

**MODIFIED HYALURONIDASES AND USES IN TREATING
HYALURONAN-ASSOCIATED DISEASES AND CONDITIONS
RELATED APPLICATIONS**

Benefit of priority is claimed to U.S. Provisional Application Serial No.
5 61/124,278 to Gregory Frost, entitled "COMBINATION THERAPY USING A
MODIFIED SOLUBLE HYALURONIDASE AND THERAPEUTIC AGENTS AND
TREATMENTS," filed on April 14, 2004; to U.S. Provisional Application Serial No.
61/130,357 to Gregory Frost, entitled "COMBINATION THERAPY USING A
SOLUBLE HYALURONIDASE AND CHEMOTHERAPEUTIC AGENTS," filed
10 on May 29, 2008; and to U.S. Provisional Application Serial No. 61/195,624 to
Gregory Frost, entitled "MODIFIED HYALURONIDASES AND USES IN
TREATING HYALURONAN-ASSOCIATED DISEASES AND CONDITIONS,"
filed on October 8, 2008.

This application is related to U.S. Patent Application Serial No. (Attorney Dkt.
15 No. 0119374-00091/ 3066), filed the same day herewith, entitled "MODIFIED
HYALURONIDASES AND USES IN TREATING HYALURONAN-
ASSOCIATED DISEASES AND CONDITIONS," which claims priority to U.S.
Provisional Application Serial Nos. 61/124,278, 61/130,357, and 61/195,624. The
subject matter of the above-noted related application is incorporated by reference in
20 its entirety.

The subject matter of each of the above-referenced applications is
incorporated by reference in its entirety.

FIELD OF THE INVENTION

Provided are combinations, compositions and kits containing a hyaluronan
25 degrading enzyme, in particular, a soluble hyaluronidase, for treatment of hyaluronan-
associated conditions, diseases and disorders. In one example, the products include
an additional agent or treatment. Such products can be used in methods for
administering the products to treat the hyaluronan-associated diseases and conditions,
for example, hyaluronan-associated cancers, for example, hyaluronan-rich tumors.
30 The methods include administration of the hyaluronan degrading enzyme
composition, such as a hyaluronidase composition, alone or in combination with other

treatments. Also provided are methods and compositions for providing sustained treatment effects in hyaluronan-associated diseases and conditions.

BACKGROUND

Hyaluronan (hyaluronic acid; HA) is a glycosaminoglycan that exists
5 predominantly in connective tissues, skin, cartilage, and in synovial fluid in mammals. Hyaluronan also is the main constituent of the vitreous of the eye. In connective tissue, the water of hydration associated with hyaluronan creates hydrated matrices between tissues. HA is found in the extracellular matrix of many cells, especially in soft connective tissues. HA has a role in various physiological
10 processes, such as in water and plasma protein homeostasis, in the intracellular matrix (Laurent TC et al (1992) FASEB J 6: 2397-2404). Certain diseases are associated with expression and/or production of hyaluronan.

Hyaluronan degrading enzymes, including hyaluronidases, are enzymes that degrade hyaluronan. Various hyaluronan degrading enzymes have been used
15 therapeutically, typically as dispersing and spreading agents in combination with other therapeutic agents. Improved compositions and methods for administration of hyaluronan degrading enzymes for treatment, particularly of hyaluronan-associated diseases and conditions, are needed.

SUMMARY

20 ~~Provided are methods for treating hyaluronan-associated diseases or~~
conditions. The methods include a step of administering a hyaluronan degrading enzyme, such as a hyaluronidase, particularly a soluble hyaluronidase, such as any of the animal or bacterial hyaluronidases. The hyaluronan degrading enzyme is modified with a polymer, such as a pegylation moiety. Exemplary of such are the hyaluronan
25 degrading enzymes are soluble human hyaluronidases. Such soluble hyaluronidases and preparations thereof are described, for example, in co-pending U.S. Patent application Serial No. 10/795,095, published as US 20040268425, U.S. Patent application Serial No. 11/065,716, published as US 20050260186, U.S. Patent application Serial No. 11/238,171, published as US 20060104968. Such soluble
30 hyaluronidases are modified with a polymer, such as a pegylation moiety. The hyaluronan degrading enzyme, such as a hyaluronidase, is modified with a polymer to

alter a property, such as, but not limited to, half-life and pharmacokinetics. The modification includes linking directly or indirectly via a linker, such as covalently or by other stable linkage, a polymer, such as dextran, a pegylation or sialation moiety, or other such polymers, such as natural or sugar polymers. In the exemplary
5 embodiments herein, the hyaluronan degrading enzyme is pegylated.

Provided herein are methods for treating a disease or condition in which a hyaluronidase substrate accumulates, and methods for treating a hyaluronan-associated disease or condition. Such methods include administering a soluble hyaluronidase that is modified by conjugation to a polymer. Also provided herein are
10 compositions containing a soluble hyaluronidase in an amount sufficient for maintaining a plasma level of the hyaluronidase enzyme in plasma at a level of at least 3 U/mL for at least a week, and combinations containing such compositions. The soluble hyaluronidase in the compositions and combinations provided herein is conjugated to a polymer.

15 Provided herein are methods for treating a disease or condition in which a hyaluronidase substrate accumulates. The methods involve administering a soluble hyaluronidase enzyme to a subject more than once a week for a predetermined number of weeks in an amount sufficient to maintain a pharmacologically active level of the hyaluronidase in the plasma of at least about or 3 U/mL to prevent resynthesis
20 of the substrate to levels prior to treatment. The hyaluronidase enzyme used in the methods is modified by conjugation to a polymer, and the predetermined number of weeks is more than one week. In some examples, the predetermined number of weeks is at least two weeks, such as two weeks, three weeks, or four weeks. Exemplary hyaluronidase substrates that accumulate in the condition or disease being treated
25 include hyaluronan. In one example, the hyaluronan expression in a sample from the subject is measured prior to treatment.

In some examples of the methods for treating a disease or condition in which a hyaluronidase substrate accumulates, after the predetermined number of weeks, administration is discontinued for a first predetermined period of time, such as at least
30 one week, and then resumed for at least one week. For example, administration can be discontinued for one week, two weeks, three weeks or four weeks, and then resumed

for at least one week. In further examples, after the first predetermined period of time, the soluble hyaluronidase is further administered to the subject more than once a week for a predetermined number of weeks in an amount sufficient to maintain a pharmacologically active level of the hyaluronidase in the plasma of at least about or
5 3 U/mL to prevent resynthesis of the substrate to levels prior to treatment. In some embodiments of any of these methods, the cycle of administration and discontinuation of administration is repeated a plurality of times.

In some embodiments of the methods provided herein, the hyaluronidase is administered in an amount sufficient to maintain a pharmacologically active level of
10 the hyaluronidase in the plasma of at least or about 3 U/mL– 12 U/mL. For example, the hyaluronidase can be administered in an amount sufficient to maintain a pharmacologically active level of the hyaluronidase in the plasma of at least at or about 5 U/mL, 6 U/mL, 7 U/mL, 8 U/mL, 9 U/mL, 10 U/mL, 15 U/mL, 20 U/mL, 30 U/mL, 40 U/mL, 45 U/mL, 50 U/mL or more. In a particular embodiment of the
15 methods, the hyaluronidase is administered in an amount sufficient to maintain a pharmacologically active level of the hyaluronidase in the plasma of at least or about 10 U/mL.

In one embodiment of the methods for treating a disease or condition in which a hyaluronidase substrate accumulates, the hyaluronidase is administered twice a
20 week. The amount of hyaluronidase administered to the subject can be, for example, 0.02 mg/kg (of the subject), 0.03 mg/kg, 0.04 mg/kg, 0.05 mg/kg, 0.06 mg/kg, 0.07 mg/kg, 0.08 mg/kg, 0.09 mg/kg, 0.1 mg/kg, 0.15 mg/kg, 0.2 mg/kg, 0.25 mg/kg, 0.30 mg/kg, 0.35 mg/kg, 0.40 mg/kg, 0.45 mg/kg, 0.5 mg/kg, 0.55 mg/kg, 0.6 mg/kg, 0.7 mg/kg, 0.8 mg/kg, 0.9 mg/kg, 1.0 mg/kg, 1.1 mg/kg, 1.2 mg/kg, 1.3 mg/kg, 1.4
25 mg/kg, 1.5 mg/kg or more. In one example, the amount of hyaluronidase administered is 0.05 mg/kg – 0.8 mg/kg. In further examples, the amount of hyaluronidase administered is or is about 50,000 Units (U); 60,000 U; 70,000 U; 80,000 U; 90,000 U; 100,000 U; 200,000 U; 300,000 U; 400,000 U; 500,000 U; 600,000 U; 700,000 U; 800,000 U; 900,000 U; 1,000,000 U; 1,500,000 U; 2,000,000 U; 2,500,000 U;
30 3,000,000 U; 3,500,000 U; 4,000,000 U or more.

Provided herein are methods for treating a hyaluronan-associated disease or condition in a subject. Such methods include the steps of (a) measuring hyaluronan expression or hyaluron in a sample from the subject; and (b) if the hyaluronan expression or hyaluron in the sample from the subject is elevated or at a level indicative of the disease or condition, administering a composition containing a soluble hyaluronidase to the subject. The soluble hyaluronidase used in these methods is modified by conjugation to a polymer. In some examples, the sample from the subject is a tissue or body fluid, such as, for example, a blood sample, tumor biopsy, cerebral spinal fluid, urine, sweat, semen or saliva sample.

In particular embodiments of the methods for treating a hyaluronan-associated disease or condition, a second and different agent for treating the disease or condition also is administered to the subject. In some examples, the second agent and the composition containing the soluble hyaluronidase are administered in a single composition. In other examples, the second agent and the composition containing the soluble hyaluronidase are administered separately, such as simultaneously, sequentially or intermittently in any order. In one embodiment, the second agent is administered after administration of the composition containing the soluble hyaluronidase. For example, the second agent can be administered after the first administration of the soluble hyaluronidase in the cycle of administration, and optionally after one or more subsequent administrations of the soluble hyaluronidase, such as after each subsequent administration of the soluble hyaluronidase, after every other subsequent administration of the soluble hyaluronidase, or once a week, once every two weeks, once every three weeks or once a month.

In some aspects of the methods for treating a hyaluronan-associated disease or condition provided herein, the second agent is administered at least 0.5 minutes, at least one minute, at least five minutes, at least fifteen minutes, at least thirty minutes, at least one hour or more than one hour after the composition containing the soluble hyaluronidase is administered. In some examples, the second agent is administered at least one or two hours, four hours, six hours, eight hours, 10 hours, 12 hours, 14 hours, 16 hours, 18 hours, 20 hours, 22 hours or 24 hours after the composition containing the soluble hyaluronidase is administered. In one example, the second agent is

administered at least forty-eight hours after the composition containing the soluble hyaluronidase. In another example, the second agent is administered at least seventy-two hours after the composition containing the soluble hyaluronidase. In further aspects of the methods provided herein for treating a hyaluronan-associated disease or condition, the expression of hyaluronan in the sample from the subject is compared to expression in a control sample or a standard.

The hyaluronidase used in the methods for treating a disease or condition in which a hyaluronidase substrate accumulates, and the methods for treating a hyaluronan-associated disease or condition, is conjugated to a polymer. In some examples, the polymer is a sialation or pegylation moiety. In some of the methods provided herein, a second agent is administered to the subject, as described above and herein. In some examples, the second agent is an anti-cancer agent or treatment, such as, for example, a chemotherapeutic agent, radiation therapy, an antibody, a peptide, a gene therapy vector, a virus or a nucleic acid. Exemplary of second agents that are used in some of the methods provided herein include anti-cancer agents, such as, for example, Acivicins; Aclarubicins; Acodazoles; Acronines; Adozelesins; Aldesleukins; Alemtuzumabs; Alitretinoin (9-Cis-Retinoic Acids); Allopurinols; Altretamines; Alvocidibs; Ambazones; Ambomycins; Ametantrones; Amifostines; Aminoglutethimides; Amsacrine; Anastrozoles; Anaxirones; Ancitabines; Anthramycins; Apaziquones; Argimenes; Arsenic Trioxides; Asparaginases; Asperlins; Atrimustines; Azacitidines; Azetepas; Azotomycins; Banoxantrones; Batabulins; Batimastats; BCG Live; Benaxibines; Bendamustines; Benzodepas; Bexarotenes; Bevacizumab; Bicalutamides; Bietaserpines; Biricodars; Bisantrones; Bisantrones; Bisnafide Dimesylates; Bizelesins; Bleomycins; Bortezomibs; Brequinars; Bropirimines; Budotitanes; Busulfans; Cactinomycins; Calusterones; Canertinibs; Capecitabines; Caracemides; Carbetimers; Carboplatins; Carboquones; Carmofurs; Carmustines with Polifeprosans; Carmustines; Carubicins; Carzelesins; Cedefingols; Celecoxibs; Cemadotins; Chlorambucils; Cioterone; Cirolemycins; Cisplatin; Cladribines; Clafenurs; Clofarabines; Crisnatols; Cyclophosphamides; Cytarabine liposomals; Cytarabines; Dacarbazines; Dactinomycins; Darbepoetin Alfa; Daunorubicin liposomals; Daunorubicins/Daunomycins; Daunorubicins;

