of fatty acid, biocompatible oil, or wax. Examples of non-polymeric biodegradable carriers include liquid oleic acid, vitamin E, peanut oil, and cottonseed oil, which are liquids that are both hydrophobic and biocompatible. In some cases, the nonpolymeric or polymeric carrier, can be a liquid at room and body temperature. In some cases, the nonpolymeric or polymeric carrier can be a solid at room and body temperature, or a solid at room temperature and a liquid at body temperature.

In another embodiment, the polymer solution can be formed into a film and the film then applied to the stent. Any of a variety of conventional methods of forming films can be used. For example, the polymer, ENPP1 agent or ENPP3 agent and solvent are preferably mixed into solution and then poured onto a smooth, flat surface such that a coating film is formed after the solution is dried to remove the solvent. The film can then be cut to fit the stent on which it is to be used. The film may then be mounted, such as by wrapping, on the outer surface of a stent.

In another embodiment, the coated stent is prepared by spraying the stent with the liquid carrier comprising the therapeutic agent such as ENPP1 agent or ENPP3 agent resulting in a coating of uniform thickness on the struts of the stent. In another embodiment, the stent may be dip coated or immersed in the coating solution comprising carrier and therapeutic agent, such that the solution completely coats the struts of the stent. Alternatively, the stent may be painted with the coating solution comprising carrier and therapeutic agent, such as with a paint brush. In each of these coating applications, the entirety of both the outer and inner surfaces of the stent are preferably coated, although only portions of either or both surfaces may be coated in some embodiments.

As discussed above, the coating composition comprises a bioactive component and a biodegradable carrier component. Preferably, the coating composition comprises from 0.1%

to 100% by weight of a biologically active component and from 1% to 99% by weight of a biodegradable carrier component. More preferably, the coating composition comprises from 0.1% to 50% by weight of a biologically active component and from 50% to 99.9% by weight of a biodegradable carrier component. The coating composition can be prepared in a number of ways including by simply mixing the bioactive component and the carrier component together to form a mixture, e.g., a solution or suspension. Alternatively, the bioactive component and the carrier component together are mixed in a suitable solvent, the coating is applied to the stent, and the solvent is removed. Preferably the coating composition is applied to the stent in its expanded state.

In addition to stents, examples of other medical devices that can be coated in accordance with aspects of the inventions disclosed herein include catheters, heart valves, pacemaker leads, annuloplasty rings and other medical implants. In other specific embodiments, coated angioplasty balloons and other coated medical devices can also comprise one of the coating compositions disclosed herein. However, stents are preferred. The coating composition may be applied to the stent (or other medical device) by any number of ways, e.g, by spraying the coating composition onto the stent, by immersing the stent in the coating composition, or by painting the stent with the coating composition. Preferably, a stent is coated in its expanded (i.e., enlarged diameter) form so that a sufficient amount of the coating composition will be applied to coat the entire surface of the expanded stent. When the stent is immersed in the coating composition, the excess coating composition on the surface of the stent may be removed, such as by brushing off the excess coating composition with a paint brush. In each of these coating applications, preferably both the outer and inner surfaces of the stent are coated.

The coating compositions described herein preferably remain on a stent, partially or in substantial part, after the stent has been introduced to the body, for at least several days , for several weeks and more preferably for several months thereby slowly releasing the therapeutic agents such as ENPP1 agent or ENPP3 agent into the blood stream.

Pharmaceutical Compositions and Formulations

The disclosure provides pharmaceutical compositions comprising a polypeptide of the disclosure within the methods described herein. Such a pharmaceutical composition is in a form suitable for administration to a subject, or the pharmaceutical composition may further comprise one or more pharmaceutically acceptable carriers, one or more additional ingredients, or some combination of these. The various components of the pharmaceutical

composition may be present in the form of a physiologically acceptable salt, such as in combination with a physiologically acceptable cation or anion, as is well known in the art.

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

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

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

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

As used herein, a "unit dose" is a discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient that would be administered to a subject or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage. The unit dosage form may be for a single daily dose or one of the 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.

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. In certain embodiments, administration of the compound of the disclosure to a subject elevates the subject's plasma PPI to a level that is close to normal, where a normal level of PPI in mammals is 1-3 μM . "Close to normal" refers to 0 to 1.2 μM or 0-40% below or above normal, 30 nM to 0.9 μM or 1-30% below or above normal, 0 to 0.6 μM or 0-20% below or above normal, or 0 to 0.3 μM or 0-10% below or above normal.

Administration of the compositions of the present disclosure to a patient, such as a mammal, such as a human, may be carried out using known procedures, at dosages and for periods of time effective to treat a disease or disorder in the patient. An effective amount of the therapeutic compound necessary to achieve a therapeutic effect may vary according to factors 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. Dosage is determined based on the biological activity of the therapeutic compound which in turn depends on the half-life and the area under the plasma time of the therapeutic compound curve. The polypeptide according to the disclosure is administered at an appropriate time interval of every 2 days, or every 4 days, or every week or every month so as to achieve a continuous level of plasma PPI that is either close to the normal (1-3 μM) level or above (30-50% higher than) normal levels of PPI. Therapeutic dosage of the polypeptides of the disclosure may also be determined based on half-life or the rate at which the therapeutic polypeptide is cleared out of the body. The polypeptide according to the disclosure is administered at appropriate time intervals of either every 2 days, or every 4 days, every week or every month so as to achieve a constant level of enzymatic activity of ENPP1 or ENPP3 polypeptides.

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 disclosure is from about 0.01 and 50 mg/kg of body weight/per day. In certain embodiments, the effective dose range for a therapeutic compound of the disclosure is from about 50 ng to 500 ng/kg, preferably 100 ng to 300 ng/kg of bodyweight. One of ordinary skill in the art would be able

to study the relevant factors and make the determination regarding the effective amount of the therapeutic compound without undue experimentation.

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

A medical doctor, e.g., physician, 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 disclosure employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

In certain embodiments, the compositions of the disclosure 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 disclosure 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. The frequency of administration of the various combination compositions of the disclosure 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 disclosure should not be construed to be limited to any particular dosage regime and the precise dosage and composition to be administered to any patient will be determined by the attending physical taking all other factors about the patient into account.

In certain embodiments, the present disclosure is directed to a packaged pharmaceutical composition comprising a container holding a therapeutically effective amount of a compound of the disclosure, 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.

Routes of Administration

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

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

EXAMPLES

The present disclosure is further exemplified by the following examples. The examples are for illustrative purpose only and are not intended, nor should they be construed as limiting the disclosure in any manner.

Mice

The tip-toe walking (*ttw/ttw*) mice and WT mice were used in the following experiments. *ttw/ttw* mice were bred onto a C57BL/6J background for more than ten generations, and *ttw/ttw* mice and wild-type (WT) littermate control (male and female) animals were generated through heterozygous mating.

Plasma collection

Whole blood from *ttw/ttw* mice and WT mice (by cardiac puncture), was collected in syringes containing trisodium ethylenediaminetetraacetic acid (EDTA) and maintained on ice until the separation of plasma and erythrocytes by centrifugation (1000×g, 4 °C, 20 min) was performed. The plasma was then depleted of platelets by filtration (2200×g, 4 °C, 20 min) through a 300,000-kDa mass cutoff filter and stored at -20 °C until further processing.

EXAMPLE 1 – Therapeutic Effect of ENPP1-Fc administration to WT and *ttw/ttw* mice

It is known that damage to a blood vessel induces an inflammatory response and endothelial activation, resulting in smooth muscle cell proliferation and narrowing of the lumen of the vessel. (Exp Mol Med. 2018 Oct 29;50(10):1-12). Carotid artery ligation in WT mice and *ttw/ttw* mice was performed to create a model of mechanical injury and was then used to study the effect of ENPP1-Fc on smooth muscle cell proliferation at the site of injury. Thus, the main aim of the experiment was to determine the therapeutic effect of ENPP1-Fc on myointimal hyperplasia in WT mice and homozygous *ttw/ttw* mice.

ttw/ttw and wildtype (WT) littermate control (male and female) animals were generated by heterozygous mating. The pups were weaned at 3 - 4 weeks of age and then maintained on normal chow diet. Animals were blindly numbered during weaning, independent on genotype. ENPP1 genotyping was then performed by the polymerase chain reaction analysis of tail DNA by following the protocols described in Okawa et al. (Okawa A, Nakamura I, Goto S, Moriya H, Nakamura Y, Ikegawa S. Mutation in Npps in a mouse model of ossification of the posterior longitudinal ligament of the spine. Nature genetics. 1998;19(3):271-3).

Left carotid artery ligation surgery may be performed on young mice, for example 6-8 week old mice. Left carotid artery ligation surgery was performed in a 7 week-old WT (n = 5) and *ttw/ttw* mice (n = 5). Mice were anesthetized by isoflurane inhalation (Forene® , Abbott GmbH & Co. KG, Wiesbaden), at an initial concentration of 1 l/min oxygen to 3 vol% isoflurane, maintaining a concentration of 0.6 l/min oxygen to 1–1.5 vol% isoflurane. Carprofen was used for analgesia (5 mg/kg bodyweight through a subcutaneous injection; Rimadyl® , Pfizer, Berlin, Germany). Left carotid arteries were exposed through a small

midline incision in the neck and ligated with a 5-0 nylon silk suture approximately 2 mm proximal from the carotid bifurcation. All animals recovered well from the procedure and showed no signs of a stroke.

Seven days after carotid artery ligation, ENPP1-Fc or vehicle is administered to a model mouse, for example, the *ttw/ttw* mouse. At 7 days after carotid ligation, intimal hyperplasia in *ttw/ttw* mice is present in vessels, but the I/M ratio is lower at 7 days compared to 14 days post-ligated *ttw/ttw* mice ($p < 0.001$ for intimal area and I/M ratio, figure 6B and 6C, respectively). Therefore, at 14 days post-ligation, arterial occlusion (blocking of the arterial lumen) is significant in control mice.

To determine whether ENPP1-Fc has a therapeutic effect if administered after the carotid ligation, 7 week-old WT and *ttw/ttw* mice were subjected to carotid ligation and allowed to recover. Both mice were then treated with either vehicle (Tris buffered saline, pH 7.4/Control cohort) or ENPP1-Fc (Experimental cohort) at 10 mg/kg bodyweight by subcutaneous injection every other day. ENPP1-Fc treatment (10 mg/kg bodyweight subcutaneously injected every other day) was initiated 7 days after carotid ligation and continued for 7 days until the carotid arteries were harvested at 14 days post ligation. Carotid arteries were fixed with 4% paraformaldehyde in PBS for morphological analyses.

Serial sections (sections of 5 μm each) were collected. For morphometrical measurements of the ligated carotid arteries, sections immediately proximal of the ligation site were taken. By using every fifth section, a total of 12 sections (every 25 μm) per animal were analyzed proximal from the ligation site, spanning a distance of approximately 250 μm . Morphometric analyses were performed by using Elastica van Gieson stain (Roth, Karlsruhe, Germany). (See Figure 2 for schematic of sections). ImageJ software was used to measure the circumference of the external elastic lamina, the internal elastic lamina and the luminal border. The medial area, the intimal area and the intima/ media ratio (I/M ratio) were calculated. Right non-ligated carotids from all mice had no measurable neointima indicating that carotid ligation mimics mechanical injury to the vasculature causing VSMC proliferation.

Statistical analyses were performed using Student's t test (unpaired two-sample testing for means). Comparisons of multiple groups used one-way ANOVA, followed by the Bonferroni's post hoc test, performed with GraphPad Prism software version 7. Probability values of $p < 0.05$ were considered significant.

ENPP1 deficiency resulted in neointimal lesion formation after carotid ligation injury in *ttw/ttw* mice and hence *ttw/ttw* mice had higher levels of VSMC proliferation when

compared with the WT mice. Representative stained sections from either 100 or 200 μ m caudal from the ligation in *ttw/ttw*-mice and WT mice showed that the carotid ligation caused intimal hyperplasia, resulting in the narrowing of the lumen, with more severe narrowing closer to the ligature (100 μ m) and less severe occlusion further away (200 μ m) (See Figure 3 and 5D).