- Decitabines; Denileukin Diftitoxes; Dexniguldipines; Dexonnaplatins; Dexrazoxanes;
 Dezaguanines; Diaziquones; Dibrospidium; Dienogests; Dinalins; Disermolides;
 Docetaxels; Dofequidars; Doxifluridines; Doxorubicin liposomals; Doxorubicin HCL;
 Docorubicin HCL liposome injection; Doxorubicins; Droloxifenes; Dromostanolone
- 5 Propionates; Duazomycins; Ecomustines; Edatrexates; Edotecarins; Eflornithines;
 Elacridars; Elinafides; Elliott's B Solutions; Elsamitrucins; Emitefurs; Enloplatins;
 Enpromates; Enzastaurins; Epiropidines; Epirubicins; Epoetin alfas; Eptaloprosts;
 Erbulozoles; Esorubicins; Estramustines; Etanidazoles; Etoglucids; Etoposide
 phosphates; Etoposide VP-16s; Etoposides; Etoprines; Exemestanes; Exisulinds;
- 10 Fadrozoles; Fazarabines; Fenretinides; Filgrastims; Floxuridines; Fludarabines;
 Fluorouracils; 5-fluorouracils; Fluoxymesterones; Flurocitabines; Fosquidones;
 Fostriecins; Fostriecins; Fotretamines; Fulvestrants; Galarubicins; Galocitabines;
 Gemcitabines; Gemtuzumabs/Ozogamicins; Geroquinols; Gimatecans; Gimeracils;
 Gloxazones; Glufosfamides; Goserelin acetates; Hydroxyureas;
- 15 Ibritumomabs/Tiuxetans; Idarubicins; Ifosfamides; Ilmofofines; Ilomastats; Imatinib
 mesylates; Imexons; Improsulfans; Indisulams; Inproquones; Interferon alfa-2as;
 Interferon alfa-2bs; Interferon Alfas; Interferon Betas; Interferon Gammas;
 Interferons; Interleukin-2s and other Interleukins (including recombinant
 Interleukins); Intoplicines; Iobenguanes [131-I]; Iproplatins; Irinotecans; Irsogladines;
- 20 Ixabepilones; Ketotrexates; L-Alanosines; Lanreotides; Lapatinibs; Ledoxantrones;
 Letrozoles; Leucovorins; Leuprolides; Leuprorelins (Leuprorelides); Levamisoles;
 Lexacalcitols; Liarozoles; Lobaplatins; Lometrexols; Lomustines/CCNUs;
 Lomustines; Lonafarnibs; Losoxantrones; Lurtotecans; Mafosfamides; Mannosulfans;
 Marimastats; Masoprocals; Maytansines; Mechlorethamines;
- 25 Meclorethamines/Nitrogen mustards; Megestrol acetates; Megestrols; Melengestrols;
 Melphalans; MelphalansIL-PAMs; Menogarils; Mepitiostanes; Mercaptopurines; 6-
 Mecaptopurine; Mesnas; Metesinds; Methotrexates; Methoxsalens; Metomidates;
 Metoprines; Meturedepas; Miboplatins; Miproxifenes; Misonidazoles; Mitindomides;
 Mitocarcins; Mitocromins; Mitoflaxones; Mitogillins; Mitoguazones; Mitomalcins;
- 30 Mitomycin Cs; Mitomycins; Mitonafides; Mitoquidones; Mitospers; Mitotanes;
 Mitoxantrones; Mitozolomides; Mivobulins; Mizoribines; Mofarotenes; Mopidamols;

- Mubritinibs; Mycophenolic Acids; Nandrolone Phenpropionates; Nedaplatins;
Nelzarabines; Nemorubicins; Nitracrine; Nocodazoles; Nofetumomabs;
Nogalamycins; Nolatrexed; Nortopixantrones; Octreotides; Oprelvekins;
Ormaplatins; Ortataxels; Oteracils; Oxaliplatin; Oxisurans; Oxophenarsines;
5 Paclitaxels; Pamidronates; Patubilones; Pegademases; Pegaspargases; Pegfilgrastims;
Peldesines; Peliomycins; Pelitrexols; Pemetrexeds; Pentamustines; Pentostatins;
Peplomycins; Perfosfamides; Perifosines; Picoplatins; Pinafides; Pipobromans;
Piposulfans; Pirfenidones; Piroxantrones; Pixantrones; Plevitrexeds; Plicamycin
Mithramycins; Plicamycins; Plomestanes; Plomestanes; Porfimer sodiums; Porfimers;
10 Porfiromycins; Prednimustines; Procarbazines; Propamidines; Prospidium;
Pumitepas; Puromycins; Pyrazofurins; Quinacrine; Ranimustines; Rasburicases;
Riboprines; Ritrosulfans; Rituximabs; Rogletimides; Roquinimex;
Rufocromomycins; Sabarubicins; Safingols; Sargramostims; Satraplatins;
Sebriplatin; Semustines; Simtrazenes; Sizofirans; Sobuzoxanes; Sorafenibs;
15 Sparfosates; Sparfosic Acids; Sparsomycins; Spirogermaniums; Spiromustines;
Spiroplatin; Spiroplatin; Squalamines; Streptonigrins; Streptovarycins;
Streptozocins; Sufosfamides; Sulofenurs; Sunitinib Malate; 6-thioguanine (6-TG);
Tacedinalines; Talcs; Talisomycins; Tallimustines; Tamoxifens; Tariquidars;
Tauromustines; Tecogalans; Tegafurs; Teloxantrones; Temoporfin; Temozolomides;
20 Teniposides/VM-26s; Teniposides; Teroxirones; Testolactones; Thiamiprines;
Thioguanines; Thiotepa; Tiamiprines; Tiazofurins; Tilomisoles; Tilorones;
Timcodars; Timonacis; Tirapazamines; Topixantrones; Topotecans; Toremifenes;
Tositumomabs; Trabectedins (Ecteinascidin 743); Trastuzumabs; Trestolones;
Tretinoin/ATRA; Triciribines; Trilostanes; Trimetrexates; Triplatin Tetranitrates;
25 Triptorelins; Trofosfamides; Tubulozoles; Ubenimex; Uracil Mustards; Uredepas;
Valrubicins; Valspodars; Vapreotides; Verteporfin; Vinblastines; Vincristines;
Vindesines; Vinepidines; Vinflunines; Vinformides; Vinglycinates; Vinleucinols;
Vinleurosines; Vinorelbines; Vinrosidines; Vintriptols; Vinzolidines; Vorozoles;
Xanthomycin As (Guamecyclines); Ziniplatins; Zilascorbs [2-H]; Zinostatin;
30 Zoledronate; Zorubicins; and Zosuquidars.

In any of methods for treating a disease or condition in which a hyaluronidase substrate accumulates and methods for treating a hyaluronan-associated disease or condition that are provided herein, the soluble hyaluronidase and/or second agent can be administered locally or systemically, such as, for example, orally, intravenously (IV), subcutaneously, intramuscularly, intra-tumorally, radermally, topically, transdermally, rectally or sub-epidermally. In particular examples of the methods provided herein, the soluble hyaluronidase and/or second agent is administered intravenously. In other examples, the soluble hyaluronidase and/or second agent is administered intra-tumorally.

Provided herein are methods for treating a disease or condition in which a hyaluronidase substrate accumulates and methods for treating a hyaluronan-associated disease or condition. In some examples, the disease or condition in which a hyaluronidase substrate accumulates is associated with high interstitial fluid pressure. In further examples, the the disease or condition treated by the methods provided herein is disc pressure, cancer or edema. The edema, for example, can be caused by organ transplant, stroke or brain trauma. In instances where the disease or condition to be treated is cancer, the cancer can be a tumor, such as a solid tumor. In some examples, the tumor has increased cellular and/or stromal expression of a hyaluronan, compared to a non-cancerous tissue of the same tissue type or compared to a non-metastatic tumor of the same tumor-type. In particular examples, the disease or condition to be treated is a late-stage cancer, a metastatic cancer and/or an undifferentiated cancer. In one example, the disease or condition is ovarian cancer, in situ carcinoma (ISC), squamous cell carcinoma (SCC), prostate cancer, pancreatic cancer, non-small cell lung cancer, breast cancer, brain cancer or colon cancer. In some examples of the methods provided herein, the treatment effects a reduction in the size of a tumor in the subject.

In some embodiments, the soluble hyaluronidase used in the methods provided is a soluble PH20, including, but not limited to, an ovine, mouse, monkey, bovine, bacterial or human PH20. In some examples, the soluble form of PH20 is a soluble PH20 that has been truncated to remove a C-terminal GPI. In some aspects of the methods provided herein, the soluble hyaluronidase has a sequence of amino acids

included in SEQ ID NO:1 or a sequence that has at least about 91% amino acid sequence identity with a sequence of amino acids included in SEQ ID NO:1, whereby the soluble hyaluronidase is soluble, N-glycosylated and neutral active. In other examples, the soluble hyaluronidase includes a sequence of amino acids set forth in
5 SEQ ID NO:1 that is truncated at an amino acid residue that is or is between amino acid residues 467 to 483. For example, the soluble hyaluronidase can include a sequence of amino acids set forth in SEQ ID NO:1 that is truncated at an amino acid residue selected from among 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482 and 483.

10 In some aspects, the soluble hyaluronidase used in the methods provided herein is secreted in CHO cells. In other aspects, the soluble hyaluronidase has the sequence of amino acids set forth as amino acids 36-467, 36-468, 36-469, 36-470, 36-471, 36-472, 36-473, 36-474, 36-475, 36-476, 36-477, 36-478, 36-479, 36-480, 36-481, 36-482, or 36-483 of SEQ ID NO:1, or has at least about 91% amino acid
15 sequence identity with a sequence of amino acids set forth as amino acids 36-467, 36-468, 36-469, 36-470, 36-471, 36-472, 36-473, 36-474, 36-475, 36-476, 36-477, 36-478, 36-479, 36-480, 36-481, 36-482, or 36-483 of SEQ ID NO:1. In particular examples, the soluble hyaluronidase is selected from among polypeptides containing a sequence of amino acids set forth in any of SEQ ID NOS: 4-9 and 46-48, and allelic
20 variants, species variants and other variants thereof. In one embodiment, the soluble hyaluronidase is a polypeptide encoded by a sequence of nucleic acids that encodes a sequence of amino acids set forth in SEQ ID NO:4. In another embodiment, the soluble hyaluronidase is selected from among polypeptides encoded by a sequence of nucleic acids that encodes a sequence of amino acids set forth in any of SEQ ID NOS:
25 4-9. In such instances, the soluble hyaluronidase can be produced by expression in CHO cells. For example, in one embodiment, the soluble hyaluronidase is designated rHuPH20. Further, in some aspects, the soluble hyaluronidase used in the methods provided herein is glycosylated.

As discussed above, in the methods provided herein for treating a disease or
30 condition in which a hyaluronidase substrate accumulates or for treating a hyaluronan-associated disease or condition, a hyaluronidase enzyme that is modified

by conjugation to a polymer is administered to a subject. In some examples, the polymer conjugated to the soluble hyaluronidase contains a pegylation moiety (PEG), such as, for example, methoxy-poly(ethylene glycol)-succinimidyl butanoate (mPEG-SBA) (5 kDa); methoxy-poly(ethylene glycol)-succinimidyl butanoate (mPEG-SBA) (20 kDa); methoxy-poly(ethylene glycol)-succinimidyl butanoate (mPEG-SBA) (30 kDa); methoxy-poly(ethylene glycol)-succinimidyl α -methylbutanoate (mPEG-SMB) (20 kDa); methoxy-poly(ethylene glycol)-succinimidyl α -methylbutanoate (mPEG-SMB) (30 kDa); methoxy-poly(ethylene glycol)-butyraldehyde (mPEG-butyr-
aldehyde) (30 kDa), methoxy-poly(ethylene glycol)-succinimidyl propionate (mPEG-SPA) (20 kDa); methoxy-poly(ethylene glycol)-succinimidyl propionate (mPEG-SPA) (30 kDa); (methoxy-poly(ethylene glycol))₂-N-hydroxysuccinimide ester (mPEG₂-NHS) (10 kDa branched); (methoxy-poly(ethylene glycol))₂-N-hydroxysuccinimide ester (mPEG₂-NHS) (20 kDa branched); (methoxy-poly(ethylene glycol))₂-N-hydroxysuccinimide ester (mPEG₂-NHS) (40 kDa branched); (methoxy-poly(ethylene glycol))₂-N-hydroxysuccinimide ester (mPEG₂-NHS) (60 kDa branched); biotin-poly(ethylene glycol)-N-hydroxysuccinimide ester (biotin-PEG-NHS) (5 kDa biotinylated); poly(ethylene glycol)-p-nitrophenyl carbonate (PEG-p-nitrophenyl-carbonate) (30 kDa); or poly(ethylene glycol)-propionaldehyde (PEG-propionaldehyde) (30 kDa). In some aspects, the the PEG is a branched or linear PEG. In a particular example, the PEG is a methoxy-PEG (mPEG), or example, a linear N-hydroxysuccinimidyl ester of methoxy poly(ethylene glycol) butanoic acid. Such PEGs can have a molecular weight of 30 or about 30 kilodaltons.

Provided herein are compositions containing a soluble hyaluronidase in an amount sufficient for maintaining a plasma level of the hyaluronidase enzyme in plasma at a level of at least 3 U/mL for at least a week, wherein the soluble hyaluronidase is conjugated to a polymer. In some aspects, the level of hyaluronidase in the plasma is at least or about 3 U/mL- 12 U/mL. Exemplary polymers conjugated to the hyaluronidase include, but are not limited to, sialation and pegylation moieties. The compositions can be administered at least twice a week for more than at least one week. In some examples, the composition contains at least or about 2.0 mg – 60 mg of the soluble hyaluronidase conjugated to a polymer, and the soluble hyaluronidase

conjugated to a polymer has a specific activity of at least or about 20,000 U/mg, 25,000 U/mg, 30,000 U/mg, 31,000 U/mg, 32,000 U/mg, 33,000 U/mg, 34,000 U/mg, 35,000 U/mg, 36,000 U/mg, 37,000 U/mg, 38, 000 U/mg, 39,000 U/mg, 40,000 U/mg, 45,000 U/mg, 50,000 U/mg, 55,000 U/mg, 60,000 U/mg or more. Further, in
5 some aspects, the composition is at least 10 mL/ per administration.

The compositions provided herein can be formulated for administration orally, intravenously (IV), subcutaneously, intramuscularly, intra-tumorally, radermally, topically, transdermally, rectally or sub-epidermally. In one example, the composition is formulated for intravenous administration. The compositions provided herein also
10 can contain histidine and/or NaCl. For example, in one embodiment, the composition is formulated with at or about 10 mM histidine and/or 130 mM NaCl. The composition can have a pH that is or is about 6.0, 6.1., 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1 or 7.2. In one example, the composition has a pH of or about 6.5.

Provided herein are combinations containing a composition containing a
15 soluble hyaluronidase in an amount sufficient for maintaining a plasma level of the hyaluronidase enzyme in plasma at a level of at least 3 U/mL for at least a week, wherein the soluble hyaluronidase is conjugated to a polymer, and a second composition containing an agent for treating a hyaluronan-associated disease or condition. In some examples, the composition containing a soluble hyaluronidase is
20 for administration at least twice a week for more than at least one week. In further aspects, the composition containing a soluble hyaluronidase contains at least or about 2.0 mg – 60 mg of the soluble hyaluronidase conjugated to a polymer; and the soluble hyaluronidase conjugated to a polymer has a specific activity of at least or about 20,000 U/mg, 25,000 U/mg, 30,000 U/mg, 31,000 U/mg, 32,000 U/mg, 33,000 U/mg, 34,000 U/mg, 35,000 U/mg, 36,000 U/mg, 37,000 U/mg, 38, 000 U/mg, 39,000
25 U/mg, 40,000 U/mg, 45,000 U/mg, 50,000 U/mg, 55,000 U/mg, 60,000 U/mg or more.

The first and second compositions of the combinations provided herein are co-formulated or provided separately. In some examples, the polymer conjugated to the
30 hyaluronidase in the combinations provided herein is a sialation or pegylation moiety. In further embodiments, the second agent in the combination is an anti-cancer agent

or treatment, such as, for example, a chemotherapeutic agent, radiation therapy an antibody, a peptide, a gene therapy vector, a virus or a nucleic acid. In particular examples, the second agent is an anti-cancer agent selected from among Acivicins; Aclarubicins; Acodazoles; Acronines; Adozelesins; Aldesleukins; Alemtuzumabs; 5 Alitretinoin (9-Cis-Retinoic Acids); Allopurinols; Altretamines; Alvocidibs; Ambazones; Ambomycins; Ametantrones; Amifostines; Aminoglutethimides; Amsacrine; Anastrozoles; Anaxirones; Ancitabines; Anthramycins; Apaziquones; Argimesnas; Arsenic Trioxides; Asparaginases; Asperlins; Atrimustines; Azacitidines; Azetepas; Azotomycins; Banoxantrones; Batabulins; Batimastats; BCG 10 Live; Benaxibines; Bendamustines; Benzodepas; Bexarotenes; Bevacizumab; Bicalutamides; Bietaserpines; Biricodars; Bisantrones; Bisantrones; Bisnafide Dimesylates; Bizelesins; Bleomycins; Bortezomibs; Brequinars; Bropirimines; Budotitanes; Busulfans; Cactinomycins; Calusterones; Canertinibs; Capecitabines; Caracemides; Carbetimers; Carboplatins; Carboquones; Carmofurs; Carmustines with 15 Polifeprosans; Carmustines; Carubicins; Carzelesins; Cedefingols; Celecoxibs; Cemadotins; Chlorambucils; Cioteronels; Cirolemycins; Cisplatins; Cladribines; Clanfenurs; Clofarabines; Crisnatols; Cyclophosphamides; Cytarabine liposomals; Cytarabines; Dacarbazines; Dactinomycins; Darbepoetin Alfas; Daunorubicin liposomals; Daunorubicins/Daunomycins; Daunorubicins; Decitabines; Denileukin 20 ~~Difitoxes; Dexniguldipines; Dexonnaplatins; Dexrazoxanes; Dezaguanines;~~ Diaziquones; Dibrospidium; Dienogests; Dinalins; Disermolides; Docetaxels; Dofequidars; Doxifluridines; Doxorubicin liposomals; Doxorubicin HCL; Docorubicin HCL liposome injection; Doxorubicins; Droloxifenes; Dromostanolone Propionates; Duazomycins; Ecomustines; Edatrexates; Edotecarins; Eflornithines; 25 Elacridars; Elinafides; Elliott's B Solutions; Elsamitrucins; Emitefurs; Enloplatins; Enpromates; Enzastaurins; Epiropidines; Epirubicins; Epoetin alfas; Eptaloprosts; Erbulozoles; Esorubicins; Estramustines; Etanidazoles; Etoglucids; Etoposide phosphates; Etoposide VP-16s; Etoposides; Etoprines; Exemestanes; Exisulinds; Fadrozoles; Fazarabines; Fenretinides; Filgrastims; Floxuridines; Fludarabines; 30 Fluorouracils; 5-fluorouracils; Fluoxymesterones; Flurocitabines; Fosquidones; Fostriecins; Fostriecins; Fotretamines; Fulvestrants; Galarubicins; Galocitabines;

- Gemcitabines; Gemtuzumabs/Ozogamicins; Geroquinols; Gimitecans; Gimeracils;
 Gloxazones; Glufosfamides; Goserelin acetates; Hydroxyureas;
 Ibritumomabs/Tiuxetans; Idarubicins; Ifosfamides; Ilmofofines; Ilomastats; Imatinib
 mesylates; Imexons; Improsulfans; Indisulams; Inproquones; Interferon alfa-2as;
 5 Interferon alfa-2bs; Interferon Alfas; Interferon Betas; Interferon Gammas;
 Interferons; Interleukin-2s and other Interleukins (including recombinant
 Interleukins); Intoplicines; Iobenguanes [¹³¹I]; Iproplatins; Irinotecans; Irsogladines;
 Ixabepilones; Ketotrexates; L-Alanosines; Lanreotides; Lapatinibs; Ledoxantrones;
 Letrozoles; Leucovorins; Leuprolides; Leuprorelins (Leuprorelides); Levamisoles;
 10 Lexacalcitols; Liarozoles; Lobaplatins; Lometrexols; Lomustines/CCNUs;
 Lomustines; Lonafarnibs; Losoxantrones; Lurtotecans; Mafosfamides; Mannosulfans;
 Marimastats; Masoprocals; Maytansines; Mechlorethamines;
 Mecllorethamines/Nitrogen mustards; Megestrol acetates; Megestrols; Melengestrols;
 Melphalans; MelphalansL-PAMs; Menogarils; Mepitiostanes; Mercaptopurines; 6-
 15 Mecaptopurine; Mesnas; Metesinds; Methotrexates; Methoxsalens; Metomidates;
 Metoprines; Meturedepas; Miboplatins; Miproxifenes; Misonidazoles; Mitindomides;
 Mitocarcins; Mitocromins; Mitoflaxones; Mitogillins; Mitoguazones; Mitomalcins;
 Mitomycin Cs; Mitomycins; Mitonafides; Mitoquidones; Mitospers; Mitotanes;
 Mitoxantrones; Mitozolomides; Mivobulins; Mizoribines; Mofarotenes; Mopidamols;
 20 Mubritinibs; Mycophenolic Acids; Nandrolone Phenpropionates; Nedaplatins;
 Nelzarabines; Nemorubicins; Nitracines; Nocodazoles; Nofetumomabs;
 Nogalamycins; Nolatrexedes; Nortopixantrones; Octreotides; Oprelvekins;
 Ormaplatins; Ortataxels; Oteracils; Oxaliplatins; Oxisurans; Oxophenarsines;
 Paclitaxels; Pamidronates; Patubilones; Pegademases; Pegaspargases; Pegfilgrastims;
 25 Peldesines; Peliomycins; Pelitrexols; Pemetrexeds; Pentamustines; Pentostatin;
 Peplomycins; Perfosfamides; Perifosines; Picoplatins; Pinafides; Pipobromans;
 Pipsulfans; Pirfenidones; Piroxantrones; Pixantrones; Plevitrexeds; Plicamycid
 Mithramycins; Plicamycins; Plomestanes; Plomestanes; Porfimer sodiums; Porfimers;
 Porfiromycins; Prednimustines; Procarbazines; Propamidines; Prospidium;
 30 Pumitepas; Puromycins; Pyrazofurins; Quinacrine; Ranimustines; Rasburicases;
 Riboprines; Ritrosulfans; Rituximabs; Rogletimides; Roquinimexs;

Rufocromomycins; Sabarubicins; Safingols; Sargramostims; Satraplatins;
Sebriplatins; Semustines; Simtrazenes; Sizofirans; Sobuzoxanes; Sorafenibs;
Sparfosates; Sparfoscic Acids; Sparsomycins; Spirogermaniums; Spiromustines;
Spiroplatins; Spiroplatins; Squalamines; Streptonigrins; Streptovarycins;
5 Streptozocins; Sufosfamides; Sulofenurs; Sunitinib Malate; 6-thioguanine (6-TG);
Tacedinalines; Talcs; Talisomycins; Tallimustines; Tamoxifens; Tariquidars;
Tauromustines; Tecogalans; Tegafurs; Teloxantrones; Temoporfin; Temozolomides;
Teniposides/VM-26s; Teniposides; Teroxirones; Testolactones; Thiamiprines;
Thioguanines; Thiotepas; Tiamiprines; Tiazofurins; Tilomisoles; Tilorones;
10 Timcodars; Timonacics; Tirapazamines; Topixantrones; Topotecans; Toremifenes;
Tositumomabs; Trabectedins (Ecteinascidin 743); Trastuzumabs; Trestolones;
Tretinoin/ATRA; Triciribines; Trilostanes; Trimetrexates; Triplatin Tetranitrates;
Triptorelins; Trofosfamides; Tubulozoles; Ubenimex; Uracil Mustards; Uredepas;
Valrubicins; Valspodars; Vapreotides; Verteporfin; Vinblastines; Vincristines;
15 Vindesines; Vinepidines; Vinflunines; Vinformides; Vinglycinates; Vinleucinols;
Vinleurosines; Vinorelbines; Vinrosidines; Vintriptols; Vinzolidines; Vorozoles;
Xanthomycin As (Guamecyclines); Zeniplatins; Zilascorbs [2-H]; Zinostatin;
Zoledronate; Zorubicins; and Zosuquidars.

In one method, a composition containing a modified hyaluronan degrading
20 enzyme is administered to a subject who has a hyaluronan-associated disease or
condition or who may have such disease or condition, followed by administering a
second and different agent or treatment for treating the disease or condition. The
second agent or treatment is administered more than twenty-four hours after
administration of the hyaluronan degrading enzyme. Administration can be effected
25 systemically or locally or by any suitable route, such as intravenously, orally,
subcutaneously or intramuscularly.

In another method for treating a hyaluronan-associated disease or condition in
a subject, a composition containing modified hyaluronan degrading enzyme is
systemically administered to a subject who has a hyaluronan-associated disease or
30 condition or who may have such disease or condition. The modified hyaluronan
degrading enzyme is administered in at least an amount that is effective to decrease

interstitial fluid pressure for more than 24 hours. Administration of the hyaluronan degrading enzyme can treat the disease or condition or can be followed by or administered with a second agent or treatment. Administration can be effected intravenously, orally, subcutaneously or intramuscularly, including, for example, intra-tumorally.

Also provided are methods for lowering interstitial fluid pressure in a subject by administering to the subject a composition containing an effective amount of a hyaluronan degrading enzyme that is modified. The amount of hyaluronan degrading enzyme lowers interstitial fluid pressure for more than 24 hours; and the amount of hyaluronan degrading enzyme is functionally equivalent to between at or about 10 units to 1,000,000 hyaluronidase units, such as at or about 10 to 50,000,000 Units, 10 to 40,000,000 Units, 10 to 36,000,000 Units, 10 to 12,000,000 Units, 10 to 1,200,000 Units, 10 to 1,000,000 Units, 10 to 500,000 Units, 100 to 100,000 Units, 500 to 50,000 Units, 1000 to 10,000 Units, 5000 to 7500 Units, 5000 Units to 50,000 Units, or 1,000 to 10,000 Units. Units are measured generally with reference to the unmodified hyaluronidase. Lowering interstitial pressure can effect treatment of a hyaluronan-associated disease or condition in a subject.

In practicing any of the methods herein, hyaluronan expression in a sample from the subject can be measured (or assessed or monitored) prior to, during or after treatment. If needed, expression of hyaluronan in a sample from the subject can be compared to expression in a control sample or to a standard. Thus provided, for example, are methods for treating a hyaluronan-associated disease or condition in a subject by first measuring hyaluronan expression in a sample from the subject; and then administering a composition containing a modified hyaluronan degrading enzyme to the subject. Administration includes local and systemic administration, such as but not limited to, orally, intravenously (IV), subcutaneously, intramuscularly, intra-tumorally, intradermally, topically, transdermally, orally, rectally or sub-epidermally. The sample, for example, is a tissue or body fluid, such as but not limited to, a blood sample, tumor biopsy, cerebral spinal fluid, urine, sweat, semen or saliva sample.

The methods can be practiced by administering a second agent or treatment that is, other than a hyaluronan degrading enzyme, and that is used for treatment of a particular disease. For example, if the disease is a tumor, then the second agent can be a chemotherapeutic and or radiation protocol/therapy. Exemplary second agents are anti-cancer agents.

Where the second agent is a drug or composition, the second agent and hyaluronidase can be administered separation, together in a single composition or simultaneously in two compositions, or intermittently or sequentially or any combination thereof. Typically, the composition containing a hyaluronan degrading enzyme is administered prior to administration of the second agent. In some embodiments, the second agent or treatment can be effected or administered before the hyaluronidase-containing compositions. In methods in which the time is not required herein to be more than 24 hours, the timing between administration of the composition containing a soluble hyaluronidase and the second agent or treatment can be within 30 seconds or 60 seconds, at least one minute, at least five minutes, at least fifteen minutes, at least thirty minutes at least one hour or more than one hour prior to (or subsequent to). The time difference can be least or two hours, four hours, six hours, eight hours, 10 hours, 12 hours, 14 hours, 16 hours, 18 hours, 20 hours, 22 hours, 24 hours, 48 hours or 72 hours prior to administration of the second agent or treatment.