In *ttw/ttw* mice the degree of intimal hyperplasia was increased, as the lumen at 200 μ m caudal from the ligation was almost completely occluded. Quantitative analyses of sequential sections of ligated common carotid arteries showed that *ttw/ttw* mice had significantly increased neointimal proliferation compared to WT mice after ligation-induced vascular remodeling for 14 days (See Figure 5A-C) but not thickened medial areas. Correspondingly, the I/M ratio of *ttw/ttw* mice was markedly increased compared with WT mice. It was expected that VSMC proliferation would be decreased in *ttw/ttw* mice upon administration of ENPP1-Fc since the mice themselves are deficient in ENPP1 protein. It was rather surprising that the VSMC proliferation in WT mice was also reduced upon ENPP1-Fc administration despite the fact that the WT mice are not deficient in ENPP1 protein. The experiment thus showed definitive evidence that raising ENPP1 protein levels to higher than normal physiological levels had a therapeutic effect of decreasing VSMC proliferation in blood vasculature caused by mechanical injury.

The results demonstrated that subcutaneous administration of recombinant ENPP1-Fc fusion protein treats intimal hyperplasia in mice models of vascular injury in both ENPP1 deficient (*ttw/ttw*) and ENPP1-non deficient (WT) mice. This surprising finding suggests that ENPP1 has therapeutic potential for treating intimal hyperplasia in patients who suffer from VSMC proliferation due to surgical tissue injury, myocardial infarction, stroke, and even non-surgical tissue injury.

EXAMPLE 2 – Prophylactic Effect of ENPP1-Fc administration to WT and *ttw/ttw* mice

The main aim of the experiment is to determine the prophylactic effect of ENPP1-Fc on intimal hyperplasia in WT mice and homozygous *ttw/ttw* mice. The scheme of prophylactic treatment using ENPP1-Fc is shown in Figure 1.

In this preventive approach, both mice (WT & *ttw/ttw* mice) were treated for 7 days prior to carotid ligation, and treatment was continued for 14 days post-surgery or carotid ligation. Left carotid artery ligation surgery was performed in a 7 week-old WT and *ttw/ttw* mice following the procedures outlined in Example I. Mice were then euthanized using CO₂ inhalation 14 days after carotid ligation following the same protocols as in Example I.

To determine the preventive effect of ENPP1 on intimal hyperplasia, both mice (WT & *ttw/ttw* mice) were treated with either vehicle (Control cohort) or ENPP1-Fc (Experimental cohort) for 7 days prior to carotid ligation, and treatment was continued for 14 days post-surgery.

14 days after surgery, both WT- and *ttw/ttw*- mice treated with ENPP1-Fc showed greatly reduced medial area (figure 4 A, $p < 0.05$ and $p < 0.01$ respectively), intimal area (figure 4 B, $p < 0.001$, both) and I/M ratio (figure 4 C, $p < 0.01$ and $p < 0.001$, respectively) compared to those treated with vehicle. Intimal and medial area as well as I/M ratio of ENPP1-Fc treated *ttw/ttw*- mice approached the same level as ENPP1-Fc treated WT-mice ($p > 0.05$), however vehicle treated *ttw/ttw*- mice developed a significantly increased intimal area and I/M ratio compared to vehicle treated WT- mice ($p < 0.01$ and $p < 0.05$, respectively).

For further investigation of apoptosis in carotids from WT- and *ttw/ttw*- mice, a sub cohort which were treated with vehicle alone was allowed to stay ligated for 21 days and TUNEL staining was performed using in situ cell death detection kit (TMR red, Roche Diagnostics GmbH, Penzberg, Germany) following the manufacturer's instructions. For negative control, staining was performed without TUNEL enzyme; for positive control, sample DNA was degraded by DNase I grade I for 10 min at room temperature.

The WT mice treated with ENPP1-Fc showed greatly reduced intimal hyperplasia compared to WT mice treated with vehicle. Likewise, the *ttw/ttw* mice treated with ENPP1-Fc showed greatly reduced intimal hyperplasia compared to *ttw/ttw* mice treated with vehicle. Histological Elastica van Gieson staining of 14 days ligated mice showed much less intimal hyperplasia in ENPP1-Fc treated WT- and *ttw/ttw*-mice than those treated with vehicle, ENPP1-Fc treated *ttw/ttw*-mice approaching the degree seen in ENPP1-Fc treated WT animals (See figure 4 G).

WT- and *ttw/ttw*- mice ligated for 21 days and preventively treated with ENPP1-Fc for 28 days also showed a greatly reduced medial area (figure 4 D, $p < 0.01$ both), intimal area (figure 4 E, $p < 0.001$ and $p < 0.01$, respectively) and I/M ratio (figure 4 F, $p < 0.001$ and $p < 0.05$, respectively) compared to those treated with vehicle. ENPP1-Fc treated WT- and *ttw/ttw*- mice approach the same level of neointimal hyperplasia, however compared to WT- and *ttw/ttw*- mice ligated for 14 days and treated for 21 days, intimal proliferation was not stopped but further progressed (I/M ratio: $p < 0.01$ and $p < 0.05$, respectively).

Interestingly, the carotids of vehicle treated *ttw/ttw*- mice ligated for 21 days had a smaller intimal area than those of vehicle treated WT-mice (figure 4 E). Histological staining of the carotids of vehicle treated *ttw/ttw*-mice ligated for 21 days revealed degraded tissue at

the intimal area, accompanied from degradation of elastic fibers (figure 7A), leading to smaller intimal areas. In the intimal area of *ttw/ttw*- mice ligated for 21 days, TUNEL staining showed increased positive staining compared to WT- mice (figure 7B), indicating increased apoptosis in the ligated arteries of *ttw/ttw*- mice treated with vehicle.

The results of quantitative analyses of the neointimal and medial areas, as well as the I/M ratio of ligated common carotid arteries obtained in vehicle-treated WT mice showed to be similar to those of WT mice without treatment. Likewise, the neointimal and medial areas, as well as the I/M ratio of ligated common carotid arteries obtained in vehicle-treated *ttw/ttw* mice showed to be similar to *ttw/ttw* mice without treatment.

The intimal area of WT mice receiving subcutaneous ENPP1-Fc was significantly reduced compared to vehicle-treated WT mice, whereas the medial area, between the external and internal lamina, remained constant. The I/M ratio showed a statistically significant decrease in ENPP1-Fc treated WT mice compared to vehicle-treated WT mice (See Figure 4) indicating that the prophylactic treatment of ENPP1-Fc prior to carotid ligation has a protective effect by lowering the level of VSMC proliferation.

Furthermore, the preventive treatment of carotid ligated *ttw/ttw*- mice led to more decreased intimal areas and I/M ratios compared to therapeutic treatment (See figure 8B and C, $p < 0.001$, both). One can therefore conclude that, in the context of ENPP1 deficiency, treatment with ENPP1-Fc is more effective when started before the onset of carotid injury, i.e., as early as possible. On the other hand, carotid ligated WT-mice did not show differences in intimal and I/M ratio between preventive and therapeutic treatment groups (figure 8B and C). This suggests that treatment with ENPP1-Fc for stopping intima proliferation is equally effective when started before or after carotid injury in wild type mice.

EXAMPLE 3 – Therapeutic Effect of ENPP3-Fc administration to WT and *ttw/ttw* mice

The main aim of the experiment is to determine the therapeutic effect of ENPP3-Fc on intimal hyperplasia in WT mice and homozygous *ttw/ttw* mice. ENPP3-Fc is prepared using previously established protocols described elsewhere. Left carotid artery ligation surgery is performed in a 6 week-old WT and *ttw/ttw* mice following protocols described in Example 1.

To determine whether ENPP3-Fc could have a therapeutic effect if administered after the carotid ligation, 6 week-old WT and *ttw/ttw* mice are subjected to carotid ligation and allowed to recover. Both mice are then treated with either vehicle (Tris buffered saline, pH 7.4/Control cohort) or ENPP3-Fc (Experimental cohort) at 10 mg/kg bodyweight by subcutaneous injection every other day. ENPP3-Fc treatment (10 mg/kg bodyweight

subcutaneously injected every other day) is initiated 7 days after carotid ligation and continued for 7 days until the carotid arteries are harvested at 14 days post ligation. Carotid arteries are fixed with 4% paraformaldehyde in PBS for morphological analyses.

Serial sections (sections of 5 μm each) are collected and analyzed following the protocols described in Example 1. Statistical analyses are performed as described in Example I. ENPP1 deficiency resulted in neointimal lesion formation after carotid ligation injury in *ttw/ttw* mice and hence *ttw/ttw* mice had higher levels of VSMC proliferation when compared with the WT mice as seen in Example I.

In *ttw/ttw* mice the degree of intimal hyperplasia increased, as the lumen at 200 μm caudal from the ligation was nearly occluded. Quantitative analyses of sequential sections of ligated common carotid arteries shows that *ttw/ttw* mice had significantly increased neointimal proliferation compared to WT mice after ligation-induced vascular remodeling for 14 days.

It is expected that VSMC proliferation will decrease in *ttw/ttw* mice upon administration of ENPP3-Fc since these mutant mice are deficient in ENPP1 protein. It is expected that the VSMC proliferation in WT mice will be reduced upon ENPP3-Fc administration. Such results will evidence that ENPP3-Fc protein has a therapeutic effect by decreasing VSMC proliferation in blood vasculature caused by mechanical injury.

The results are expected to demonstrate that subcutaneous administration of recombinant ENPP3-Fc fusion protein can treat intimal hyperplasia in mice models of vascular injury in both ENPP1 deficient (*ttw/ttw*) and ENPP1 non-deficient (WT) mice. Thus, ENPP3-Fc may serve as a therapeutic for treating intimal hyperplasia in patients who suffer from VSMC proliferation caused due to surgical tissue injury, myocardial infarction, stroke, and even non-surgical tissue injury.

EXAMPLE 4 – Prophylactic Effect of ENPP3-Fc administration to WT and *ttw/ttw* mice

The main aim of the experiment is to determine the prophylactic effect of ENPP3-Fc on intimal hyperplasia in WT mice and homozygous *ttw/ttw* mice. The scheme of prophylactic treatment using ENPP3-Fc is similar to the schematic shown in Figure 1.

In this preventive approach, both mice (WT & *ttw/ttw* mice) are treated for 7 days prior to carotid ligation, and treatment is continued for 14 days post-surgery or carotid ligation. Left carotid artery ligation surgery is performed in a 6 week-old WT and *ttw/ttw* mice following the procedures outlined in Example I. Mice are then euthanized using CO₂ inhalation 14 days after carotid ligation following the same protocols as in Example I.

To determine the preventive effect of ENPP3 on intimal hyperplasia, both mice (WT & *ttw/ttw* mice) are treated with either vehicle (Control cohort) or ENPP3-Fc (Experimental cohort) for 7 days prior to carotid ligation, and treatment continued for 14 days post-surgery. The WT mice treated with ENPP3-Fc are expected to show greatly reduced intimal hyperplasia in comparison to WT mice treated with vehicle. Likewise, the *ttw/ttw* mice treated with ENPP3-Fc are expected to show greatly reduced intimal hyperplasia compared to *ttw/ttw* mice treated with vehicle.

The results of quantitative analyses of the neointimal and medial areas, as well as the I/M ratio of ligated common carotid arteries obtained in vehicle-treated WT mice are expected to be similar to those of WT mice without treatment. Likewise, the neointimal and medial areas, as well as the I/M ratio of ligated common carotid arteries obtained in vehicle-treated *ttw/ttw* mice are expected to be similar to those of *ttw/ttw* mice without treatment.

The intimal area of WT mice receiving subcutaneous ENPP3-Fc is expected to be significantly reduced compared to vehicle-treated WT mice, whereas the medial area, between the external and internal lamina, is expected to be constant. The I/M ratio is expected to show a statistically significant decrease in ENPP3-Fc treated WT mice compared to vehicle-treated WT mice indicating that the prophylactic treatment of ENPP3-Fc prior to carotid ligation will have a protective effect by lowering the level of VSMC proliferation. Thus, ENPP3-Fc administration is expected to prevent and effectively treat myointimal proliferation and stenosis in carotid ligated WT mice in addition to carotid ligated *ttw/ttw* mice. The experiment is expected to demonstrate that administration of ENPP3 prior to and after carotid ligation protects against intimal hyperplasia even in WT mice.

EXAMPLE 5 – ENPP1 Eluting coated stent for the treatment of Atherosclerotic Blood Vessels.

Atherosclerosis is the most common inflammatory disease of arterial vessels, which can lead to life-threatening myocardial infarction or ischemic stroke. The main aim of the experiment is to determine the ability of ENPP1 or ENPP1-Fc eluting stents to inhibit neointima formation and inflammation thereby reducing thrombosis and/or vessel occlusion which increases the risk of hemorrhagic complications post cardiac surgery.