Exemplary second agents and treatments include, for example, anticancer agents are a chemotherapeutic agent, radiation therapy, an antibody, a peptide, a gene therapy vector, a virus, such as an oncolytic virus, and a nucleic acids, such as gene therapy vectors that deliver an anti-cancer protein or other therapeutic protein. Other exemplary second agents or treatments include, but are not limited to, an analgesic agent, an anti-inflammatory agent, an antimicrobial agent, an amoebicidal agent, a trichomonocidal agent, an anti-parkinson's disease agent, an anti-malarial agent, an anticonvulsant agent, an anti-depressant agent, an anti-arthritis agent, an anti-fungal agent, an antihypertensive agent, an antipyretic agent, an anti-parasitic agent, an antihistamine agent, an alpha-adrenergic agonist agent, an alpha blocker agent, an anesthetic agent, a bronchial dilator agent, a biocide agent, a bactericide agent, a

bacteriostatic agent, a beta adrenergic blocker agent, a calcium channel blocker agent, a cardiovascular drug agent, a contraceptive agent, a cosmetic or aesthetic agent, a decongestant agent, a diuretic agent, a depressant agent, a diagnostic agent, a therapeutic antibody, an electrolyte agent, a hypnotic agent, a hormone agent, a hyperglycemic agent, a muscle relaxant agent, a muscle contractant agent, an ophthalmic agent, a parasympathomimetic agent, a psychic energizer agent, a sedative agent, a sleep inducer, a sympathomimetic agent, a tranquilizer agent, a urinary agent, a vaginal agent, a viricide agent, a vitamin agent, a non-steroidal anti-inflammatory agent, and an angiotensin converting enzyme inhibitor agent. Exemplary of such agents are: Acivicins; Aclarubicins; Acodazoles; Acronines; Adozelesins; Aldesleukins; Alemtuzumabs; Alitretinoin (9-Cis-Retinoic Acids); Allopurinols; Altretamines; Alvocidibs; Ambazones; Ambomycins; Ametantrones; Amifostines; Aminoglutethimides; Amsacrine; Anastrozoles; Anaxirones; Ancitabines; Anthramycins; Apaziquones; Argimesnas; Arsenic Trioxides; Asparaginases; Asperlins; Atrimustines; Azacitidines; Azetepas; Azotomycins; Banoxantrones; Batabulins; Batimastats; BCG Live; Benaxibines; Bendamustines; Benzodepas; Bexarotenes; Bevacizumab; Bicalutamides; Bietaserpines; Biricodars; Bisantrones; Bisnafide Dimesylates; Bizelesins; Bleomycins; Bortezomibs; Brequinars; Bropirimines; Budotitanes; Busulfans; Cactinomycins; Calusterones; Canertinibs; Capecitabines; Caracemides; Carbetimers; Carboplatins; Carboquones; Carmofurs; Carmustines with Polifeprosans; Carmustines; Carubicins; Carzelesins; Cedefingols; Celecoxibs; Cemadotins; Chlorambucils; Cioterone; Cirolemycins; Cisplatin; Cladribines; Clafenurs; Clofarabines; Crisnatols; Cyclophosphamides; Cytarabine liposomals; Cytarabines; Dacarbazines; Dactinomycins; Darbepoetin Alfa; Daunorubicin liposomals; Daunorubicins/Daunomycins; Daunorubicins; Decitabines; Denileukin Diftitoxes; Dexniguldipines; Dexonnaplatins; Dexrazoxanes; Dezaguanines; Diaziquones; Dibrospidium; Dienogests; Dinalins; Disermolides; Docetaxels; Dofequidars; Doxifluridines; Doxorubicin liposomals; Doxorubicin HCL; Docorubicin HCL liposome injection; Doxorubicins; Droloxifenes; Dromostanolone Propionates; Duazomycins; Ecomustines; Edatrexates; Edotecarins; Eflornithines; Elacridars; Elinafides; Elliott's B Solutions; Elsamitrucins; Emitefurs; Enloplatin;

Enpromates; Enzastaurins; Epiropidines; Epirubicins; Epoetin alfas; Eptaloprosts;
 Erbulozoles; Esorubicins; Estramustines; Etanidazoles; Etoglucids; Etoposide
 phosphates; Etoposide VP-16s; Etoposides; Etoprines; Exemestanes; Exisulinds;
 Fadrozoles; Fazarabines; Fenretinides; Filgrastims; Floxuridines; Fludarabines;
 5 Fluorouracils; 5-fluorouracils; Fluoxymesterones; Flurocitabines; Fosquidones;
 Fostriecins; Fostriecins; Fotretamines; Fulvestrants; Galarubicins; Galocitabines;
 Gemcitabines; Gemtuzumabs/Ozogamicins; Geroquinols; Gimatacans; Gimeracils;
 Gloxazones; Glufosfamides; Goserelin acetates; Hydroxyureas;
 Ibritumomabs/Tiuxetans; Idarubicins; Ifosfamides; Ilmofofines; Ilomastats; Imatinib
 10 mesylates; Imexons; Improsulfans; Indisulams; Inproquones; Interferon alfa-2as;
 Interferon alfa-2bs; Interferon Alfas; Interferon Betas; Interferon Gammas;
 Interferons; Interleukin-2s and other Interleukins (including recombinant
 Interleukins); Intoplicines; Iobenguanes [131-I]; Iproplatins; Irinotecans; Irsogladines;
 Ixabepilones; Ketotrexates; L-Alanosines; Lanreotides; Lapatinibs; Ledoxantrones;
 15 Letrozoles; Leucovorins; Leuprolides; Leuprorelins (Leuprorelides); Levamisoles;
 Lexacalcitols; Liarozoles; Lobaplatins; Lometrexols; Lomustines/CCNUs;
 Lomustines; Lonafernibs; Losoxantrones; Lurtotecans; Mafosfamides; Mannosulfans;
 Marimastats; Masoprocals; Maytansines; Mechlorethamines;
 Mecllorethamines/Nitrogen mustards; Megestrol acetates; Megestrols; Melengestrols;
 20 Melphalans; MelphalansIL-PAMs; Menogarils; Mepitiostanes; Mercaptopurines; 6-
 Mecaptopurine; Mesnas; Metesinds; Methotrexates; Methoxsalens; Metomidates;
 Metoprines; Meturedepas; Miboplatins; Miproxifenes; Misonidazoles; Mitindomides;
 Mitocarcins; Mitocromins; Mitoflaxones; Mitogillins; Mitoguazones; Mitomalcins;
 Mitomycin Cs; Mitomycins; Mitonafides; Mitoquidones; Mitospers; Mitotanes;
 25 Mitoxantrones; Mitozolomides; Mivobulins; Mizoribines; Mofarotenes; Mopidamols;
 Mubritinibs; Mycophenolic Acids; Nandrolone Phenpropionates; Nedaplatins;
 Nelzarabines; Nemorubicins; Nitracrine; Nocodazoles; Nofetumomabs;
 Nogalamycins; Nolatrexeds; Nortopixantrones; Octreotides; Oprelvekins;
 Ormaplatins; Ortataxels; Oteracils; Oxaliplatins; Oxisurans; Oxophenarsines;
 30 Paclitaxels; Pamidronates; Patubilones; Pegademases; Pegaspargases; Pegfilgrastims;
 Peldesines; Peliomycins; Pelitrexols; Pemetrexeds; Pentamustines; Pentostatins;

- Peplomycins; Perfosfamides; Perifosines; Picoplatins; Pinafides; Pipobromans;
Piposulfans; Pirfenidones; Piroxantrones; Pixantrones; Plevitrexeds; Plicamycid
Mithramycins; Plicamycins; Plomestanes; Plomestanes; Porfimer sodiums; Porfimers;
Porfiromycins; Prednimustines; Procarbazines; Propamidines; Prospidiums;
5 Pumitepas; Puromycins; Pyrazofurins; Quinacrine; Ranimustines; Rasburicases;
Riboprines; Ritrosulfans; Rituximabs; Rogletimides; Roquinimexs;
Rufocromomycins; Sabarubicins; Safingols; Sargramostims; Satraplatins;
Sebriplatin; Semustines; Simtrazenes; Sizofirans; Sobuzoxanes; Sorafenibs;
Sparfosates; Sparfosic Acids; Sparsomycins; Spirogermaniums; Spiromustines;
10 Spiroplatin; Spiroplatin; Squalamines; Streptonigrins; Streptovaryns;
Streptozocins; Sufosfamides; Sulofenurs; Sunitinib Malate; 6-thioguanine (6-TG);
Tacedinalines; Talcs; Talisomycins; Tallimustines; Tamoxifens; Tariquidars;
Tauromustines; Tecogalans; Tegafurs; Teloxantrones; Temoporfin; Temozolomides;
Teniposides/VM-26s; Teniposides; Teroxirones; Testolactones; Thiamiprines;
15 Thioguanines; Thiotepas; Tiamiprines; Tiazofurins; Tilomisoles; Tilorones;
Timcodars; Timonacis; Tirapazamines; Topixantrones; Topotecans; Toremifenes;
Tositumomabs; Trabectedins (Ecteinascidin 743); Trastuzumabs; Trestolones;
Tretinoin/ATRA; Triciribines; Trilostanes; Trimetrexates; Triplatin Tetranitrates;
Triptorelins; Trofosfamides; Tubulozoles; Ubenimexs; Uracil Mustards; Uredepas;
20 Valrubicins; Valspodars; Vapreotides; Verteporfin; Vinblastines; Vincristines;
Vindesines; Vinepidines; Vinflunines; Vinformides; Vinglycinates; Vinleucinols;
Vinleurosines; Vinorelbines; Vinrosidines; Vintriptols; Vinzolidines; Vorozoles;
Xanthomycin As (Guamecyclines); Zeniplatins; Zilascorbs [2-H]; Zinostatins;
Zoledronate; Zorubicins; and Zosuquidars.
- 25 Hyaluronan-associated diseases or conditions include, for example, diseases or
conditions associated with or including high interstitial fluid pressure, such as disc
pressure, cancer and edema. Edema can result from or be manifested in, for example,
from organ transplant, stroke or brain trauma. Cancers, include solid and
lymphatic/blood tumors and metastatic disease, and undifferentiated tumors. The
30 tumors amenable to treatment typically exhibit cellular and/or stromal expression of a
hyaluronan, compared to a non-cancerous tissue of the same tissue type or compared

to a non-metastatic tumor of the same tumor-type. Cancers include any one or more of ovarian cancer, in situ carcinoma (ISC), squamous cell carcinoma (SCC), prostate cancer, pancreatic cancer, other gastric cancers, non-small cell lung cancer, breast cancer, brain cancer and colon cancer.

5 As noted, the hyaluronan degrading enzymes for use in the methods, compositions and combinations herein, include soluble hyaluronidases, including non-human animal hyaluronidases, bacterial hyaluronidases and human hyaluronidases. Exemplary of soluble hyaluronidases are soluble active portions of PH20, such as ovine, bovine, and human PH20. To render the PH20 soluble, such as human PH20,
10 the PH20 is truncated to remove a C-terminal GPI anchor attachment signal sequence. With reference to human PH20, truncation ends at any of residues 467-482 or corresponding to any of residues 467-482 of SEQ ID NO:1 in the human, allelic or species variants or other variants. For example, the hyaluronan degrading enzyme can be selected from among polypeptides containing a sequence of amino acids set forth in
15 any of SEQ ID NOS: 4-9 and 47-48, and allelic variants, species variants and other variants thereof. The hyaluronan degrading enzyme can be one that is encoded by a sequence of nucleic acids that encodes a sequence of amino acids set forth in SEQ ID NO:3 or any of SEQ ID NOS: 4-9 and 47-48, such as the sequence of nucleic acids set forth in SEQ ID NO: 49. They can be the hyaluronan degrading enzymes that have
20 such sequences of amino acids. The hyaluronan degrading enzyme is selected from among the other variants thereof. The other variants are selected from among polypeptides having at least 60%, 65, 70, 75, 80, 85, 88%, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% or more sequence identity along their full length to a contiguous sequence of amino acids from the that set forth in SEQ ID NO:1, 4-9 and 47-48 as long as the
25 polypeptide is soluble and exhibits hyaluronidase activity (i.e. can degrade hyaluronan), which can be assessed by methods known to one of skill in the art, including methods described herein.

 In particular, the hyaluronan degrading enzyme can be polypeptide that is produced by expression of the nucleic acid molecule that encodes amino acids 1-482
30 or 36-482 (*i.e.* , as set forth in SEQ ID NO:1) of the human PH20 soluble hyaluronidase or allelic or species or other variants thereof. Expression can include

expression and secretion in cells, such as CHO cells. For example, the composition is designated recombinant human PH20 (rHuPH20), which is produced by expression of nucleic acid encoding amino acids 36-482, linked to nucleic acid encoding a signal sequence for secretion, such as amino acids 1-35, in CHO cells. rHuPH20 is isolated from the medium. Following isolation it is modified, such as by reaction with one or more pegylation moieties. (PEG) or other polymer. Other hyaluronan degrading enzymes can be similarly modified and can be produced by recombinant expression or isolated from natural sources. Such methods and products are known to those of skill in the art.

Exemplary polymers for modification of the hyaluronan degrading enzyme include, but are not limited to, methoxy-poly(ethylene glycol)-succinimidyl butanoate (mPEG-SBA) (5 kDa); methoxy-poly(ethylene glycol)-succinimidyl butanoate (mPEG-SBA) (20 kDa); methoxy-poly(ethylene glycol)-succinimidyl butanoate (mPEG-SBA) (30 kDa); methoxy-poly(ethylene glycol)-succinimidyl α -methylbutanoate (mPEG-SMB) (20 kDa); methoxy-poly(ethylene glycol)-succinimidyl α -methylbutanoate (mPEG-SMB) (30 kDa); methoxy-poly(ethylene glycol)-butyraldehyde (mPEG-butyraldehyde) (30 kDa), methoxy-poly(ethylene glycol)-succinimidyl propionate (mPEG-SPA) (20 kDa); methoxy-poly(ethylene glycol)-succinimidyl propionate (mPEG-SPA) (30 kDa); (methoxy-poly(ethylene glycol))₂-N-hydroxysuccinimide ester (mPEG₂-NHS) (10 kDa branched); (methoxy-poly(ethylene glycol))₂-N-hydroxysuccinimide ester (mPEG₂-NHS) (20 kDa branched); (methoxy-poly(ethylene glycol))₂-N-hydroxysuccinimide ester (mPEG₂-NHS) (40 kDa branched); (methoxy-poly(ethylene glycol))₂-N-hydroxysuccinimide ester (mPEG₂-NHS) (60 kDa branched); biotin-poly(ethylene glycol)-N-hydroxysuccinimide ester (biotin-PEG-NHS) (5 kDa biotinylated); poly(ethylene glycol)-p-nitrophenyl carbonate (PEG-p-nitrophenyl-carbonate) (30 kDa); and poly(ethylene glycol)-propionaldehyde (PEG-propionaldehyde) (30 kDa).