Without being bound to any one theory, it is expected that inducing the overexpression of ENPP1 or ENPP1-Fc at the site of the implanted stent would result in one or more (i) a decrease in platelet activation, (ii) a reduction in restenosis and inflammatory

responses, and (iii) a decrease in VSMC proliferation, following stent implantation. This therapy is based on the delivery of ENPP1 mRNA (or ENPP1-Fc mRNA or ENPP1-Albumin mRNA) to the endothelial cells, which then in turn express the ENPP1 protein at the site of the stent implant after mRNA translation.

Production of ENPP1 mRNA

pcDNA 3.3 plasmid (Eurofins Genomics GmbH, Ebersberg, Germany) containing ENPP1 DNA templates is amplified using the HotStar HiFidelity Polymerase Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The PCR product (PCR cyclor: Eppendorf, Wesseling, Germany) is purified with the Qiaquick PCR Purification Kit (Qiagen). In vitro transcribed mRNA is generated with the MEGascript1 T7 Kit (Ambion, Glasgow, Scotland) according to the manufacturer's instructions.

To modify the mRNA a 3'-0-Mem7 G(5')ppp(5')G RNA Cap Structure Analog (New England Biolabs, Frankfurt, Germany) is added to the reaction as well as pseudouridine-5'-triphosphate and 5-methylcytidine-5'-triphosphate (TriLink Biotech, San Diego, CA, USA), which are substituted for UTP and CTP, respectively. For RNase inhibition 1 µl of RNase inhibitor (Thermo Scientific, Waltham) is added per reaction. The in vitro transcribed mRNA is then purified with the RNeasy Kit (Qiagen). The purified mRNA is dephosphorilized using the Antarctic Phosphatase Kit (New England Biolabs) and once again purified with the RNeasy Kit (Qiagen). The same procedure is repeated to generate enhanced green fluorescent protein (eGFP) mRNA using eGFP DNA. (*Avci-Adali M, Behring A, Keller T, Krajewski S, Schlensak C, Wendel HP (2014), Optimized conditions for successful transfection of human endothelial cells with in vitro synthesized and modified mRNA for induction of protein expression. J Biol Eng 8: 8*).

The functionality of the generated ENPP1 mRNA is validated by measuring free phosphate after hydrolysis of ATP by transfected HEK293 cells. ENPP1 mRNA transfected HEK293 cells are incubated with 20 µM ATP (mölLab, Langenfeld, Germany) or PBS as control for 10 min at 37°C on a shaking platform (Polymax 1040, Heidolph, Schwabach, Germany). The ATP substrate degrades over time in the presence of ENPP1, with the accumulation of the enzymatic product AMP. Using varying concentrations of ATP substrate, the initial rate velocities for ENPP1 are derived in the presence of ATP, and the data is fit to a curve to derive the enzymatic rate constants.

Stent Coating

In order to develop a bioactive stent coating, which allows local delivery of ENPP1 mRNA and transfection of endothelial cells in vivo, the generated ENPP1 mRNA is first coated on thermanox plastic slides. The stent coating is thus simulated using thermanox plastic slides (Nunc, Thermo scientific, USA). First, 100.000 HEK293 cells per well are seeded on a 12-well plate.

After 24 hours, 2 µl Lipofectamin as well as 10 µg ENPP1 mRNA are mixed with 50 µl Opti-MEM and incubated at room temperature for 20 min. Meanwhile, 10 µl from a polylactic-co-glycolic-acid (PLGA) (Evoniks, Darmstadt) stock solution (20 mg/ml)) is diluted in 990 µl ethyl acetate (final concentration 200 µg/ml). Then 200 µl of the PLGA solution are mixed with the transfection complexes.

The thermanox slides are coated with the solution in a step-by-step approach at room temperature. eGFP mRNA and sterilized water are used as controls. The HEK293 cells are supplied with a new medium before the dried slides are plated face down onto the cells. The cells are incubated with the slides at 37°C and 5% CO₂ for 24 hrs, 48 hrs and 72 hrs and then analyzed using a FACScan cytometer.

The expression of ENPP1 of HEK293 cells was measured using flow cytometry. The ENPP1 coated thermanox slide exposed cells and control cells are stained with anti-ENPP1-fluorescein isothiocyanate (FITC) antibody. Flow cytometric analysis of the HEK293 cells after incubation with the ENPP1 mRNA/PLGA covered thermanox slides are expected to show that the ENPP1 mRNA is released from the PLGA coating, whereby increase in ENPP1 expression is expected to be detectable after 24 hours, 48 hours and 72 hours post exposure to slides.

Compared to control HEK293 cells, (which were exposed thermanox slides coated with Lipofectamine alone) 0.5-1 µg of the ENPP1 mRNA is expected to be sufficient to induce increase of the ENPP1 protein expression in HEK cells exposed to ENPP1 mRNA coated thermanox slides even after 24 hours of exposure.

Without being bound to any one theory, it is proposed herein that the ENPP1 expressed at the site of the stent implant is expected to prevent intimal proliferation and reduce platelet occlusion thereby the risk of hemorrhagic complications post cardiac surgery as seen from the results of Examples 1 and 2.

EXAMPLE 5 – Preparation and implantation of ENPP1 Eluting coated stent for the treatment of Atherosclerotic Blood Vessels

An ENPP1 agent coated stent is prepared and then implanted in a coronary artery. In this example, a juvenile pig animal model is used for implanting the ENPP1-coated stent to determine the efficacy of an ENPP1 coated stent to inhibit neointima formation, restenosis and inflammation.

Preparation of ENPP1 coated stent

Any stent is amenable to be coated with ENPP1 agent. Common examples of commercial sources that sell stents for use include Abbot, Boston Scientific, Medtronic, Alvimedica, Lepu Medical Technology, Cordis, Balton or Biotronik.

For example, a plain stent such as a bare metal stent can be converted to ENPP1 coated eluting stent by placing a polymeric film comprising ENPP1 mRNA inside the stent or by spraying a polymeric or nonpolymeric solution comprising ENPP1 mRNA or ENPP1 polypeptide on to the stent surface.

Some examples of ENPP1 polymeric film are shown below, the ENPP1 polymeric film can be placed inside stents to create ENPP1 coated eluting stents. Optionally nonpolymeric carrier such as Vitamin E, Vitamin E acetate, Vitamin E succinate, oleic acid, peanut oil and cottonseed oil can be added to the solution improve the stability of ENPP1 agent in the polymeric film

- (a) ENPP1 agent coating composition (A) – 10 mg PCL (poly caprolactone) polymer and 100 µg ENPP1 mRNA (or ENPP1-Fc mRNA or ENPP1-Albumin mRNA) are dissolved in sterile double distilled water at room temperature. The solution is poured onto a glass plate and the solvent is allowed to evaporate for 12-24 hours. After almost complete removal of the solvent, the ENPP1-loaded PCL film is removed from the glass plate and is cut to 1.5 cm by 1.5 cm size. The ENPP1 mRNA (or ENPP1-Fc mRNA or ENPP1-Albumin mRNA) comprising polymeric film is then mounted on the stainless stent. The same process can be repeated for preparing a stent coated with a vector expressing ENPP1 polypeptide (ENPP1 or ENPP1-Fc or ENPP1-Albumin) by using 50 µg of vector DNA.
- (b) ENPP1 agent coating composition (B) – 10 mg EVA (ethylene-vinyl acetate) polymer and 100 µg ENPP1 mRNA (or ENPP1-Fc mRNA or ENPP1-Albumin mRNA) are dissolved in sterile double distilled water at room temperature. The solution is poured onto a glass plate and the solvent is allowed to evaporate for 12-24 hours. After almost complete removal of the solvent, the ENPP1-mRNA (or

ENPP1-Fc mRNA or ENPP1-Albumin mRNA) loaded EVA film is removed from the glass plate and was cut to 1.5 cm by 1.5 cm size. The ENPP1 mRNA (or ENPP1-Fc mRNA or ENPP1-Albumin mRNA) comprising polymeric film is then mounted on the stainless stent. The same process can be repeated for preparing a stent coated with a vector expressing ENPP1 polypeptide (ENPP1 or ENPP1-Fc or ENPP1-Albumin) by using 50 µg of vector DNA.

Some examples of ENPP1 comprising spray solutions are shown below, the spray solutions can be applied onto stents to create ENPP1 coated eluting stents. Optionally nonpolymeric carrier such as Vitamin E, Vitamin E acetate, Vitamin E succinate, oleic acid, peanut oil and cottonseed oil can be added to the spray solution improve the stability of ENPP1 agent.

- (c) ENPP1 agent coating composition (C)- 10 mg PCL (poly caprolactone) polymer and 100 µg ENPP1 mRNA is dissolved in sterile double distilled water at room temperature. 100 µl polymeric PCL solution comprising the ENPP1 mRNA (or ENPP1-Fc mRNA or ENPP1-Albumin mRNA) is sprayed onto a stent (6 mmx20 mm) using a semi-automated nebulizer apparatus. The nebulizer spray system provides means of rotating and traversing the length of the stent at a controlled rate. The traversing component of the apparatus contained a glass nebulizer system that applies nebulized polycaprolactone solution to the stent at a rate of 3 ml per minute. Once applied, the polymer coating is "reflowed" by application of 60° C heated air for approximately 5 seconds. The process of reflowing the polymer provides better adherence to the stent surface. The same process can be repeated for preparing a stent coated with a vector expressing ENPP1 polypeptide (ENPP1 or ENPP1-Fc or ENPP1-Albumin) by using 50 µg of vector DNA.
- (d) ENPP1 agent coating composition (D)-A 1% solution of uncured two-part silicone rubber is dissolved in trichloroethylene and then sprayed on to the stent using a nebulizer spray system as described above in (C). The coated stent is dried at room temperature for 15 minutes to allow the trichloroethylene to evaporate. The coated stent comprising silicone is heated in a vacuum oven for a period of four hours in order to crosslink the silicone coating. The coated stents are removed from the oven and allowed to cool for a period of 1 hour. 100 µg ENPP1 mRNA is dissolved in sterile double distilled water at room temperature. A volume of 100

μ l of ENPP1 comprising spray solution is applied to the silicone coating of each stent in dropwise fashion. The crosslinked silicone absorbs the solution, where the solvent subsequently evaporates at room temperature, leaving behind the ENPP1 mRNA (or ENPP1-Fc mRNA or ENPP1-Albumin mRNA) entrapped within the silicone. The same process can be repeated for preparing a stent coated with a vector expressing ENPP1 polypeptide (ENPP1 or ENPP1-Fc or ENPP1-Albumin) by using 50 μ g of vector DNA. The solvent subsequently evaporates at room temperature, leaving behind the ENPP1 encoding vector entrapped within the silicone.

- (e) ENPP1 agent coating composition (E)-10 mg PCL (poly caprolactone) polymer and ENPP1 polypeptide (any one of ENPP1 or ENPP1-Fc or ENPP1-albumin) is dissolved in sterile double distilled water at room temperature to reach an ENPP1 polypeptide concentration of 10 mg/ml. 100 μ l polymeric PCL solution comprising the ENPP1 polypeptide (10 mg/ml) is sprayed onto a stent as described in (C)
- (f) ENPP1 agent coating composition (F)- The coated stent comprising silicone are prepared as discussed in (d). The coated stents are removed from the oven and allowed to cool for a period of 1 hour. ENPP1 polypeptide (ENPP1 or ENPP1-Fc or ENPP1-Albumin) is dissolved in a sterile double distilled water at room temperature to reach an ENPP1 polypeptide concentration of 10 mg/ml. A volume of 100 μ l of ENPP1 comprising spray solution (10 mg/ml) is applied to the silicone coating of each stent in dropwise fashion. The crosslinked silicone absorbs the solution, where the solvent subsequently evaporates at room temperature, leaving behind the ENPP1 mRNA entrapped within the silicone.

Animal Model

Thirty 4-to-5-month-old juvenile pigs with the weight of 25–35 kg are procured from commercial sources. Thirty stainless steel vents are obtained from one or more commercial sources such as Abbot, Boston Scientific, Medtronic, Alvimedica, Lepu Medical Technology, Cordis, Balton or Biotronik. Thirty stainless steel stents thus obtained are coated with ENPP1 mRNA following the protocol shown above for coating. Thirty bare metal stents (BMSs) are obtained from Abbott to be used as control set. The ENPP1 coated stent is then sterilized using ethylene oxide, compressed, and mounted on a balloon angioplasty catheter. It is then deployed at a site in an artery using standard balloon angioplasty techniques.

The stents are randomly assigned and placed in the left anterior descending, circumflex, or right coronary arteries (one stent per artery) of 30 pigs, one coated stent per pig. The pigs are then maintained on 75 mg clopidogrel and 100 mg aspirin per day and sacrificed after 7 days and 14 days, respectively.

Seven or 14 days after stent implantation, the animals are euthanized using intravenous injection of pentobarbital euthanasia solution (100 mg/kg), and the stented coronary arteries were harvested. The arteries are sectioned into 3 to 5 mm segments from the proximal, middle, and distal part of the stents, fixed in 4% formalin for 48 h, and embedded in paraffin. The sections are subjected to histology and morphometrical measurements to determine intimal, medial area and I/M ratios following the protocols described in Example 1. The intimal area of arterial sections obtained from pigs receiving ENPP1 coated stents is expected to be significantly reduced compared to arterial sections from pigs having non-eluting stainless-steel bare mesh stent. The I/M ratio is expected to show a statistically significant decrease in the arterial sections of pigs with ENPP1 coated stents compared to pigs with non-eluting stainless-steel stents. Thus, in situ administration of ENPP1 agent by using ENPP1 coated stents is expected to prevent and effectively treat myointimal proliferation and/or restenosis at the site of injury.

EXAMPLE 6 – Preparation and implantation of ENPP3 Eluting coated stent for the treatment of Atherosclerotic Blood Vessels

An ENPP3 agent coated stent is prepared and then implanted in a coronary artery. In this example, a juvenile pig animal model is used for implanting the ENPP3-coated stent to determine the efficacy of an ENPP3 coated stent to inhibit neointima formation, restenosis and inflammation.

Preparation of ENPP3 coated stent

Any stent is amenable to be coated with ENPP3 agent. Common examples of commercial sources that sell stents for use include Abbot, Boston Scientific, Medtronic, Alvimedica, Lepu Medical Technology, Cordis, Balton or Biotronik.

For example, a plain stent such as a bare metal stent can be converted to ENPP3 coated stent by placing a polymeric film comprising ENPP3 mRNA inside the stent or by spraying a polymeric or nonpolymeric solution comprising ENPP3 mRNA or ENPP3 polypeptide on to the stent surface.

Some examples of ENPP3 polymeric film are shown below, the ENPP3 polymeric film can be placed inside stents to create ENPP3 coated eluting stents. Optionally nonpolymeric carrier such as Vitamin E, Vitamin E acetate, Vitamin E succinate, oleic acid, peanut oil and cottonseed oil can be added to the solution improve the stability of ENPP3 agent in the polymeric film

- (a) ENPP3 agent coating composition (A) – 10 mg PCL (poly caprolactone) polymer and 100 µg ENPP3 mRNA (or ENPP3-Fc mRNA or ENPP3-Albumin mRNA) are dissolved in sterile double distilled water at room temperature. The solution is poured onto a glass plate and the solvent is allowed to evaporate for 12-24 hours. After almost complete removal of the solvent, the ENPP3-loaded PCL film is removed from the glass plate and is cut to 1.5 cm by 1.5 cm size. The ENPP3 mRNA (or ENPP3-Fc mRNA or ENPP3-Albumin mRNA) comprising polymeric film is then mounted on the stainless stent. The same process can be repeated for preparing a stent coated with a vector expressing ENPP3 polypeptide (ENPP3 or ENPP3- Fc or ENPP3-Albumin) by using 50 µg of vector DNA.
- (b) ENPP3 agent coating composition (B) – 10 mg EVA (ethylene-vinyl acetate) polymer and 100 µg ENPP3 mRNA (or ENPP3-Fc mRNA or ENPP3-Albumin mRNA) are dissolved in sterile double distilled water at room temperature. The solution is poured onto a glass plate and the solvent is allowed to evaporate for 12-24 hours. After almost complete removal of the solvent, the ENPP3-mRNA (or ENPP3-Fc mRNA or ENPP3-Albumin mRNA) loaded EVA film is removed from the glass plate and was cut to 1.5 cm by 1.5 cm size. The ENPP3 mRNA (or ENPP3-Fc mRNA or ENPP3-Albumin mRNA) comprising polymeric film is then mounted on the stainless stent. The same process can be repeated for preparing a stent coated with a vector expressing ENPP3 polypeptide (ENPP3 or ENPP3-Fc or ENPP3-Albumin) by using 50 µg of vector DNA.

Some examples of ENPP3 comprising spray solutions are shown below, the spray solutions can be applied onto stents to create ENPP3 coated eluting stents. Optionally nonpolymeric carrier such as Vitamin E, Vitamin E acetate, Vitamin E succinate, oleic acid, peanut oil and cottonseed oil can be added to the spray solution improve the stability of ENPP3 agent.

- (c) ENPP3 agent coating composition (C)- 10 mg PCL (poly caprolactone) polymer and 100 µg ENPP3 mRNA(or ENPP3-Fc mRNA or ENPP3-Albumin mRNA) is dissolved in sterile double distilled water at room temperature. 100 µl polymeric PCL solution comprising the ENPP3 mRNA (or ENPP3-Fc mRNA or ENPP3-Albumin mRNA) is sprayed onto a stent (6 mmx20 mm) using a semi-automated nebulizer apparatus as described above in Example 5. The same process can be repeated for preparing a stent coated with a vector expressing ENPP3 polypeptide (ENPP3 or ENPP3-Fc or ENPP3-Albumin) by using 50 µg of vector DNA.
- (d) ENPP3 agent coating composition (D)-A 1% solution of uncured two-part silicone rubber is dissolved in trichloroethylene and then sprayed on to the stent using a nebulizer spray system as described above in Example 5. The coated stents are removed from the oven and allowed to cool for a period of 1 hour. 100 µg ENPP3 mRNA (or ENPP3-Fc mRNA or ENPP3-Albumin mRNA) is dissolved in sterile double distilled water at room temperature. A volume of 100 µl of ENPP3 mRNA (or ENPP3-Fc mRNA or ENPP3-Albumin mRNA) comprising spray solution is applied to the silicone coating of each stent in dropwise fashion. The crosslinked silicone absorbs the solution, where the solvent subsequently evaporates at room temperature, leaving behind the ENPP3 mRNA (or ENPP3-Fc mRNA or ENPP3-Albumin mRNA) entrapped within the silicone. The same process can be repeated for preparing a stent coated with a vector expressing ENPP3 polypeptide (ENPP3 or ENPP3-Fc or ENPP3-Albumin) by using 50 µg of vector DNA. The solvent subsequently evaporates at room temperature, leaving behind the ENPP3 encoding vector entrapped within the silicone.
- (e) ENPP3 agent coating composition (E)-10 mg PCL (poly caprolactone) polymer and ENPP3 polypeptide (any one of ENPP3 or ENPP3-Fc or ENPP3-albumin) is dissolved in sterile double distilled water at room temperature to reach an ENPP3 polypeptide concentration of 10 mg/ml. 100µl polymeric PCL solution comprising the ENPP3 polypeptide (10 mg/ml) is sprayed onto a stent as described in Example 5
- (f) ENPP3 agent coating composition (F)- The coated stent comprising silicone are prepared as describe in Example 5 The coated stents are removed from the oven and allowed to cool for a period of 1 hour. ENPP3 polypeptide (any one of ENPP3, ENPP3-Fc, ENPP3-Albumin) is dissolved in a sterile double distilled water at room temperature to reach an ENPP3 polypeptide concentration of 10

mg/ml. A volume of 100 µl of ENPP3 comprising spray solution (10 mg/ml) is applied to the silicone coating of each stent in dropwise fashion. The crosslinked silicone absorbs the solution, where the solvent subsequently evaporates at room temperature, leaving behind the ENPP3 polypeptide entrapped within the silicone.

Animal Model

Thirty 4-to-5-month-old juvenile pigs with the weight of 25–35 kg are procured from commercial sources as described in Example 5. Thirty stainless steel vents are obtained from commercial sources. Thirty stainless steel stents thus obtained are coated with ENPP3 mRNA following the protocol shown above for coating. Thirty bare metal stents (BMSs) are obtained from Abbott to be used as control set. The ENPP3 coated stent is then sterilized using ethylene oxide, compressed, and mounted on a balloon angioplasty catheter. It is then deployed at a site in an artery using standard balloon angioplasty techniques.

The stents are randomly assigned and placed in the left anterior descending, circumflex, or right coronary arteries (one stent per artery) of 30 pigs, one coated stent per pig. The pigs are then maintained on 75 mg clopidogrel and 100 mg aspirin per day and sacrificed after 7 days and 14 days, respectively. Seven or 14 days after stent implantation, the animals are euthanized using intravenous injection of pentobarbital euthanasia solution (100 mg/kg), and the stented coronary arteries were harvested. The arteries are sectioned into 3 to 5 mm segments from the proximal, middle, and distal part of the stents, fixed in 4% formalin for 48 h, and embedded in paraffin.

The sections are subjected to histology and morphometrical measurements to determine intimal, medial area and I/M ratios following the protocols described in Example 1. The intimal area of arterial sections obtained from pigs receiving ENPP3 coated stents is expected to be significantly reduced compared to arterial sections from pigs having non-eluting stainless-steel bare mesh stent. The I/M ratio is expected to show a statistically significant decrease in the arterial sections of pigs with ENPP3 eluting stents compared to pigs with non-eluting stainless-steel stents. Thus, in situ administration of ENPP3 agent by using ENPP3 coated eluting stents is expected to prevent and effectively treat myointimal proliferation and/or restenosis at the site of injury.

INCORPORATION BY REFERENCE

The disclosure of each and every U.S. and foreign patent and pending patent application and publication referred to herein is specifically incorporated herein by reference in its entirety, as are the contents of Sequence Listing and Figures.

EQUIVALENTS

Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents of the specific embodiments described herein. Such equivalents are intended to be encompassed by the following claims. Any combination of the embodiments disclosed in the any plurality of the dependent claims or Examples is contemplated to be within the scope of the disclosure.

OTHER EMBODIMENTS

From the foregoing description, it will be apparent that variations and modifications may be made to the disclosure described herein to adopt it to various usages and conditions, including the use of different signal sequences to express functional variants of ENPP1 or ENPP3 or combinations thereof in different viral vectors having different promoters or enhancers or different cell types known in art to treat any diseases characterized by the presence of pathological calcification or ossification are within the scope according to the disclosure. Other embodiments according to the disclosure are within the following claims.

Recitation of a listing of elements in any definition of a variable herein includes definitions of that variable as any single element or combination (or sub combination) of listed elements. Recitation of an embodiment herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

All publications and patent applications mentioned in the specification are indicative of the level of skill of those skilled in the art to which this disclosure pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Other embodiments are within the following claims.

What is claimed is:

1. A method for reducing and/or preventing progression of vascular smooth muscle cell proliferation in a subject having a tissue injury, the method comprising: administering to the subject an amount of an ENPP1 agent effective to reduce and/or prevent progression of vascular smooth muscle cell proliferation at a site of injury in the subject, wherein the subject is not ENPP1 deficient, thereby to reduce and/or prevent progression of vascular smooth muscle cell proliferation.
2. The method of claim 1, wherein the tissue injury comprises injury to an artery.
3. The method of claim 1 or 2, wherein the tissue injury comprises stent placement in an artery.
4. The method of any one of claims 1-3, wherein the subject is at risk of developing restenosis.
5. The method of any one of claims 1-3, wherein the subject suffers from restenosis.
6. The method of claim 5, wherein the subject suffers from restenosis in an artery.
7. A method for reducing and/or preventing progression of vascular smooth muscle cell proliferation in a subject who has a condition requiring surgery at a surgical site, the method comprising: administering to the subject an amount of an ENPP1 agent effective to reduce and/or prevent progression of vascular smooth muscle cell proliferation at said surgical site in the subject, wherein the subject is not ENPP1 deficient, thereby to reduce and/or prevent progression of vascular smooth muscle cell proliferation at said surgical site.
8. The method of claim 7, wherein the agent is administered to the subject prior to, during and/or after surgery.
9. The method of claim 7 or 8, further comprising performing the surgery.
10. The method of any one of claims 7-9, wherein the surgery comprises artery bypass grafting.
11. The method of any one of claims 7-10, wherein the surgery comprises placement of an arterial stent.
12. The method of any one of claims 7-11, wherein the surgery comprises angioplasty.
13. A method for ameliorating a myocardial infarction or a stroke in a subject suffering therefrom, the method comprising: administering to the subject an amount of an

ENPP1 agent effective to ameliorate a myocardial infarction or stroke, thereby to ameliorate said myocardial infarction or stroke.

14. A method for reducing and/or preventing progression of vascular smooth muscle cell proliferation in a subject suffering a myocardial infarction or a stroke, the method comprising: administering to the subject an amount of an ENPP1 agent effective to reduce and/or prevent progression of vascular smooth muscle cell proliferation in vasculature associated with a myocardial infarction or stroke, thereby to reduce and/or prevent progression of vascular smooth muscle cell proliferation in vasculature of said subject associated with myocardial infarction or stroke.