PEG moieties are well known, and include those that are commercially available or that can be synthesized. PEG moieties can be branched or linear PEG, such as a methoxy-PEG (mPEG), including, for example, a linear N-hydroxysuccinimidyl ester of methoxy poly(ethylene glycol) butanoic acid. The

molecular weight of the PEG can be anything suitable, such as 30 or about 30 kilodaltons.

When the hyaluronan degrading enzyme is modified, the specific activity can be reduced. To compensate, a greater amount (weight) of the modified species is employed. For example, the specific activity of a PEGylated rHuPH20 can be 2- or 3- or 4-fold less or about 2- or 3- or 4-fold less than the specific activity of a native rHuPH20. Dosages are adjusted accordingly to deliver the desired units. Dosages depend upon the disease or condition and particular hyaluronan degrading enzyme, such as the particular hyaluronidase, and modification thereof. Typical dosages are at or about 10 to 50,000,000 Units, 10 to 40,000,000 Units, 10 to 36,000,000 Units, 10 to 12,000,000 Units, 10 to 1,200,000 Units, 10 to 1,000,000 Units, 10 to 500,000 Units, 100 to 100,000 Units, 500 to 50,000 Units, 1000 to 10,000 Units, 5000 to 7500 Units, 5000 Units to 50,000 Units, or 1,000 to 10,000 Units of soluble hyaluronidase. In instances where the hyaluronan degrading enzyme is not a hyaluronidase, typical dosages are functionally equivalent to at or about 10 to 50,000,000 hyaluronidase Units, 10 to 40,000,000 Units, 10 to 36,000,000 Units, 10 to 12,000,000 Units, 10 to 1,200,000 Units, 10 to 1,000,000 Units, 10 to 500,000 Units, 100 to 100,000 Units, 500 to 50,000 Units, 1000 to 10,000 Units, 5000 to 7500 Units, 5000 Units to 50,000 Units, or 1,000 to 10,000 hyaluronidase Units. Such dosages are selected to or can be used to effect at least a 25 %, about 25 %, 50 % or about 50 % decrease of interstitial fluid pressure in a tissue of the subject, which can be maintained for more than about or 1 or 2 hours, or at least 8 or at least about 8 hours, at least 24 or at least about 24 hours, at least 48 or at least about 48 hours or at least 72 or at least about 72 hours, following the administration.

Administration of the modified hyaluronan degrading enzyme composition, such as a modified hyaluronidase composition, can effect changes in vascular volume, such as in a tumor. Such changes can be at least 2-fold or about 2-fold or at least 3-fold or about 3-fold increase in the vascular volume of a tissue in the subject. Administration of the composition containing a hyaluronan degrading enzyme can effect at least a 25 % or about 25 % or at least a 50 % or about 50 % reduction in water content in a tissue of the subject. These changes can be maintained or

manifested for at least 8 or about 8 hours, at least 24 or about 24 hours, at least 48 or about 48 hours, or at least 72 or about 72 hours following administration of the composition containing the hyaluronan degrading enzyme.

Administration of the composition containing a hyaluronan degrading enzyme can effect a decrease in the percentage of hyaluronan positive cells in a tissue of the subject. The hyaluronan-positive cells can occur in a tumor, such as a solid tumor. Treatment can effect a reduction in the size of a tumor in the subject.

Administration of the composition containing a hyaluronan degrading enzyme can be manifested and/or observed as a hyaluronidase activity in the blood of the subject. The half-life of the hyaluronidase in the blood of the subject is at least 1 or about 1, 5 or about 5, 8 or about 8, 10 or about 10, 15 or about 15, 24 or about 24, 48 or about 48, or 72 or about 72 hours. For example, where the administration of the composition containing a hyaluronan degrading enzyme results in hyaluronidase activity in the blood of the subject, one milliliter of plasma from the subject has at least 5 % or about 5 % of the total hyaluronidase activity administered to the patient for at least 1 or about 1, 5 or about 5, 8 or about 8, 10 or about 10, 15 or about 15, 24 or about 24, 48 or about 48, or 72 or about 72 hours.

Also provided are combinations that contain:

a first composition containing an effective amount of a hyaluronan degrading enzyme for lowering interstitial fluid pressure, where the amount lowers interstitial fluid pressure for more than 24 hours; and an amount of hyaluronan degrading enzyme is functionally equivalent to between at or about 10 to 1,000,000, about 10 to 50,000,000 Units, 10 to 40,000,000 Units, 10 to 36,000,000 Units, 10 to 12,000,000 Units, 10 to 1,200,000 Units, 10 to 1,000,000 Units, 10 to 500,000 Units, 100 to 100,000 Units, 500 to 50,000 Units, 1000 to 10,000 Units, 5000 to 7500 Units, 5000 Units to 50,000 Units, or 1,000 to 10,000 hyaluronidase; and a second composition containing an agent for treating a hyaluronan-associated disease or condition.

The hyaluronan degrading enzymes are modified by conjugation to a polymer, such as one that increases half-life of the hyaluronidase. Exemplary polymers, include dextran, a sialation or pegylation moiety and/or combinations thereof. The hyaluronan degrading enzymes can be soluble hyaluronidases, which can include

those selected from among non-human animal hyaluronidases, human hyaluronidases and bacterial hyaluronidases as described above, and include the soluble hyaluronidase is a soluble form of a PH20 described above for use in the methods. The second agent or treatment includes any set forth above for use in the methods.

5 By virtue of methods herein, cancers and other diseases that contain pericellular matrices that rich in proteoglycans that contain hyaluronan are treated. Such cancers and other diseases are amenable to treatment with modified soluble hyaluronidases and other agents as described herein (above and below). As detailed herein, contact with a modified hyaluronan degrading enzyme, such pegylated
10 rHuPH20, results in collapse of the pericellular coats. As exemplified pegylated rHuPH20 (PEGrHuPH20) reduced tumor IFP in a dose dependent fashion, achieving more than 85% reduction in IFP following IV administration. Peritumoral HA remained depleted over 3 days after a single dose of PEGrHuPH20. Along with histologic collapse of pericellular hyaluronan surrounding the tumor cells, tumor
15 water content significantly decreased over 3 days, consistent with changes detected in the tumor by Apparent Diffusion Coefficient (ADC) MRI and IFP monitoring. Furthermore, as exemplified a 3.5-fold selective increase in tumor vascular volume was achieved within 8 hours post-dosing as a result of vascular decompression of blood vessels within the tumor. This was confirmed by histology and ultrasound.
20 Such hyaluronan in the tumor microenvironment can be targeted with soluble modified hyaluronidases, such as pegylated rHuPH20 (PEGrHuPH20). As exemplified also, co-administration of pegylated rHuPH20 with a chemotherapeutic agent, such as docetaxel or liposomal doxorubicin, can increase the anti-tumor activity of the chemotherapeutic agent in animal models compared to when the
25 chemotherapeutic agent is administered alone.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 depicts the tumor volume in nude mice inoculated intramuscularly with human PC3 prostate cancer cells to establish tumors (PC3 Prostate Carcinoma Model). Following inoculation, the mice were subjected to treatment regimens in
30 which doses of either active pharmaceutical ingredient (API) buffer, 30 mg/kg docetaxel, 10 mg/kg docetaxel, PEGylated rHuPH20 (P) or PEGylated rHuPH20

plus 10 mg/kg docetaxel (T+P) were administered. The tumor volumes were measured at various time points to assess the effect of co-administration of PEGylated rHuPH20 with docetaxel on the anti-tumor activity of docetaxel.

5 **Figure 2** depicts the percentage of mice survival to 1500 mm³ tumor volume at various points following intramuscular injection of human PC3 prostate cancer cells, followed by treatment with either buffer (control mice), docetaxel, PEGylated rHuPH20 or PEGylated rHuPH20/docetaxel.

10 **Figure 3** depicts the tumor volume in nude mice inoculated intramuscularly with human PC3 prostate cancer cells to establish tumors (PC3 Prostate Carcinoma Model). Following inoculation, the mice were subjected to treatment regimens in which doses of either API buffer, PEGylated rHuPH20 (P), liposomal doxorubicin (D) or PEGylated rHuPH20 plus liposomal doxorubicin (D+P) were administered. The tumor volumes were measured at various time points to assess the effect of co-administration of PEGylated rHuPH20 with liposomal doxorubicin on the anti-tumor activity of liposomal doxorubicin.

Figure 4 depicts the body weight change (in percentage) following administration of 3000, 7000, 10000, or 30000 Units of PEGylated rHuPH20 (P) on days 0, 3, 7, 10, 14, 17, 21, 24.

20 **Figure 5** depicts the body weight change (in percentage) following co-administration of 10 mg/kg docetaxel with either 3000, 7000, or 10000 Units of PEGylated rHuPH20 (T+P) to nude mice on days 0, 7, 14 and 21. These mice also received either 3000, 7000, or 10000 Units of PEGylated rHuPH20 on days 3, 10, 17 and 24. The body weight change of mice that received only API buffer or 10 mg/kg docetaxel also are depicted.

25 **Figure 6** depicts the number of granulocytes in the blood of nude mice administered various doses of either PEGylated rHuPH20 with 10 mg/kg Taxotere® (docetaxel), PEGylated rHuPH20 alone, Taxotere® (docetaxel) alone or API buffer alone. Mice in group 1 received API buffer on days 0, 3, 7, 10, 14, 17, 21, 24; Groups 2-5 received PEGylated rHuPH20 at a dose of either 3000, 7000, 10000 or 30000 units/mouse, respectively, on days 0, 3, 7, 10, 14, 17, 21, 24; Groups 6-8 were co-administered Taxotere® (docetaxel) with either 3000, 7000, 10000 units/mouse

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PEGylated rHuPH20, respectively, on days 0, 7, 14, 21 and then PEGylated rHuPH20 alone on days 3, 10, 17, 24; Groups 9 and 10 received 10 mg/kg Taxotere® (docetaxel) or 30 mg/kg Taxotere® (docetaxel), respectively, on days 0, 7 and 14. The number of granulocytes in the blood at various time points was then assessed.

5 **Figure 7** depicts the albumin levels in the serum of mice nude mice administered various doses of either PEGylated rHuPH20 with 10 mg/kg Taxotere® (docetaxel), PEGylated rHuPH20 alone, Taxotere® (docetaxel) alone or API buffer alone. Mice in group 1 received API buffer on days 0, 3, 7, 10, 14, 17, 21, 24; Groups 2-5 received PEGylated rHuPH20 at a dose of either 3000, 7000, 10000 or 30000
10 units/mouse, respectively, on days 0, 3, 7, 10, 14, 17, 21, 24; Groups 6-8 were co-administered Taxotere® (docetaxel) with either 3000, 7000, 10000 units/mouse PEGylated rHuPH20, respectively, on days 0, 7, 14, 21 and then PEGylated rHuPH20 alone on days 3, 10, 17, 24; Groups 9 and 10 received 10 mg/kg Taxotere® (docetaxel) or 30 mg/kg Taxotere® (docetaxel), respectively, on days 0, 7 and 14.

15 **Figure 8** depicts the effects of repeated administration of PEGylated rHuPH20, alone, in the HA-rich human prostate tumor xenograft model, PC3. As described in Example 16A, mice were injected on days 0, 3, 5, 7, 10, 12, 14 and 17, with control buffer and various amounts (enzymatic units (U)) of PEGylated HuPH20. tumor volume (mm^3) was measured over the course of the study in each group of
20 animals, at days 2, 4, 7, 11, 14 and 18 by capturing images using the Visual Sonics® ultrasound system and using an ultrasound imaging software program. These results also are set forth in Table 29.

Figure 9 depicts the tumor volume and percent survival in three different tumor models (PC3, 4T1-GFP, Mat LyLu), having varying degrees of hyaluronan
25 (HA) tumor expression (+++, ++ and +), following administration of API buffer or 3000 U PEGylated rHuPH20. The results are described in Example 17C, and also are set forth in Tables 33-37. As noted in Example 17, the effects was assessed in each model by determining the percentage of “surviving” animals at each time-point in each group. For this study, a tumor volume of greater than or equal to 1500 mm^3 was
30 selected as an endpoint, which was considered analogous to a moribund (non-surviving) state and animals with tumor volumes below 1500 mm^3 were considered

surviving, while animals with tumor volumes 1500 mm³ or greater were considered morbid.

Figure 10 depicts the percentage of mice surviving at various points following treatment with PEGylated rHuPH20 or control buffer in a PC3 brain tumor model, as described in Example 18. Survival was determined by assessing the number of mice that were alive at the indicated time.

Figure 11 depicts the percentage of mice surviving at various points following treatment with control buffer, irradiation, or combination therapy of irradiation and PEGylated rHuPH20 in a PC3 brain tumor model, as described in Example 18. Survival was determined by assessing the number of mice that were alive at the indicated time.

Figure 12 depicts a PK regression curve after administration of PEGylated rHuPH20 in mice.

DETAILED DESCRIPTION

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I. EXAMPLES

A. DEFINITIONS

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which the invention(s) belong. All patents, patent applications, published applications and publications, Genbank sequences, databases, websites and other published materials referred to throughout the entire disclosure herein, unless noted otherwise, are incorporated by reference in their entirety. In the event that there is a plurality of definitions for terms herein, those in this section prevail. Where reference is made to

a URL or other such identifier or address, it understood that such identifiers can change and particular information on the internet can come and go, but equivalent information can be found by searching the internet. Reference thereto evidences the availability and public dissemination of such information.

5 As used herein, dosing regime refers to the amount of agent, for example, the composition containing a soluble hyaluronidase or other agent, administered, and the frequency of administration. The dosing regime is a function of the disease or condition to be treated, and thus can vary.

 As used herein, frequency of administration refers to the time between
10 successive administrations of treatment. For example, frequency can be days, weeks or months. For example, frequency can be more than once weekly, for example, twice a week, three times a week, four times a week, five times a week, six times a week or daily. Frequency also can be one, two, three or four weeks. The particular frequency is function of the particular disease or condition treated. Generally,
15 frequency is more than once weekly, and generally is twice weekly.

 As used herein, a "cycle of administration" refers to the repeated schedule of the dosing regime of administration of the enzyme and/or a second agent that is repeated over successive administrations. For example, an exemplary cycle of administration is a 28 day cycle with administration twice weekly for three weeks,
20 ~~followed by one week of discontinued dosing.~~

 As used herein, "predetermined" with reference to a number of weeks of administration or discontinued administration refers to a period of time that is decided or established in advance. The period of time can be empirically determined and is a function of the disease or condition, the severity of the condition, the particular
25 patient and other factors within the level of skill of a treating physician.

 As used herein, a hyaluronan degrading enzyme refers to an enzyme that catalyzes the cleavage of a hyaluronan polymer (also referred to as hyaluronic acid or HA) into smaller molecular weight fragments. Exemplary of hyaluronan degrading enzymes are hyaluronidases, and particular chondroitinases and lyases that have the
30 ability to depolymerize hyaluronan. Exemplary chondroitinases that are hyaluronan degrading enzymes include, but are not limited to, chondroitin ABC lyase (also

known as chondroitinase ABC), chondroitin AC lyase (also known as chondroitin sulfate lyase or chondroitin sulfate eliminase) and chondroitin C lyase. Chondroitin ABC lyase comprises two enzymes, chondroitin-sulfate-ABC endolyase (EC 4.2.2.20) and chondroitin-sulfate-ABC exolyase (EC 4.2.2.21). An exemplary chondroitin-sulfate-ABC endolyases and chondroitin-sulfate-ABC exolyases include, but are not limited to, those from *Proteus vulgaris* and *Flavobacterium heparinum* (the *Proteus vulgaris* chondroitin-sulfate-ABC endolyase is set forth in SEQ ID NO:98; Sato *et al.* (1994) *Appl. Microbiol. Biotechnol.* 41(1):39-46). Exemplary chondroitinase AC enzymes from the bacteria include, but are not limited to, those from *Flavobacterium heparinum* *Victivallis vadensis*, set forth in SEQ ID NO:99, and *Arthrobacter* *aureus* (Tkalec *et al.* (2000) *Applied and Environmental Microbiology* 66(1):29-35; Ernst *et al.* (1995) *Critical Reviews in Biochemistry and Molecular Biology* 30(5):387-444). Exemplary chondroitinase C enzymes from the bacteria include, but are not limited to, those from *Streptococcus* and *Flavobacterium* (Hibi *et al.* (1989) *FEMS-Microbiol-Lett.* 48(2):121-4; Michelacci *et al.* (1976) *J. Biol. Chem.* 251:1154-8; Tsuda *et al.* (1999) *Eur. J. Biochem.* 262:127-133).

As used herein, hyaluronidase refers to a class of hyaluronan degrading enzymes. Hyaluronidases include bacterial hyaluronidases (EC 4.2.2.1 or EC 4.2.99.1), hyaluronidases from leeches, other parasites, and crustaceans (EC 3.2.1.36), and mammalian-type hyaluronidases (EC 3.2.1.35). Hyaluronidases include any of non-human origin including, but not limited to, murine, canine, feline, leporine, avian, bovine, ovine, porcine, equine, piscine, ranine, bacterial, and any from leeches, other parasites, and crustaceans. Exemplary non-human hyaluronidases include, hyaluronidases from cows (SEQ ID NOS:10, 11, 64 and BH55 (U.S. Pat. Nos. 5,747,027 and 5,827,721), yellow jacket wasp (SEQ ID NOS:12 and 13), honey bee (SEQ ID NO:14), white-face hornet (SEQ ID NO:15), paper wasp (SEQ ID NO:16), mouse (SEQ ID NOS:17-19, 31), pig (SEQ ID NOS:20-21), rat (SEQ ID NOS:22-24, 30), rabbit (SEQ ID NO:25), sheep (SEQ ID NOS:26, 27, 63 and 65), chimpanzee (SEQ ID NO:101), Rhesus monkey (SEQ ID NO:102), orangutan (SEQ ID NO:28), cynomolgus monkey (SEQ ID NO:29), guinea pig (SEQ ID NO:32), *Arthrobacter sp.* (strain FB24) (SEQ ID NO:67), *Bdellovibrio bacteriovorus* (SEQ ID NO:68),

Propionibacterium acnes (SEQ ID NO:69), *Streptococcus agalactiae* ((SEQ ID NO:70); 18RS21 (SEQ ID NO:71); serotype Ia (SEQ ID NO:72); serotype III (SEQ ID NO:73), *Staphylococcus aureus* (strain COL (SEQ ID NO:74); strain MRSA252 (SEQ ID NOS:75 and 76); strain MSSA476 (SEQ ID NO:77); strain NCTC 8325 (SEQ ID NO:78); strain bovine RF122 (SEQ ID NOS:79 and 80); strain USA300 (SEQ ID NO:81), *Streptococcus pneumoniae* ((SEQ ID NO:82); strain ATCC BAA-255 / R6 (SEQ ID NO:83); serotype 2, strain D39 / NCTC 7466 (SEQ ID NO:84), *Streptococcus pyogenes* (serotype M1) (SEQ ID NO:85); serotype M2, strain MGAS10270 (SEQ ID NO:86); serotype M4, strain MGAS10750 (SEQ ID NO:87); serotype M6 (SEQ ID NO:88); serotype M12, strain MGAS2096 (SEQ ID NOS:89 and 90); serotype M12, strain MGAS9429 (SEQ ID NO:91); serotype M28 (SEQ ID NO:92); *Streptococcus suis* (SEQ ID NOS:93-95); *Vibrio fischeri* (strain ATCC 700601/ ES114 (SEQ ID NO:96)), and the *Streptomyces hyaluronolyticus* hyaluronidase enzyme, which is specific for hyaluronic acid and does not cleave chondroitin or chondroitin sulfate (Ohya, T. and Kaneko, Y. (1970) *Biochim. Biophys. Acta* 198:607). Hyaluronidases also include those of human origin. Exemplary human hyaluronidases include HYAL1 (SEQ ID NO:36), HYAL2 (SEQ ID NO:37), HYAL3 (SEQ ID NO:38), HYAL4 (SEQ ID NO:39), and PH20 (SEQ ID NO:1). Also included amongst hyaluronidases are soluble hyaluronidases, including, ovine and bovine PH20, soluble human PH20 and soluble rHuPH20. Examples of commercially available bovine or ovine soluble hyaluronidases Vitrase® (ovine hyaluronidase) and Amphadase® (bovine hyaluronidase).

Reference to hyaluronan degrading enzymes includes precursor hyaluronan degrading enzyme polypeptides and mature hyaluronan degrading enzyme polypeptides (such as those in which a signal sequence has been removed), truncated forms thereof that have activity, and includes allelic variants and species variants, variants encoded by splice variants, and other variants, including polypeptides that have at least 40%, 45%, 50%, 55%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the precursor polypeptides set forth in SEQ ID NOS: 1 and 10-48, 63-65, 67-102, or the mature form thereof. For example, reference to hyaluronan degrading enzyme also includes the human PH20 precursor

polypeptide variants set forth in SEQ ID NOS:50-51. Hyaluronan degrading enzymes also include those that contain chemical or posttranslational modifications and those that do not contain chemical or posttranslational modifications. Such modifications include, but are not limited to, pegylation, albumination, glycosylation, farnesylation, carboxylation, hydroxylation, phosphorylation, and other polypeptide modifications known in the art.

As used herein, a soluble hyaluronidase refers to a polypeptide characterized by its solubility under physiologic conditions. Soluble hyaluronidases can be distinguished, for example, by its partitioning into the aqueous phase of a Triton X-114 solution warmed to 37°C (Bordier et al., (1981) J. Biol. Chem., 256:1604-7). Membrane-anchored, such as lipid anchored hyaluronidases, will partition into the detergent rich phase, but will partition into the detergent-poor or aqueous phase following treatment with Phospholipase-C. Included among soluble hyaluronidases are membrane anchored hyaluronidases in which one or more regions associated with anchoring of the hyaluronidase to the membrane has been removed or modified, where the soluble form retains hyaluronidase activity. Soluble hyaluronidases include recombinant soluble hyaluronidases and those contained in or purified from natural sources, such as, for example, testes extracts from sheep or cows. Exemplary of such soluble hyaluronidases are soluble human PH20. Other soluble hyaluronidases include ovine (SEQ ID NOS:27, 63, 65) and bovine (SEQ ID NOS:11, 64) PH20.

As used herein, soluble human PH20 or sHuPH20 include mature polypeptides lacking all or a portion of the glycosylphosphatidylinositol (GPI) attachment site at the C-terminus such that upon expression, the polypeptides are soluble. Exemplary sHuPH20 polypeptides include mature polypeptides having an amino acid sequence set forth in any one of SEQ ID NOS:4-9 and 47-48. The precursor polypeptides for such exemplary sHuPH20 polypeptides include a signal sequence. Exemplary of the precursors are those set forth in SEQ ID NOS:3 and 40-46, each of which contains a 35 amino acid signal sequence at amino acid positions 1-35. Soluble HuPH20 polypeptides also include those degraded during or after the production and purification methods described herein.

As used herein, soluble recombinant human PH20 (rHuPH20) refers to a soluble form of human PH20 that is recombinantly expressed in Chinese Hamster Ovary (CHO) cells. Soluble rHuPH20 is encoded by nucleic acid that includes the signal sequence and is set forth in SEQ ID NO:49. Also included are DNA molecules that are allelic variants thereof and other soluble variants. The nucleic acid encoding soluble rHuPH20 is expressed in CHO cells which secrete the mature polypeptide. As produced in the culture medium, there is heterogeneity at the C-terminus so that the product includes a mixture of species that can include any one or more of SEQ ID NOS. 4-9 in various abundance. Corresponding allelic variants and other variants also are included, including those corresponding to the precursor human PH20 polypeptides set forth in SEQ ID NOS:50-51. Other variants can have 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity with any of SEQ ID NOS.4-9 and 47-48 as long they retain a hyaluronidase activity and are soluble.

As used herein, a hyaluronidase substrate refers to a substrate (e.g. protein or polysaccharide) that is cleaved and/or depolymerized by a hyaluronidase enzyme. Generally, a hyaluronidase substrate is a glycosaminoglycan. An exemplary hyaluronidase substrate is hyaluronan (HA).

As used herein, a hyaluronan-associated disease, disorder or condition refers to any disease or condition in which hyaluronan levels are elevated as cause, consequence or otherwise observed in the disease or condition. Hyaluronan-associated diseases and conditions are associated with elevated hyaluronan expression in a tissue or cell, increased interstitial fluid pressure, decreased vascular volume, and/or increased water content in a tissue. Hyaluronan-associated diseases, disorders or conditions can be treated by administration of a composition containing a hyaluronan degrading enzyme, such as a hyaluronidase, for example, a soluble hyaluronidase, either alone or in combination with or in addition to another treatment and/or agent. Exemplary diseases and conditions, include, but are not limited to, hyaluronan-rich cancers, for example, tumors, including solid tumors such as late-stage cancers, a metastatic cancers, undifferentiated cancers, ovarian cancer, in situ carcinoma (ISC), squamous cell carcinoma (SCC), prostate cancer, pancreatic cancer,

non-small cell lung cancer, breast cancer, colon cancer and other cancers. Also exemplary of hyaluronan-associated diseases and conditions are diseases that are associated with elevated interstitial fluid pressure, such as diseases associated with disc pressure, and edema, for example, edema caused by organ transplant, stroke, 5 brain trauma or other injury. Exemplary hyaluronan-associated diseases and conditions include diseases and conditions associated with elevated interstitial fluid pressure, decreased vascular volume, and/or increased water content in a tissue, including cancers, disc pressure and edema. In one example, treatment of the hyaluronan-associated condition, disease or disorder includes amelioration, reduction, 10 or other beneficial effect on one or more of increased interstitial fluid pressure (IFP), decreased vascular volume, and increased water content in a tissue.

As used herein, elevated hyaluronan levels refers to amounts of hyaluronan in particular tissue, body fluid or cell, dependent upon the disease or condition. consequence or otherwise observed in the disease. For example, as consequence of 15 the presence of a hyaluronan-rich tumor, hyaluronan (HA) levels can be elevated in body fluids, such as blood, urine, saliva and serum, and/or in the tumorous tissue or cell. The level can be compared to a standard or other suitable control, such as a comparable sample from a subject who does not have the HA-associated disease.

As used herein, a polymer that is conjugated to a hyaluronan degrading 20 enzyme, such as a hyaluronidase, refers to any polymer that is covalently or otherwise stably linked, directly or via a linker, to a hyaluronan degrading enzyme. Such polymers, typically increase serum half-life, and include, but are not limited to sialic moieties, pegylation moieties, dextran, and sugar and other moieties, such as for glycosylation.

25 As used herein, a native hyaluronan degrading enzyme, e.g. a native soluble hyaluronidase, is a hyaluronan degrading enzyme that has not been modified with a polymer, for example, a pegylation moiety (PEG) or sialation moiety. Hence, a native hyaluronan degrading enzyme is unmodified. Typically, native hyaluronan degrading enzymes, such as native soluble hyaluronidases, have decreased half-life in a 30 biological environment (e.g. in a subject) compared to hyaluronan degrading enzymes that have been modified by conjugation of a polymer, e.g. PEG.

As used herein, specific activity refers to Units of activity per mg protein. The milligrams of hyaluronidase is defined by the absorption of a solution of at 280 nm assuming a molar extinction coefficient of approximately 1.7, in units of $M^{-1} cm^{-1}$.