15. The method of claim 13 or 14, wherein the subject is not ENPP1 deficient.

16. The method of any one of claims 1-15, wherein the ENPP1 agent comprises an ENPP1 polypeptide.

17. The method of any one of claims 1-15, wherein the ENPP1 agent is a nucleic acid encoding an ENPP1 polypeptide.

18. The method of any one of claims 1-15, wherein the ENPP1 agent comprises a viral vector comprising a nucleic acid encoding an ENPP1 polypeptide.

19. The method of any one of claims 16-18, wherein the ENPP1 polypeptide comprises the extracellular domain of ENPP1.

20. The method of any one of claims 16-18, wherein the ENPP1 polypeptide comprises the catalytic domain of ENPP1.

21. The method of any one of claims 16-18, wherein the ENPP1 polypeptide comprises amino acids 99 to 925 of SEQ ID NO:1.

22. The method of any one of claims 16-18, wherein the ENPP1 polypeptide comprises a heterologous protein.

23. The method of claim 22, wherein the heterologous protein increases the circulating half-life of the ENPP1 polypeptide in mammal.

24. The method of claim 22 or 23, wherein the heterologous protein is an Fc region of an immunoglobulin molecule.

25. The method of claim 24, wherein the immunoglobulin molecule is an IgG1 molecule.

26. The method of claim 22 or 23, wherein the heterologous protein is an albumin molecule.

27. The method of any one of claims 22-26, wherein the heterologous protein is carboxy-terminal to the ENPP1 polypeptide.

28. The method of any one of claims 22-27, wherein ENPP1 agent comprises a linker.
29. The method of claim 28, wherein the linker separates the ENPP1 polypeptide and the heterologous protein.
30. The method of claim 28 or 29, wherein the linker comprises the following amino acid sequence: (GGGS)_n, wherein n is an integer from 1 to 10.
31. The method according to any one of claims 1-30, wherein the ENPP1 agent is subcutaneously administered to the subject.
32. The method according to any one of claims 1-30, wherein the ENPP1 agent is intravenously administered to the subject.
33. A method for reducing and/or preventing progression of vascular smooth muscle cell proliferation in a subject having a tissue injury, the method comprising: administering to the subject an amount of an ENPP3 agent effective to reduce and/or prevent progression of vascular smooth muscle cell proliferation at a site of injury in the subject, thereby to reduce and/or prevent progression of vascular smooth muscle cell proliferation.
34. The method of claim 33, wherein the tissue injury comprises injury to an artery.
35. The method of claim 33 or 34, wherein the tissue injury comprises stent placement in an artery.
36. The method of any one of claims 33-35, wherein the subject is at risk of developing restenosis.
37. The method of any one of claims 33-36, wherein the subject suffers from restenosis.
38. The method of claim 37, wherein the subject suffers from restenosis in an artery.
39. A method for reducing and/or preventing progression of vascular smooth muscle cell proliferation in a subject who has a condition requiring surgery at a surgical site, the method comprising: administering to the subject an amount of an ENPP3 agent effective to reduce and/or prevent progression of vascular smooth muscle cell proliferation at said surgical site in the subject, thereby to reduce and/or prevent progression of vascular smooth muscle cell proliferation at said surgical site.
40. The method of claim 39, wherein the agent is administered to the subject prior to, during and/or after surgery.
41. The method of claim 39 or 40, further comprising performing the surgery.

42. The method of any one of claims 39-41, wherein the surgery comprises artery bypass grafting.

43. The method of any one of claims 39-42, wherein the surgery comprises placement of an arterial stent.

44. The method of any one of claims 39-43, wherein the surgery comprises angioplasty.

45. A method for ameliorating a myocardial infarction or a stroke in a subject suffering therefrom, the method comprising: administering to the subject an amount of an ENPP3 agent effective to ameliorate a myocardial infarction or stroke, thereby to ameliorating said myocardial infarction or stroke.

46. A method for reducing and/or preventing progression of vascular smooth muscle cell proliferation in a subject suffering a myocardial infarction or a stroke, the method comprising: administering to the subject an amount of an ENPP3 agent effective to reduce and/or prevent progression of vascular smooth muscle cell proliferation in vasculature associated with a myocardial infarction or stroke, thereby to reduce and/or prevent progression of vascular smooth muscle cell proliferation in vasculature of said subject associated with myocardial infarction or stroke.

47. The method of any one of claims 33-46, wherein the subject is not ENPP1 deficient.

48. The method of any one of claims 33-47, wherein the ENPP3 agent comprises an ENPP3 polypeptide.

49. The method of any one of claims 33-47, wherein the ENPP3 agent is a nucleic acid encoding an ENPP3 polypeptide.

50. The method of any one of claims 33-47, wherein the ENPP3 agent comprises a viral vector comprising a nucleic acid encoding an ENPP3 polypeptide.

51. The method of any one of claims 33-50, wherein the ENPP3 polypeptide comprises the extracellular domain of ENPP3.

52. The method of any one of claims 33-51, wherein the ENPP3 polypeptide comprises the catalytic domain of ENPP3.

53. The method of any one of claims 33-51, wherein the ENPP3 polypeptide comprises amino acids 49-875 of SEQ ID NO: 7.

54. The method of any one of claims 33-53, wherein the ENPP3 polypeptide comprises a heterologous protein.

55. The method of claim 54, wherein the heterologous protein increases the circulating half-life of the ENPP3 polypeptide in mammal.
56. The method of claim 54 or 55, wherein the heterologous protein is an Fc region of an immunoglobulin molecule.
57. The method of claim 56, wherein the immunoglobulin molecule is an IgG1 molecule.
58. The method of claim 54 or 55, wherein the heterologous protein is an albumin molecule.
59. The method of any one of claims 54-58, wherein the heterologous protein is carboxy-terminal to the ENPP3 polypeptide.
60. The method of any one of claims 33-59, wherein ENPP3 agent comprises a linker.
61. The method of claim 60, wherein the linker separates the ENPP3 polypeptide and the heterologous protein.
62. The method of claim 60 or 61, wherein the linker comprises the following amino acid sequence: (GGGS)_n, wherein n is an integer from 1 to 10.
63. The method according to any one of claims 33-62, wherein the ENPP3 agent is subcutaneously administered to the subject.
64. The method according to any one of claims 33-62, wherein the ENPP3 agent is intravenously administered to the subject.
65. A coated stent comprising:
a vascular stent; and
a coating on the stent, the coating comprising an ENPP1 agent; and
a carrier for said ENPP1 agent,
wherein said coating is configured to release said ENPP1 agent from the stent at a rate of 1-10 µg/ml per day.
66. The coated stent of claim 65, said ENPP1 agent in an amount between 1 wt % and 50 wt %, based on a total weight of the coating.
67. The coated stent of claim 66, wherein said ENPP1 agent is selected from a group consisting of: ENPP1, ENPP1-Fc, ENPP1-Albumin, and ENPP1 mRNA.
68. The coated stent of claim 65, wherein the carrier is non-reactive with said ENPP1 agent.

69. The coated stent of claim 65, wherein the carrier comprises a polymeric carrier that is physically bound to said ENPP1 agent.
70. The coated stent of claim 65, wherein the carrier comprises a polymeric carrier that is chemically bound to said ENPP1 agent.
71. The coated stent of claim 65, wherein the carrier comprises a polymeric biodegradable carrier.
72. The coated stent of claim 65, wherein the carrier comprises a nonpolymeric carrier.
73. The coated stent of claim 72, wherein the nonpolymeric carrier is selected from a group consisting of: Vitamin E, Vitamin E acetate, Vitamin E succinate, oleic acid, peanut oil and cottonseed oil.
74. The coated stent of claim 65, wherein the carrier is a liquid at body temperature.
75. The coated stent of claim 65, wherein the carrier is a solid at body temperature.
76. A method for reducing and/or preventing progression of vascular smooth muscle cell proliferation in a subject having a tissue injury, the method comprising: implanting an arterial stent coated with an ENPP1 agent into an artery of the subject proximal to said tissue injury, wherein said implanted stent is configured to release said ENPP1 agent in an amount effective to reduce and/or prevent progression of vascular smooth muscle cell proliferation at a site of injury in the subject, wherein the subject is not ENPP1 deficient, thereby to reduce and/or prevent progression of vascular smooth muscle cell proliferation at said site of injury in said subject.
77. The method of claim 76, wherein the tissue injury comprises stent placement in an artery.
78. The method of claim 76 or 77, wherein the tissue injury is due to a prior placement of a non-eluting arterial stent in said artery or due to a prior placement of an eluting arterial stent in said artery which elutes therapeutic agents other than said ENPP1 agent.
79. The method of any one of claims 76-78, wherein the subject is at risk of developing restenosis.

80. A method for reducing and/or preventing progression of vascular smooth muscle cell proliferation in a subject who has a condition requiring surgery at a surgical site, the method comprising: implanting an arterial stent coated with an ENPP1 agent into an artery proximal to said surgical site in the subject, wherein said implanted stent is configured to release said ENPP1 agent in an amount effective to reduce and/or prevent progression of vascular smooth muscle cell proliferation, wherein the subject is not ENPP1 deficient, thereby to reduce and/or prevent progression of vascular smooth muscle cell proliferation at said surgical site.

81. The method of claim 80, wherein the agent is administered to the subject prior to, during and/or after surgery.

82. The method of claim 80 or 81, further comprising performing the surgery.

83. The method of any one of claims 80-82 wherein the surgery comprises artery bypass grafting.

84. The method of claim 80, wherein the condition requiring surgery is due to a prior placement of a non-eluting arterial stent in said artery.

85. The method of claim 80, wherein the condition requiring surgery is due to a prior placement of an eluting arterial stent in said artery which elutes therapeutic agents other than said ENPP1 agent.

86. The method of any one of claims 80-85, wherein the surgery comprises angioplasty.

87. A method for ameliorating a myocardial infarction or a stroke in a subject suffering therefrom, the method comprising: implanting an arterial stent coated with an ENPP1 agent into an artery of said subject, wherein said implanted stent is configured to release said ENPP1 agent in an amount effective to ameliorate a myocardial infarction or stroke, thereby to ameliorating said myocardial infarction or stroke.

88. A method for reducing and/or preventing progression of vascular smooth muscle cell proliferation in a subject suffering a myocardial infarction or a stroke, the method comprising: implanting an arterial stent coated with an ENPP1 agent into an artery of a subject, wherein said implanted stent is configured to release said ENPP1 agent in an amount effective to reduce and/or prevent progression of vascular smooth muscle cell proliferation in vasculature associated with a myocardial infarction or stroke, thereby to reduce and/or

prevent progression of vascular smooth muscle cell proliferation in vasculature of said subject associated with myocardial infarction or stroke.

89. The method of claim 87 or 88, wherein the subject is not ENPP1 deficient.
90. The method of any one of claims 77-89, wherein the ENPP1 agent comprises an ENPP1 polypeptide.
91. The method of any one of claims 77-89, wherein the ENPP1 agent is a nucleic acid encoding an ENPP1 polypeptide.
92. The method of any one of claims 77-89, wherein the ENPP1 agent comprises a viral vector comprising a nucleic acid encoding an ENPP1 polypeptide.
93. The method of any one of claims 90-92, wherein the ENPP1 polypeptide comprises the extracellular domain of ENPP1.
94. The method of any one of claims 90-92, wherein the ENPP1 polypeptide comprises the catalytic domain of ENPP1.
95. The method of any one of claims 90-92, wherein the ENPP1 polypeptide comprises amino acids 99 to 925 of SEQ ID NO:1.
96. The method of any one of claims 90-92, wherein the ENPP1 polypeptide comprises a heterologous protein.
97. The method of claim 96, wherein the heterologous protein increases the circulating half-life of the ENPP1 polypeptide in mammal.
98. The method of claim 96 or 97, wherein the heterologous protein is an Fc region of an immunoglobulin molecule.
99. The method of claim 98, wherein the immunoglobulin molecule is an IgG1 molecule.
100. The method of claim 96 or 97, wherein the heterologous protein is an albumin molecule.
101. The method of any one of claims 96-100, wherein the heterologous protein is carboxy-terminal to the ENPP1 polypeptide.