As used herein, activity refers to a functional activity or activities of a polypeptide or portion thereof associated with a full-length (complete) protein. Functional activities include, but are not limited to, biological activity, catalytic or enzymatic activity, antigenicity (ability to bind or compete with a polypeptide for binding to an anti-polypeptide antibody), immunogenicity, ability to form multimers, and the ability to specifically bind to a receptor or ligand for the polypeptide.

As used herein, hyaluronidase activity refers to the ability to enzymatically catalyze the cleavage of hyaluronic acid. The United States Pharmacopeia (USP) XXII assay for hyaluronidase determines hyaluronidase activity indirectly by measuring the amount of higher molecular weight hyaluronic acid, or hyaluronan, (HA) substrate remaining after the enzyme is allowed to react with the HA for 30 min at 37°C (USP XXII-NF XVII (1990) 644-645 United States Pharmacopeia Convention, Inc, Rockville, MD). A Reference Standard solution can be used in an assay to ascertain the relative activity, in units, of any hyaluronidase. *In vitro* assays to determine the hyaluronidase activity of hyaluronidases, such as soluble rHuPH20, are known in the art and described herein. Exemplary assays include the microturbidity assay described below (see e.g. Example 3) that measures cleavage of hyaluronic acid by hyaluronidase indirectly by detecting the insoluble precipitate formed when the uncleaved hyaluronic acid binds with serum albumin. Reference Standards can be used, for example, to generate a standard curve to determine the activity in Units of the hyaluronidase being tested.

As used herein, a unit of activity of a hyaluronidase refers to a U.S.P. National Formulary (NF XIII) unit (NFU), as determined by comparing to standard hyaluronidase samples (e.g., USP or WHO standard), for example, using the turbidity reducing ELISA-based assay described in Example 2 herein, whereby turbidity reducing units are related to the NFU, and U.S.P. unit through a standard curve of a sample of hyaluronidase (e.g., USP or WHO standard) standardized through the U.S.P. Thus, the enzyme activities as determined in Example 2 are relative TRU (see,

for example, Dorfman et al., 1948, J. Biol. Chem. 172:367). A hyaluronidase unit is normalized to the standard activity. Hence, for example, pegylated hyaluronidase can exhibit lower activity/mg. For purposes herein, dosages reference units. Units/mg (standard activity) of a particular modified hyaluronidase can be determined empirically if needed.

As used herein, "functionally equivalent amount" or grammatical variations thereof, with reference to a hyaluronan degrading enzyme, refers to the amount of hyaluronan degrading enzyme that achieves the same effect as an amount of a reference enzyme, such as a hyaluronidase. For example, the activity of any hyaluronan degrading enzyme can be compared to the activity of rHuPH20 to determine the functionally equivalent amount of a hyaluronan degrading enzyme that would achieve the same effect as a known amount of rHuPH20. For example, the ability of a hyaluronan degrading enzyme to act as a spreading or diffusing agent can be assessed by injecting it into the lateral skin of mice with trypan blue (see e.g. U.S. Pat. Publication No. 20050260186), and the amount of hyaluronan degrading enzyme required to achieve the same amount of diffusion as, for example, 100 units of a Hyaluronidase Reference Standard, can be determined. The amount of hyaluronan degrading enzyme required is, therefore, functionally equivalent to 100 units. In another example, the ability of a hyaluronan degrading enzyme to increase the *in vivo* activity of a co-administered agent (e.g. the anti-tumor activity of a chemotherapeutic agent) can be assessed in animal models or human subjects, such as described in Example 14, and the amount of hyaluronan degrading enzyme required to achieve the same increase in the activity of the co-administered agent as, for example, the administered quantity of rHuPH20, can be determined.

As used herein, the residues of naturally occurring α -amino acids are the residues of those 20 α -amino acids found in nature which are incorporated into protein by the specific recognition of the charged tRNA molecule with its cognate mRNA codon in humans.

As used herein, nucleic acids include DNA, RNA and analogs thereof, including peptide nucleic acids (PNA) and mixtures thereof. Nucleic acids can be single or double-stranded. When referring to probes or primers, which are optionally

labeled, such as with a detectable label, such as a fluorescent or radiolabel, single-stranded molecules are contemplated. Such molecules are typically of a length such that their target is statistically unique or of low copy number (typically less than 5, generally less than 3) for probing or priming a library. Generally a probe or primer contains at least 14, 16 or 30 contiguous nucleotides of sequence complementary to or identical to a gene of interest. Probes and primers can be 10, 20, 30, 50, 100 or more nucleic acids long.

As used herein, a peptide refers to a polypeptide that is from 2 to 40 amino acids in length.

As used herein, the amino acids which occur in the various sequences of amino acids provided herein are identified according to their known, three-letter or one-letter abbreviations (Table 1). The nucleotides which occur in the various nucleic acid fragments are designated with the standard single-letter designations used routinely in the art.

As used herein, an "amino acid" is an organic compound containing an amino group and a carboxylic acid group. A polypeptide contains two or more amino acids. For purposes herein, amino acids include the twenty naturally-occurring amino acids, non-natural amino acids and amino acid analogs (i.e., amino acids wherein the α -carbon has a side chain).

As used herein, "amino acid residue" refers to an amino acid formed upon chemical digestion (hydrolysis) of a polypeptide at its peptide linkages. The amino acid residues described herein are presumed to be in the "L" isomeric form. Residues in the "D" isomeric form, which are so designated, can be substituted for any L-amino acid residue as long as the desired functional property is retained by the polypeptide.

NH₂ refers to the free amino group present at the amino terminus of a polypeptide. COOH refers to the free carboxy group present at the carboxyl terminus of a polypeptide. In keeping with standard polypeptide nomenclature described in *J. Biol. Chem.*, 243: 3557-3559 (1968), and adopted 37 C.F.R., §§ 1.821-1.822, abbreviations for amino acid residues are shown in Table 1:

Table 1 – Table of Correspondence

SYMBOL	
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1-Letter	3-Letter	AMINO ACID
Y	Tyr	Tyrosine
G	Gly	Glycine
F	Phe	Phenylalanine
M	Met	Methionine
A	Ala	Alanine
S	Ser	Serine
I	Ile	Isoleucine
L	Leu	Leucine
T	Thr	Threonine
V	Val	Valine
P	Pro	proline
K	Lys	Lysine
H	His	Histidine
Q	Gln	Glutamine
E	Glu	glutamic acid
Z	Glx	Glu and/or Gln
W	Trp	Tryptophan
R	Arg	Arginine
D	Asp	aspartic acid
N	Asn	asparagine
B	Asx	Asn and/or Asp
C	Cys	Cysteine
X	Xaa	Unknown or other

It should be noted that all amino acid residue sequences represented herein by formulae have a left to right orientation in the conventional direction of amino-terminus to carboxyl-terminus. In addition, the phrase "amino acid residue" is

5 broadly defined to include the amino acids listed in the Table of Correspondence (Table 1) and modified and unusual amino acids, such as those referred to in 37 C.F.R. §§ 1.821-1.822, and incorporated herein by reference. Furthermore, it should be noted that a dash at the beginning or end of an amino acid residue sequence indicates a peptide bond to a further sequence of one or more amino acid residues, to
10 an amino-terminal group such as NH₂ or to a carboxyl-terminal group such as COOH.

As used herein, "naturally occurring amino acids" refer to the 20 L-amino acids that occur in polypeptides.

As used herein, "non-natural amino acid" refers to an organic compound that has a structure similar to a natural amino acid but has been modified structurally to
15 mimic the structure and reactivity of a natural amino acid. Non-naturally occurring amino acids thus include, for example, amino acids or analogs of amino acids other

than the 20 naturally-occurring amino acids and include, but are not limited to, the D-isostereomers of amino acids. Exemplary non-natural amino acids are described herein and are known to those of skill in the art.

As used herein, a DNA construct is a single or double stranded, linear or
5 circular DNA molecule that contains segments of DNA combined and juxtaposed in a manner not found in nature. DNA constructs exist as a result of human manipulation, and include clones and other copies of manipulated molecules.

As used herein, a DNA segment is a portion of a larger DNA molecule having specified attributes. For example, a DNA segment encoding a specified polypeptide
10 is a portion of a longer DNA molecule, such as a plasmid or plasmid fragment, which, when read from the 5' to 3' direction, encodes the sequence of amino acids of the specified polypeptide.

As used herein, the term polynucleotide means a single- or double-stranded polymer of deoxyribonucleotides or ribonucleotide bases read from the 5' to the 3'
15 end. Polynucleotides include RNA and DNA, and can be isolated from natural sources, synthesized *in vitro*, or prepared from a combination of natural and synthetic molecules. The length of a polynucleotide molecule is given herein in terms of nucleotides (abbreviated "nt") or base pairs (abbreviated "bp"). The term nucleotides is used for single- and double-stranded molecules where the context permits. When
20 ~~the term is applied to double-stranded molecules it is used to denote overall length~~
and will be understood to be equivalent to the term base pairs. It will be recognized by those skilled in the art that the two strands of a double-stranded polynucleotide can differ slightly in length and that the ends thereof can be staggered; thus all nucleotides within a double-stranded polynucleotide molecule can not be paired. Such unpaired
25 ends will, in general, not exceed 20 nucleotides in length.

As used herein, "similarity" between two proteins or nucleic acids refers to the relatedness between the sequence of amino acids of the proteins or the nucleotide sequences of the nucleic acids. Similarity can be based on the degree of identity and/or homology of sequences of residues and the residues contained therein.
30 Methods for assessing the degree of similarity between proteins or nucleic acids are known to those of skill in the art. For example, in one method of assessing sequence

similarity, two amino acid or nucleotide sequences are aligned in a manner that yields a maximal level of identity between the sequences. "Identity" refers to the extent to which the amino acid or nucleotide sequences are invariant. Alignment of amino acid sequences, and to some extent nucleotide sequences, also can take into account
5 conservative differences and/or frequent substitutions in amino acids (or nucleotides). Conservative differences are those that preserve the physico-chemical properties of the residues involved. Alignments can be global (alignment of the compared sequences over the entire length of the sequences and including all residues) or local (the alignment of a portion of the sequences that includes only the most similar region
10 or regions).

"Identity" per se has an art-recognized meaning and can be calculated using published techniques. (See, e.g.: *Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of*
15 *Sequence Data*, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exists a number of methods to measure identity between two polynucleotide or polypeptides, the term "identity" is
20 well known to skilled artisans (Carillo, H. & Lipton, D., *SIAM J Applied Math* 48:1073 (1988)).

As used herein, homologous (with respect to nucleic acid and/or amino acid sequences) means about greater than or equal to 25% sequence homology, typically greater than or equal to 25%, 40%, 50%, 60%, 70%, 80%, 85%, 90% or 95%
25 sequence homology; the precise percentage can be specified if necessary. For purposes herein the terms "homology" and "identity" are often used interchangeably, unless otherwise indicated. In general, for determination of the percentage homology or identity, sequences are aligned so that the highest order match is obtained (see, e.g.: *Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New
30 York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data, Part I*,

Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; Carillo *et al.* (1988) *SIAM J Applied Math* 48:1073). By sequence homology,
5 the number of conserved amino acids is determined by standard alignment algorithms programs, and can be used with default gap penalties established by each supplier. Substantially homologous nucleic acid molecules would hybridize typically at moderate stringency or at high stringency all along the length of the nucleic acid of interest. Also contemplated are nucleic acid molecules that contain degenerate
10 codons in place of codons in the hybridizing nucleic acid molecule.

Whether any two molecules have nucleotide sequences or amino acid sequences that are at least 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% "identical" or "homologous" can be determined using known computer algorithms such as the "FASTA" program, using for example, the default parameters as in
15 Pearson *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:2444 (other programs include the GCG program package (Devereux, J., *et al.*, *Nucleic Acids Research* 12(I):387 (1984)), BLASTP, BLASTN, FASTA (Atschul, S.F., *et al.*, *J Molec Biol* 215:403 (1990)); Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo *et al.* (1988) *SIAM J Applied Math* 48:1073). For example,
20 the BLAST function of the National Center for Biotechnology Information database can be used to determine identity. Other commercially or publicly available programs include, DNASTar "MegAlign" program (Madison, WI) and the University of Wisconsin Genetics Computer Group (UWG) "Gap" program (Madison WI). Percent homology or identity of proteins and/or nucleic acid molecules can be
25 determined, for example, by comparing sequence information using a GAP computer program (*e.g.*, Needleman *et al.* (1970) *J. Mol. Biol.* 48:443, as revised by Smith and Waterman ((1981) *Adv. Appl. Math.* 2:482). Briefly, the GAP program defines similarity as the number of aligned symbols (*i.e.*, nucleotides or amino acids), which are similar, divided by the total number of symbols in the shorter of the two sequences.
30 Default parameters for the GAP program can include: (1) a unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) and the weighted com-

parison matrix of Gribskov *et al.* (1986) *Nucl. Acids Res.* 14:6745, as described by Schwartz and Dayhoff, eds., *ATLAS OF PROTEIN SEQUENCE AND STRUCTURE*, National Biomedical Research Foundation, pp. 353-358 (1979); (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no
5 penalty for end gaps.

Therefore, as used herein, the term "identity" or "homology" represents a comparison between a test and a reference polypeptide or polynucleotide. As used herein, the term at least "90% identical to" refers to percent identities from 90 to 99.99 relative to the reference nucleic acid or amino acid sequence of the polypeptide.

10 Identity at a level of 90% or more is indicative of the fact that, assuming for exemplification purposes a test and reference polypeptide length of 100 amino acids are compared. No more than 10% (i.e., 10 out of 100) of the amino acids in the test polypeptide differs from that of the reference polypeptide. Similar comparisons can be made between test and reference polynucleotides. Such differences can be
15 represented as point mutations randomly distributed over the entire length of a polypeptide or they can be clustered in one or more locations of varying length up to the maximum allowable, e.g. 10/100 amino acid difference (approximately 90% identity). Differences are defined as nucleic acid or amino acid substitutions, insertions or deletions. At the level of homologies or identities above about 85-90%,
20 the result should be independent of the program and gap parameters set, such high levels of identity can be assessed readily, often by manual alignment without relying on software.