102. The method of any one of claims 96-101, wherein ENPP1 agent comprises a linker.
103. The method of claim 102, wherein the linker separates the ENPP1 polypeptide and the heterologous protein.
104. The method of claim 102 or 103, wherein the linker comprises the following amino acid sequence: (GGGGS) n , wherein n is an integer from 1 to 10.
105. A method for reducing and/or preventing progression of vascular smooth muscle cell proliferation in a subject having a tissue injury, the method comprising: implanting an arterial stent coated with an ENPP3 agent into an artery of a subject proximal to said tissue injury, wherein said implanted stent is configured to release said ENPP3 agent in an amount effective to reduce and/or prevent progression of vascular smooth muscle cell proliferation at a site of injury in the subject, thereby to reduce and/or prevent progression of vascular smooth muscle cell proliferation at said site of injury in said subject.
106. The method of claim 105, wherein the tissue injury comprises injury to an artery.
107. The method of claim 105 or 106, wherein the tissue injury comprises stent placement in an artery.
108. The method of any one of claims 105-107, wherein the subject is at risk of developing restenosis.
109. A method for reducing and/or preventing progression of vascular smooth muscle cell proliferation in a subject who has a condition requiring surgery at a surgical site, the method comprising: implanting an arterial stent coated with an ENPP3 agent into an artery proximal to said surgical site in the subject, wherein said implanted stent is configured to release said ENPP3 agent in an amount effective to reduce and/or prevent progression of vascular smooth muscle cell proliferation, thereby to reduce and/or prevent progression of vascular smooth muscle cell proliferation at said surgical site.
110. The method of claim 109, wherein the agent is administered to the subject prior to, during and/or after surgery.
111. The method of claim 109 or 110, further comprising performing the surgery.

112. The method of any one of claims 109-111 wherein the surgery comprises artery bypass grafting.
113. The method of claim 109, wherein the condition requiring surgery is due to a prior placement of a non-eluting arterial stent in said artery.
114. The method of claim 109, wherein the condition requiring surgery is due to a prior placement of an eluting arterial stent in said artery which elutes therapeutic agents other than said ENPP3 agent.
115. The method of any one of claims 109-114, wherein the surgery comprises angioplasty.
116. A method for ameliorating a myocardial infarction or a stroke in a subject suffering therefrom, the method comprising: implanting an arterial stent coated with an ENPP3 agent into an artery of said subject, wherein said implanted stent is configured to release said ENPP3 agent in an amount effective to ameliorate a myocardial infarction or stroke, thereby to ameliorating said myocardial infarction or stroke.
117. A method for reducing and/or preventing progression of vascular smooth muscle cell proliferation in a subject suffering a myocardial infarction or a stroke, the method comprising: implanting an arterial stent coated with an ENPP3 agent into an artery of a subject, wherein said implanted stent is configured to release said ENPP3 agent in an amount effective to reduce and/or prevent progression of vascular smooth muscle cell proliferation in vasculature associated with a myocardial infarction or stroke, thereby to reduce and/or prevent progression of vascular smooth muscle cell proliferation in vasculature of said subject associated with myocardial infarction or stroke.
118. The method of any one of claims 105 -117 wherein the subject is not ENPP1 deficient.
119. The method of any one of claims 105-118, wherein the ENPP3 agent comprises an ENPP3 polypeptide.
120. The method of any one of claims 105-119, wherein the ENPP3 agent is a nucleic acid encoding an ENPP3 polypeptide.

121. The method of any one of claims 105-120, wherein the ENPP3 agent comprises a viral vector comprising a nucleic acid encoding an ENPP3 polypeptide.
122. The method of any one of claims 105-121, wherein the ENPP3 polypeptide comprises a heterologous protein.
123. The method of any one of claims 105-121, wherein the ENPP3 polypeptide comprises the extracellular domain of ENPP3.
124. The method of any one of claims 105-123, wherein the ENPP3 polypeptide comprises the catalytic domain of ENPP3.
125. The method of any one of claims 105-124, wherein the ENPP3 polypeptide comprises amino acids 49-875 of SEQ ID NO: 7.
126. The method of any one of claims 119-125, wherein the ENPP3 polypeptide comprises a heterologous protein
127. The method of claim 126, wherein the heterologous protein increases the circulating half-life of the ENPP3 polypeptide in mammal.
128. The method of claim 126 or 127, wherein the heterologous protein is an Fc region of an immunoglobulin molecule.
129. The method of claim 128, wherein the immunoglobulin molecule is an IgG1 molecule.
130. The method of claim 126 or 127, wherein the heterologous protein is an albumin molecule.
131. The method of any one of claims 124-130, wherein the heterologous protein is carboxy-terminal to the ENPP3 polypeptide.
132. The method of any one of claims 105-131, wherein ENPP3 agent comprises a linker.
133. The method of claim 132, wherein the linker separates the ENPP3 polypeptide and the heterologous protein.

134. The method of claim 132 or 133, wherein the linker comprises the following amino acid sequence: (GGGGS)_n, wherein n is an integer from 1 to 10.

135. A coated stent comprising:

a vascular stent; and

a coating on the stent, the coating comprising an ENPP3 agent; and

a carrier for said ENPP3 agent,

wherein said coating is configured to release said ENPP3 agent from the stent at a rate of 1-10 µg/ml per day.

136. The coated stent of claim 135, said ENPP3 agent in an amount between 1 wt % and 50 wt %, based on a total weight of the coating.

137. The coated stent of claim 136, wherein said ENPP3 agent is selected from a group consisting of: ENPP3, ENPP3-Fc, ENPP3-Albumin, and ENPP3 mRNA.

138. The coated stent of claim 135, wherein the carrier is non-reactive with said ENPP3 agent.

139. The coated stent of claim 135, wherein the carrier comprises a polymeric carrier that is physically bound to said ENPP3 agent.

140. The coated stent of claim 135, wherein the carrier comprises a polymeric carrier that is chemically bound to said ENPP3 agent.

141. The coated stent of claim 135, wherein the carrier comprises a polymeric biodegradable carrier.

142. The coated stent of claim 135, wherein the carrier comprises a nonpolymeric carrier.

143. The coated stent of claim 142, wherein the nonpolymeric carrier is selected from a group consisting of: Vitamin E, Vitamin E acetate, Vitamin E succinate, oleic acid, peanut oil and cottonseed oil.

144. The coated stent of claim 135, wherein the carrier is a liquid at body temperature.

145. The coated stent of claim 135, wherein the carrier is a solid at body temperature.

146. The method of any of the aforesaid claims, wherein the ENPP1 agent comprises ENPP1 variants that retain enzymatic activity.
147. The method of any of the aforesaid claims, wherein the ENPP3 agent comprises ENPP3 variants that retain enzymatic activity.
148. The coated stent of any one of claims 65-75, wherein the ENPP1 agent comprises ENPP1 variants that retain enzymatic activity.
149. The coated stent of any one of claims 135-145, wherein the ENPP3 agent comprises ENPP3 variants that retain enzymatic activity.

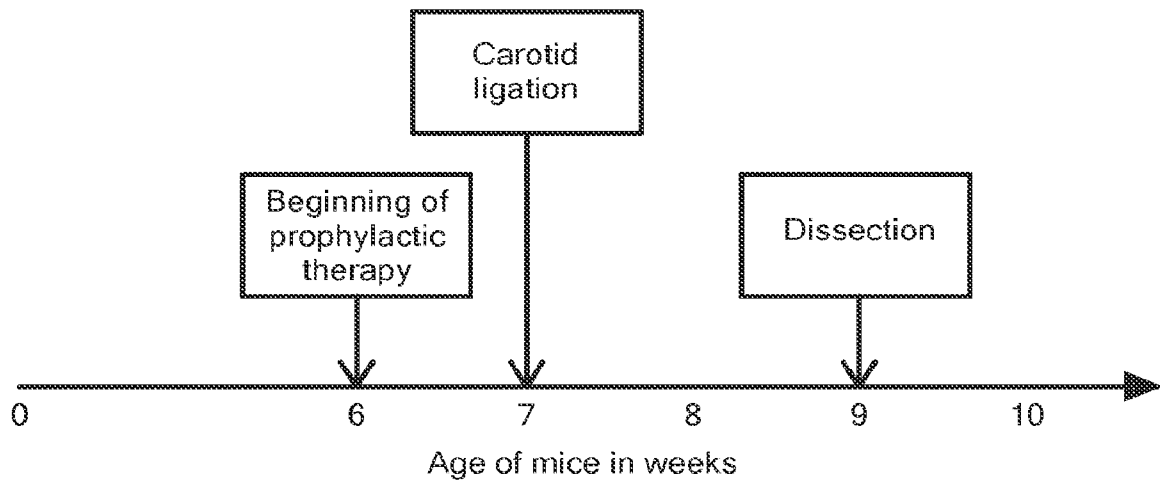
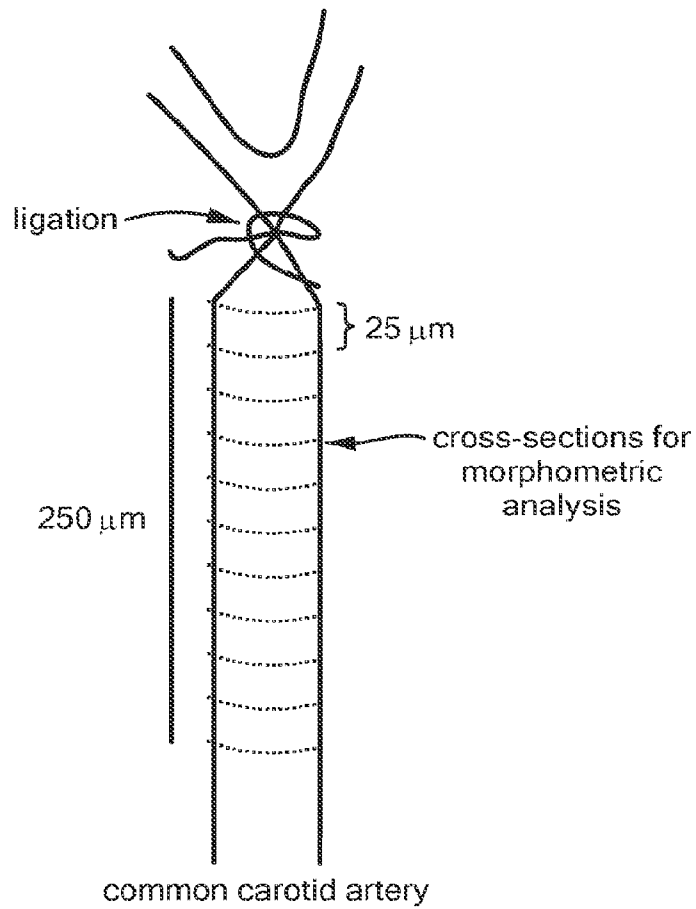
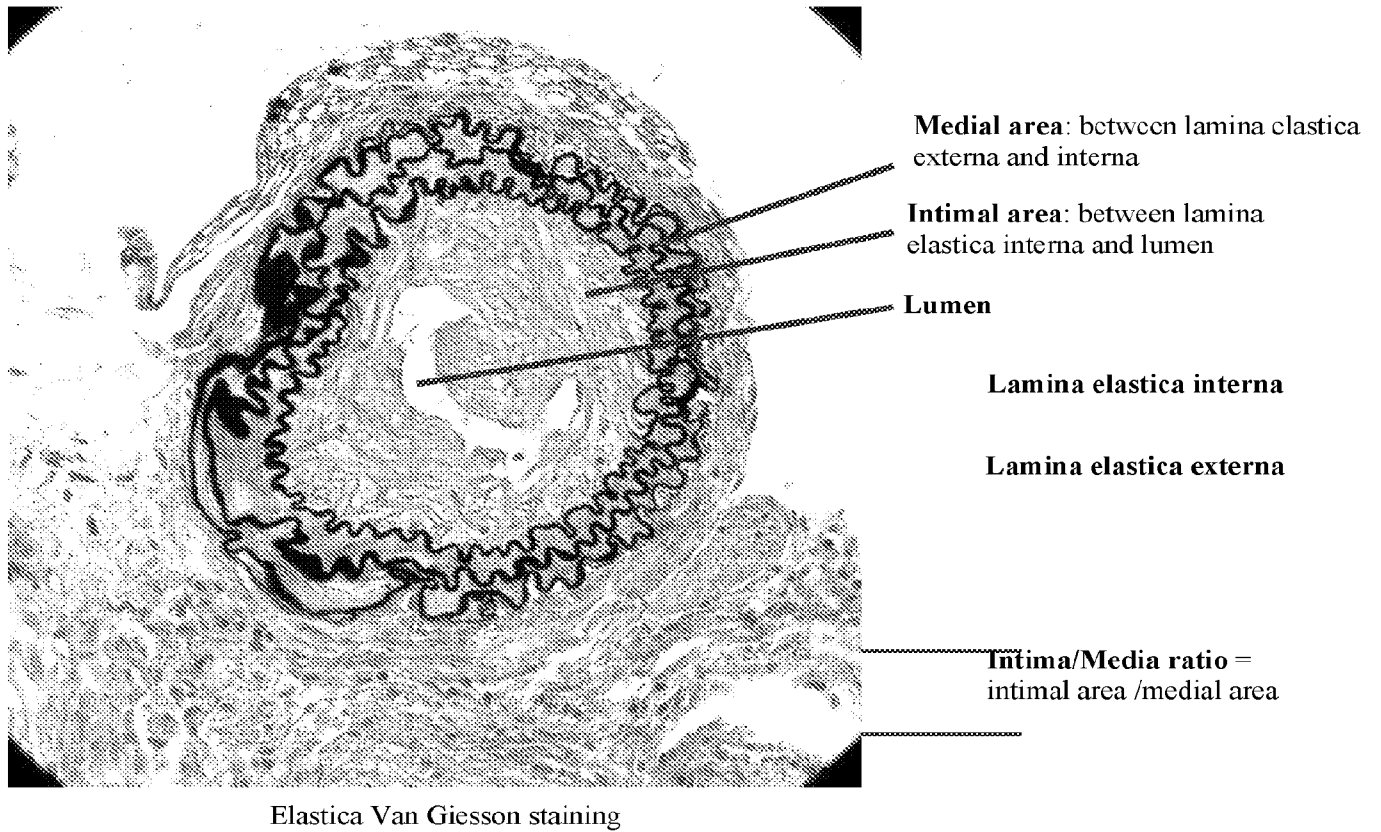
*Fig. 1**Fig. 2A*

FIG. 2B



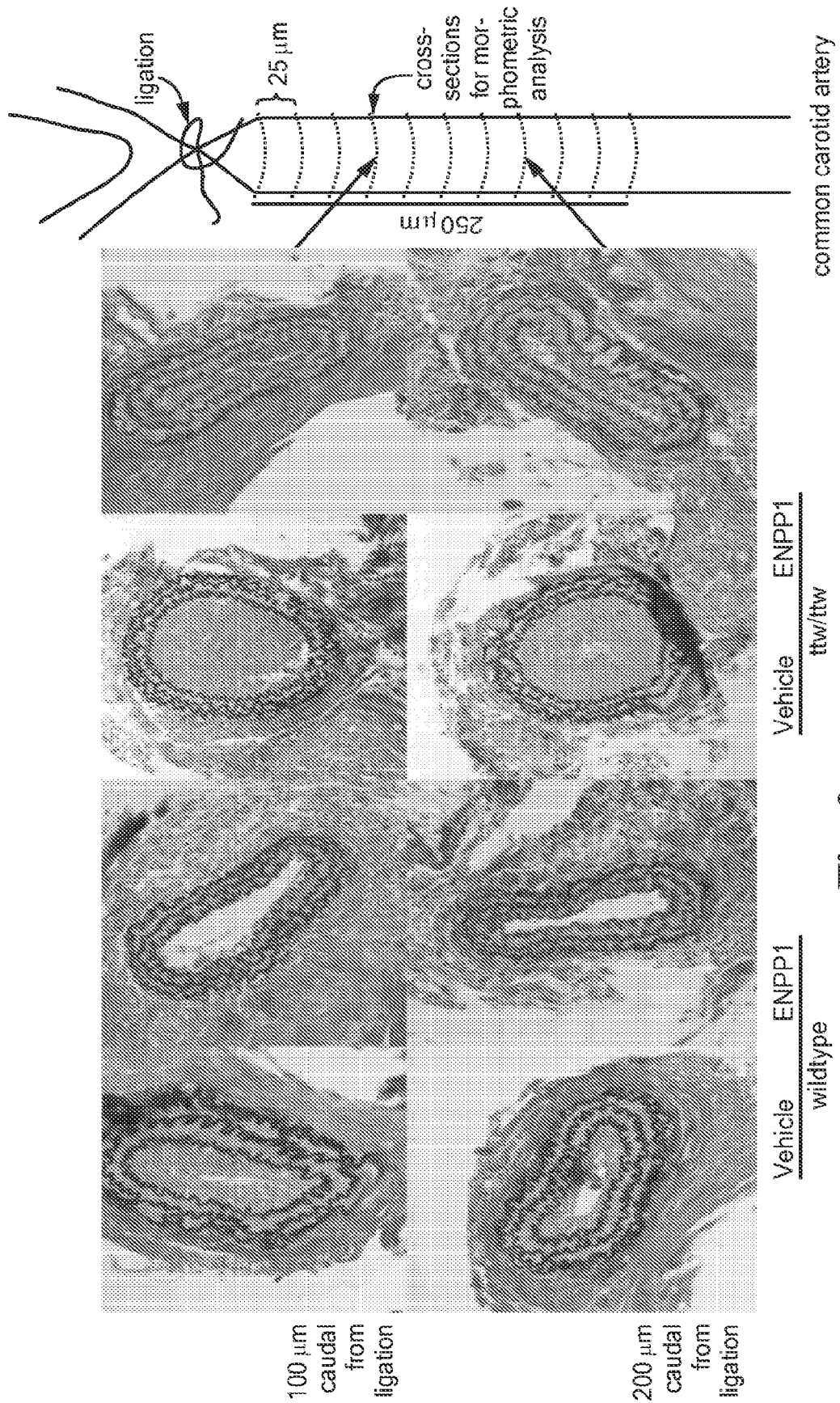


Fig. 3

FIG. 4 (A-F)Prophylactic (preventive) Treatment

ENPP1-Fc treatment was started 7 days prior to carotid ligation, and serial sections of the left carotid arteries were taken 14 (A-C) or 21 (D-F) days after carotid ligation.

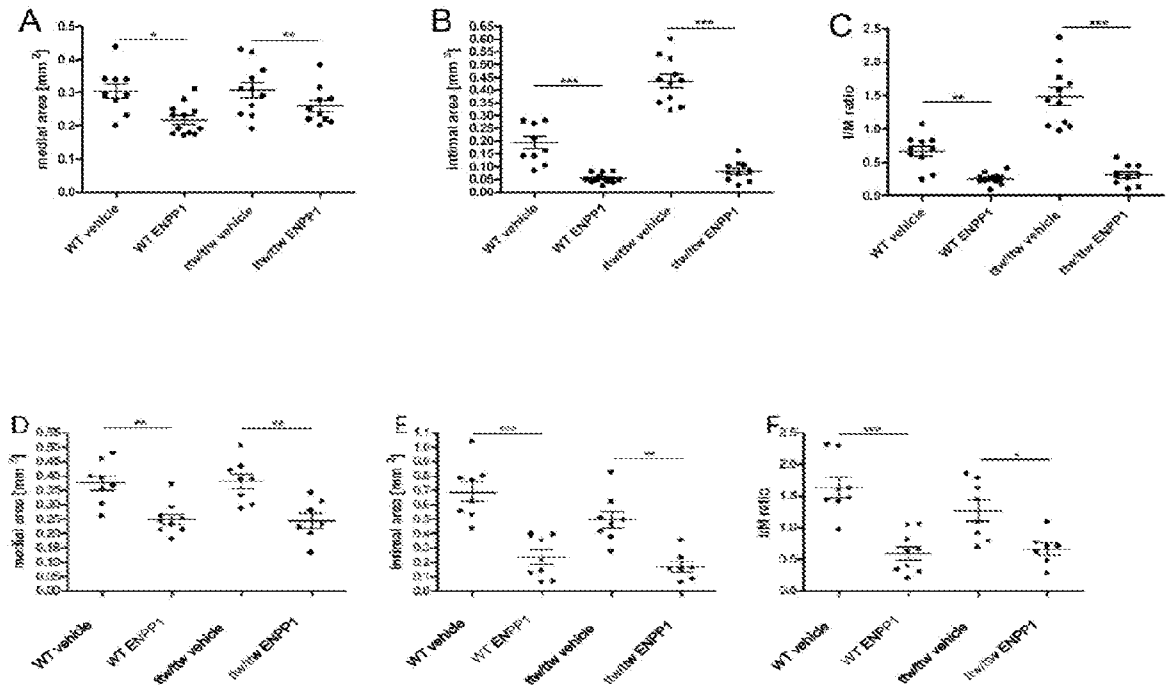


FIG. 4G

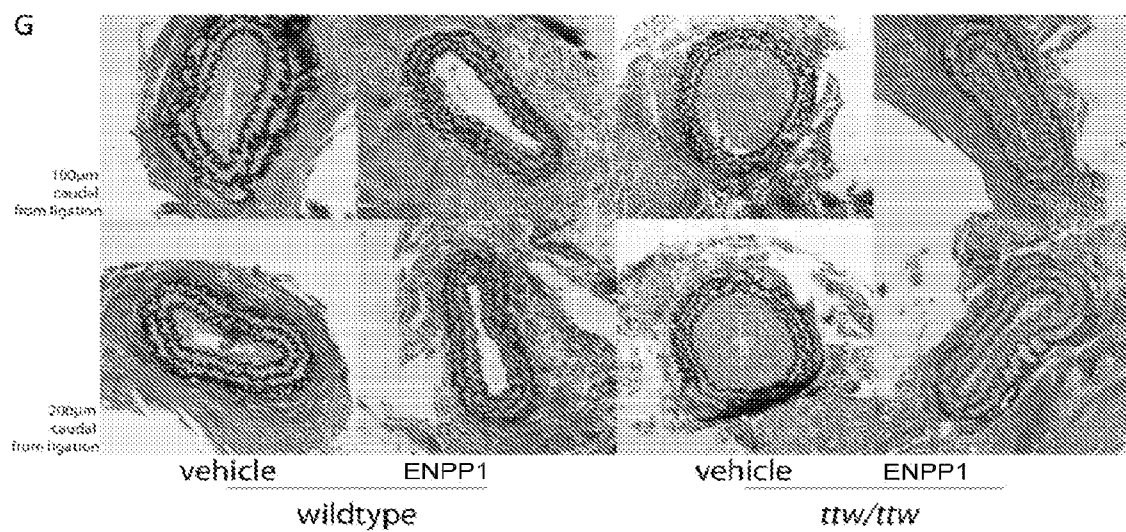


FIG. 5

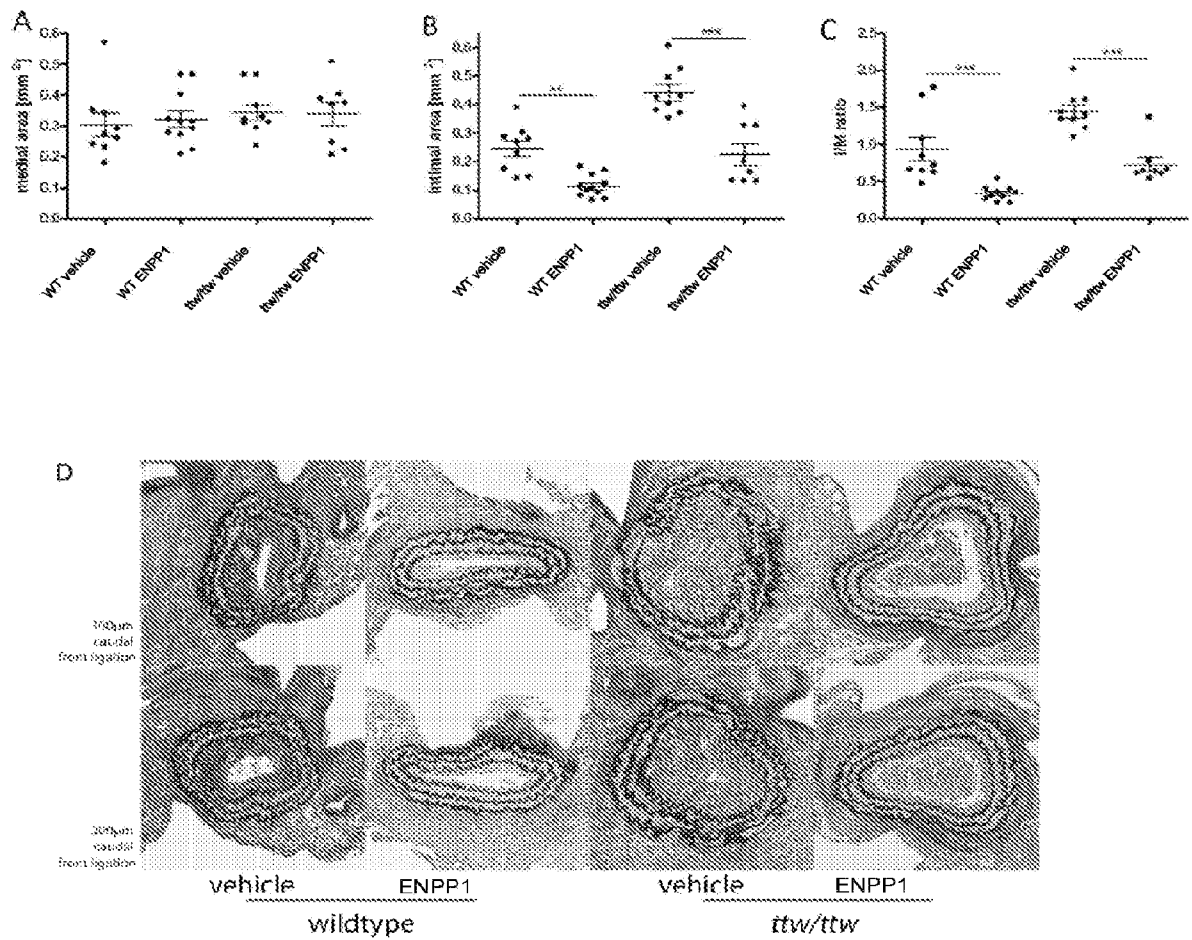
Therapeutic Treatment

FIG. 6

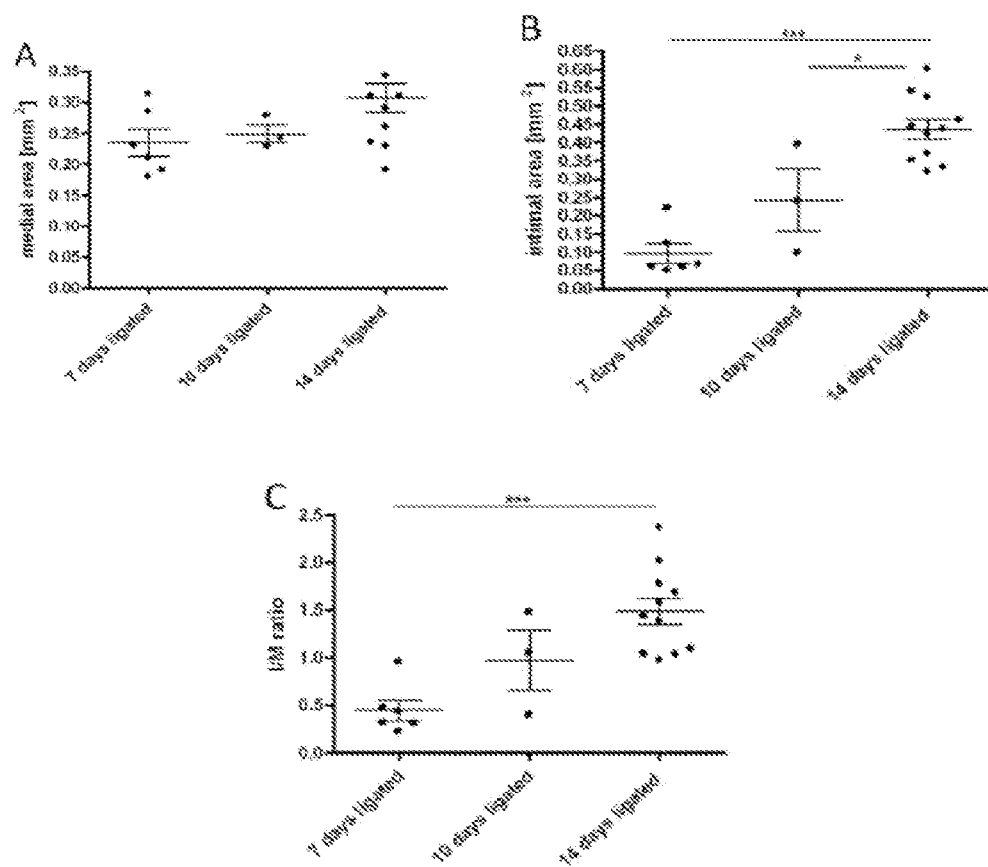


FIG. 7

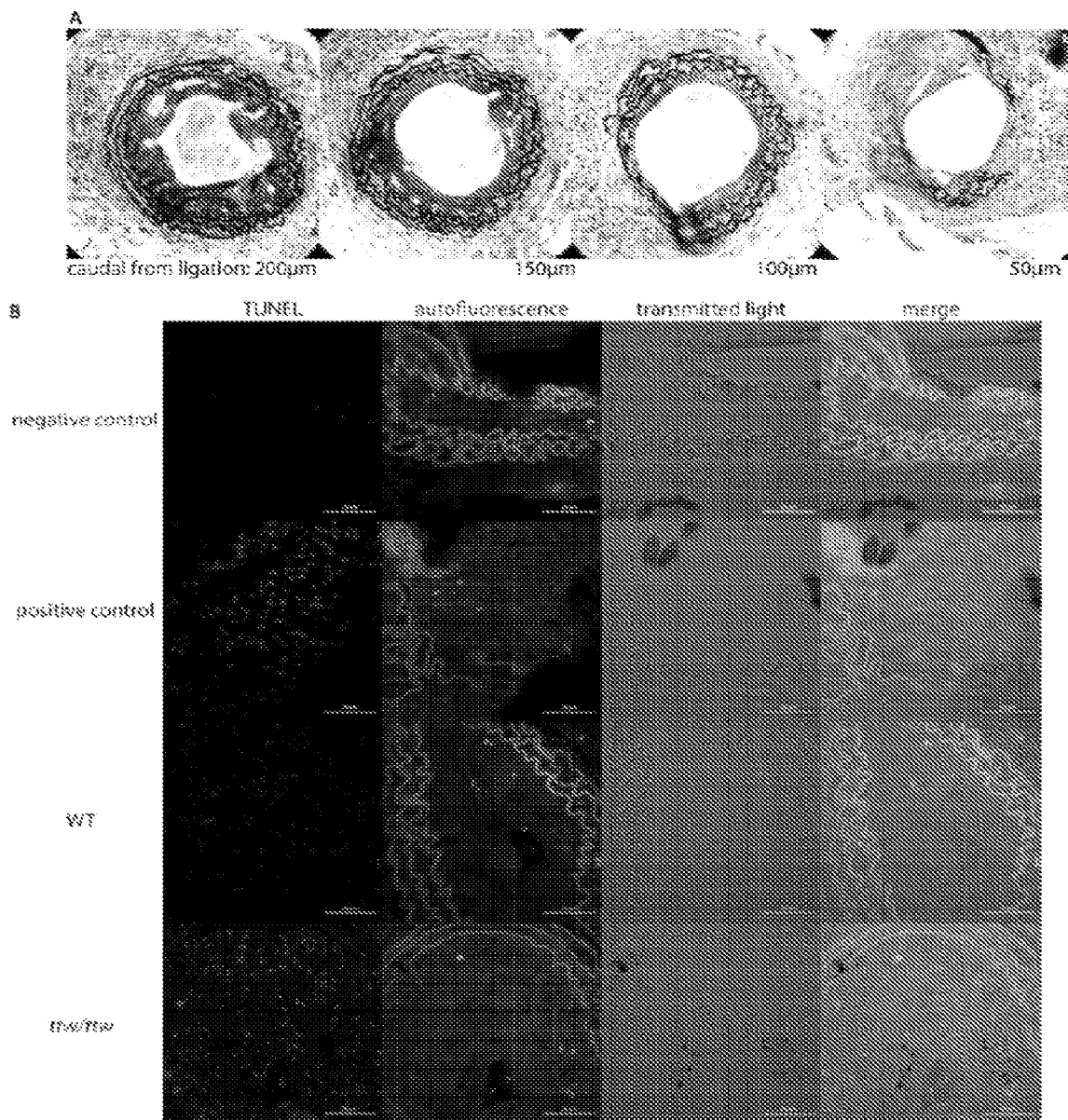
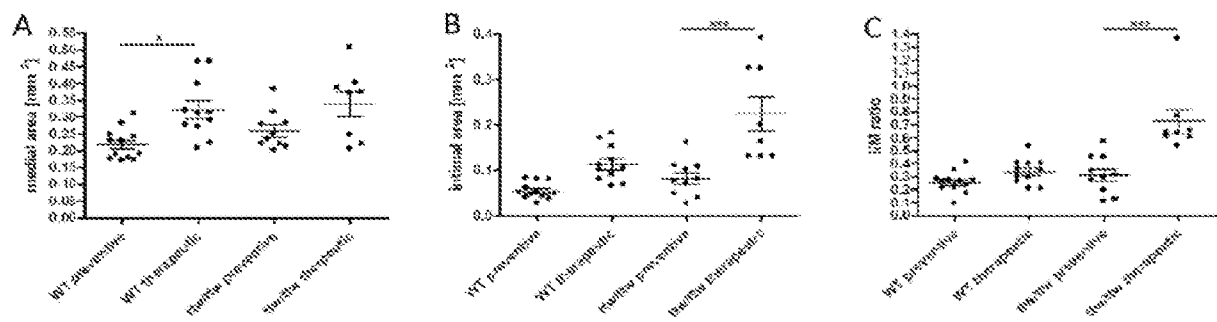
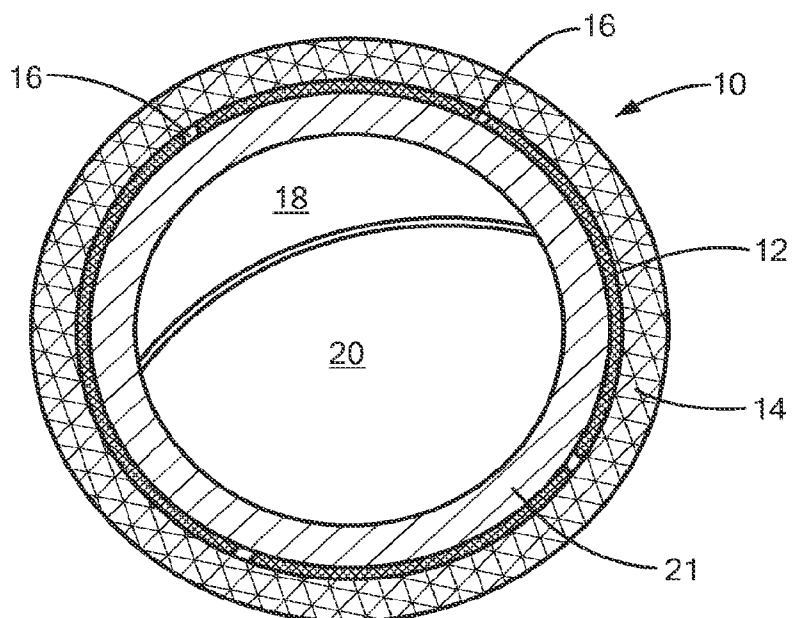
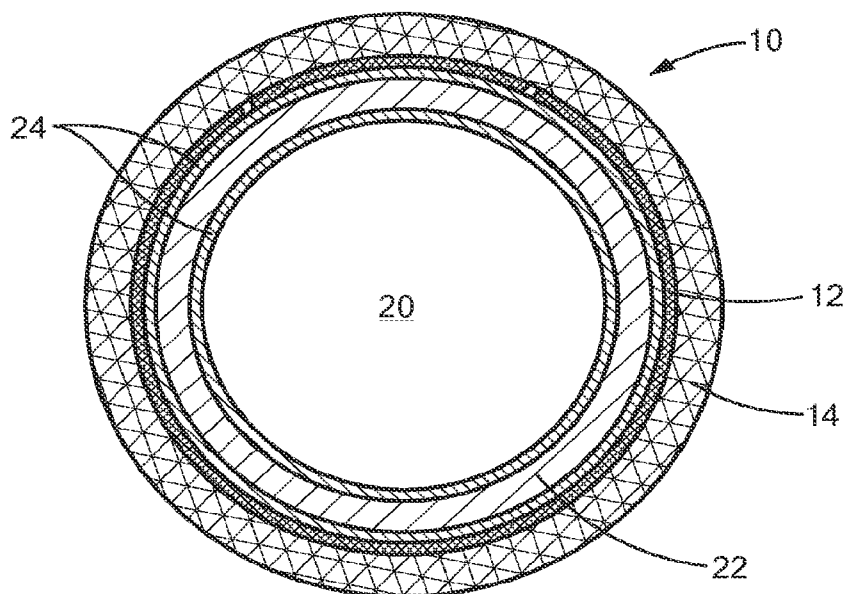


FIG. 8



*Fig. 9A**Fig. 9B*