As used herein, an aligned sequence refers to the use of homology (similarity and/or identity) to align corresponding positions in a sequence of nucleotides or
25 amino acids. Typically, two or more sequences that are related by 50% or more identity are aligned. An aligned set of sequences refers to 2 or more sequences that are aligned at corresponding positions and can include aligning sequences derived from RNAs, such as ESTs and other cDNAs, aligned with genomic DNA sequence.

As used herein, "primer" refers to a nucleic acid molecule that can act as a
30 point of initiation of template-directed DNA synthesis under appropriate conditions (e.g., in the presence of four different nucleoside triphosphates and a polymerization

agent, such as DNA polymerase, RNA polymerase or reverse transcriptase) in an appropriate buffer and at a suitable temperature. It will be appreciated that a certain nucleic acid molecules can serve as a "probe" and as a "primer." A primer, however, has a 3' hydroxyl group for extension. A primer can be used in a variety of methods, including, for example, polymerase chain reaction (PCR), reverse-transcriptase (RT)-PCR, RNA PCR, LCR, multiplex PCR, panhandle PCR, capture PCR, expression PCR, 3' and 5' RACE, in situ PCR, ligation-mediated PCR and other amplification protocols.

As used herein, "primer pair" refers to a set of primers that includes a 5' (upstream) primer that hybridizes with the 5' end of a sequence to be amplified (e.g. by PCR) and a 3' (downstream) primer that hybridizes with the complement of the 3' end of the sequence to be amplified.

As used herein, "specifically hybridizes" refers to annealing, by complementary base-pairing, of a nucleic acid molecule (e.g. an oligonucleotide) to a target nucleic acid molecule. Those of skill in the art are familiar with *in vitro* and *in vivo* parameters that affect specific hybridization, such as length and composition of the particular molecule. Parameters particularly relevant to *in vitro* hybridization further include annealing and washing temperature, buffer composition and salt concentration. Exemplary washing conditions for removing non-specifically bound nucleic acid molecules at high stringency are 0.1 x SSPE, 0.1% SDS, 65°C, and at medium stringency are 0.2 x SSPE, 0.1% SDS, 50°C. Equivalent stringency conditions are known in the art. The skilled person can readily adjust these parameters to achieve specific hybridization of a nucleic acid molecule to a target nucleic acid molecule appropriate for a particular application. Complementary, when referring to two nucleotide sequences, means that the two sequences of nucleotides are capable of hybridizing, typically with less than 25%, 15% or 5% mismatches between opposed nucleotides. If necessary, the percentage of complementarity will be specified. Typically the two molecules are selected such that they will hybridize under conditions of high stringency.

As used herein, substantially identical to a product means sufficiently similar so that the property of interest is sufficiently unchanged so that the substantially identical product can be used in place of the product.

As used herein, it also is understood that the terms “substantially identical” or
5 “similar” varies with the context as understood by those skilled in the relevant art.

As used herein, an allelic variant or allelic variation references any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation arises naturally through mutation, and can result in phenotypic polymorphism within populations. Gene mutations can be silent (no change in the
10 encoded polypeptide) or can encode polypeptides having altered amino acid sequence. The term “allelic variant” also is used herein to denote a protein encoded by an allelic variant of a gene. Typically the reference form of the gene encodes a wildtype form and/or predominant form of a polypeptide from a population or single reference member of a species. Typically, allelic variants, which include variants between and
15 among species typically have at least 80%, 90% or greater amino acid identity with a wildtype and/or predominant form from the same species; the degree of identity depends upon the gene and whether comparison is interspecies or intraspecies. Generally, intraspecies allelic variants have at least about 80%, 85%, 90% or 95% identity or greater with a wildtype and/or predominant form, including 96%, 97%,
20 98%, 99% or greater identity with a wildtype and/or predominant form of a polypeptide. Reference to an allelic variant herein generally refers to variations in proteins among members of the same species.

As used herein, “allele,” which is used interchangeably herein with “allelic variant” refers to alternative forms of a gene or portions thereof. Alleles occupy the
25 same locus or position on homologous chromosomes. When a subject has two identical alleles of a gene, the subject is said to be homozygous for that gene or allele. When a subject has two different alleles of a gene, the subject is said to be heterozygous for the gene. Alleles of a specific gene can differ from each other in a single nucleotide or several nucleotides, and can include substitutions, deletions and
30 insertions of nucleotides. An allele of a gene also can be a form of a gene containing a mutation.

As used herein, species variants refer to variants in polypeptides among different species, including different mammalian species, such as mouse and human.

As used herein, a splice variant refers to a variant produced by differential processing of a primary transcript of genomic DNA that results in more than one type
5 of mRNA.

As used herein, modification, when used in reference to modification of a sequence of amino acids, is used to describe modifications of a polypeptide or a sequence of nucleotides in a nucleic acid molecule and includes deletions, insertions, and replacements of amino acids and nucleotides, respectively. Methods of
10 modifying a polypeptide are routine to those of skill in the art, such as by using recombinant DNA methodologies.

As used herein, the term promoter means a portion of a gene containing DNA sequences that provide for the binding of RNA polymerase and initiation of transcription. Promoter sequences are commonly, but not always, found in the 5'
15 non-coding region of genes.

As used herein, isolated or purified polypeptide or protein or biologically-active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue from which the protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized.

20 Preparations can be determined to be substantially free if they appear free of readily detectable impurities as determined by standard methods of analysis, such as thin layer chromatography (TLC), gel electrophoresis and high performance liquid chromatography (HPLC), used by those of skill in the art to assess such purity, or sufficiently pure such that further purification would not detectably alter the physical
25 and chemical properties, such as enzymatic and biological activities, of the substance. Methods for purification of the compounds to produce substantially chemically pure compounds are known to those of skill in the art. A substantially chemically pure compound, however, can be a mixture of stereoisomers. In such instances, further purification might increase the specific activity of the compound.

30 The term substantially free of cellular material includes preparations of proteins in which the protein is separated from cellular components of the cells from

which it is isolated or recombinantly-produced. In one embodiment, the term substantially free of cellular material includes preparations of enzyme proteins having less than about 30% (by dry weight) of non-enzyme proteins (also referred to herein as a contaminating protein), generally less than about 20% of non-enzyme proteins or 5 10% of non-enzyme proteins or less than about 5% of non-enzyme proteins. When the enzyme protein is recombinantly produced, it also is substantially free of culture medium, *i.e.*, culture medium represents less than about or at 20%, 10% or 5% of the volume of the enzyme protein preparation.

As used herein, the term substantially free of chemical precursors or other 10 chemicals includes preparations of enzyme proteins in which the protein is separated from chemical precursors or other chemicals that are involved in the synthesis of the protein. The term includes preparations of enzyme proteins having less than about 30% (by dry weight) 20%, 10%, 5% or less of chemical precursors or non-enzyme chemicals or components.

15 As used herein, synthetic, with reference to, for example, a synthetic nucleic acid molecule or a synthetic gene or a synthetic peptide refers to a nucleic acid molecule or polypeptide molecule that is produced by recombinant methods and/or by chemical synthesis methods.

As used herein, production by recombinant means by using recombinant DNA 20 ~~methods means the use of the well known methods of molecular biology for~~ expressing proteins encoded by cloned DNA.

As used herein, vector (or plasmid) refers to discrete elements that are used to introduce a heterologous nucleic acid into cells for either expression or replication thereof. The vectors typically remain episomal, but can be designed to effect 25 integration of a gene or portion thereof into a chromosome of the genome. Also contemplated are vectors that are artificial chromosomes, such as yeast artificial chromosomes and mammalian artificial chromosomes. Selection and use of such vehicles are well known to those of skill in the art.

As used herein, an expression vector includes vectors capable of expressing 30 DNA that is operatively linked with regulatory sequences, such as promoter regions, that are capable of effecting expression of such DNA fragments. Such additional

segments can include promoter and terminator sequences, and optionally can include other regulatory sequences, such as, but not limited to, one or more origins of replication, one or more selectable markers, an enhancer and a polyadenylation signal. Expression vectors are generally derived from plasmid or viral DNA, or can contain elements of both. Thus, an expression vector refers to a recombinant DNA or RNA construct, such as a plasmid, a phage, recombinant virus or other vector that, upon introduction into an appropriate host cell, results in expression of the cloned DNA. Appropriate expression vectors are well known to those of skill in the art and include those that are replicable in eukaryotic cells and/or prokaryotic cells and those that remain episomal or those which integrate into the host cell genome.

As used herein, vector also includes "virus vectors" or "viral vectors." Viral vectors are engineered viruses that are operatively linked to exogenous genes to transfer (as vehicles or shuttles) the exogenous genes into cells.

As used herein, operably or operatively linked when referring to DNA segments means that the segments are arranged so that they function in concert for their intended purposes, e.g., transcription initiates in the promoter and proceeds through the coding segment to the terminator.

As used herein the term assessing is intended to include quantitative and qualitative determination in the sense of obtaining an absolute value for the activity of a protease, or a domain thereof, present in the sample, and also of obtaining an index, ratio, percentage, visual or other value indicative of the level of the activity.

Assessment can be direct or indirect and the chemical species actually detected need not of course be the proteolysis product itself but can for example be a derivative thereof or some further substance. For example, detection of a cleavage product of a complement protein, such as by SDS-PAGE and protein staining with Coomassie blue.

As used herein, biological activity refers to the *in vivo* activities of a compound or physiological responses that result upon *in vivo* administration of a compound, composition or other mixture. Biological activity, thus, encompasses therapeutic effects and pharmaceutical activity of such compounds, compositions and mixtures. Biological activities can be observed in *in vitro* systems designed to test or

use such activities. Thus, for purposes herein a biological activity of a protease is its catalytic activity in which a polypeptide is hydrolyzed.

As used herein equivalent, when referring to two sequences of nucleic acids, means that the two sequences in question encode the same sequence of amino acids or equivalent proteins. When equivalent is used in referring to two proteins or peptides, it means that the two proteins or peptides have substantially the same amino acid sequence with only amino acid substitutions that do not substantially alter the activity or function of the protein or peptide. When equivalent refers to a property, the property does not need to be present to the same extent (*e.g.*, two peptides can exhibit different rates of the same type of enzymatic activity), but the activities are usually substantially the same.

As used herein, “modulate” and “modulation” or “alter” refer to a change of an activity of a molecule, such as a protein. Exemplary activities include, but are not limited to, biological activities, such as signal transduction. Modulation can include an increase in the activity (*i.e.*, up-regulation or agonist activity) a decrease in activity (*i.e.*, down-regulation or inhibition) or any other alteration in an activity (such as a change in periodicity, frequency, duration, kinetics or other parameter). Modulation can be context dependent and typically modulation is compared to a designated state, for example, the wildtype protein, the protein in a constitutive state, or the protein as expressed in a designated cell type or condition.

As used herein, a composition refers to any mixture. It can be a solution, suspension, liquid, powder, paste, aqueous, non-aqueous or any combination thereof.

As used herein, a combination refers to any association between or among two or more items. The combination can be two or more separate items, such as two compositions or two collections, can be a mixture thereof, such as a single mixture of the two or more items, or any variation thereof. The elements of a combination are generally functionally associated or related.

As used herein, a kit is a packaged combination that optionally includes other elements, such as additional reagents and instructions for use of the combination or elements thereof.

As used herein, "disease or disorder" refers to a pathological condition in an organism resulting from cause or condition including, but not limited to, infections, acquired conditions, genetic conditions, and characterized by identifiable symptoms. Diseases and disorders of interest herein are hyaluronan-associated diseases and disorders.

As used herein, "treating" a subject with a disease or condition means that the subject's symptoms are partially or totally alleviated, or remain static following treatment. Hence treatment encompasses prophylaxis, therapy and/or cure. Prophylaxis refers to prevention of a potential disease and/or a prevention of worsening of symptoms or progression of a disease. Treatment also encompasses any pharmaceutical use of a modified interferon and compositions provided herein.

As used herein, a pharmaceutically effective agent, includes any therapeutic agent or bioactive agents, including, but not limited to, for example, anesthetics, vasoconstrictors, dispersing agents, conventional therapeutic drugs, including small molecule drugs and therapeutic proteins.

As used herein, treatment means any manner in which the symptoms of a condition, disorder or disease or other indication, are ameliorated or otherwise beneficially altered.

As used herein therapeutic effect means an effect resulting from treatment of a subject that alters, typically improves or ameliorates the symptoms of a disease or condition or that cures a disease or condition. A therapeutically effective amount refers to the amount of a composition, molecule or compound which results in a therapeutic effect following administration to a subject.

As used herein, the term "subject" refers to an animal, including a mammal, such as a human being.

As used herein, a patient refers to a human subject.

As used herein, amelioration of the symptoms of a particular disease or disorder by a treatment, such as by administration of a pharmaceutical composition or other therapeutic, refers to any lessening, whether permanent or temporary, lasting or transient, of the symptoms that can be attributed to or associated with administration of the composition or therapeutic.

As used herein, prevention or prophylaxis refers to methods in which the risk of developing disease or condition is reduced.

As used herein, a “therapeutically effective amount” or a “therapeutically effective dose” refers to the quantity of an agent, compound, material, or composition containing a compound that is at least sufficient to produce a therapeutic effect. Hence, it is the quantity necessary for preventing, curing, ameliorating, arresting or partially arresting a symptom of a disease or disorder.

As used herein, unit dose form refers to physically discrete units suitable for human and animal subjects and packaged individually as is known in the art.

As used herein, a single dosage formulation refers to a formulation for direct administration.

As used herein, an “article of manufacture” is a product that is made and sold. As used throughout this application, the term is intended to encompass hyaluronan degrading enzyme, such as hyaluronidase, and second agent compositions contained in articles of packaging.

As used herein, fluid refers to any composition that can flow. Fluids thus encompass compositions that are in the form of semi-solids, pastes, solutions, aqueous mixtures, gels, lotions, creams and other such compositions.

As used herein, a “kit” refers to a combination of compositions provided herein and another item for a purpose including, but not limited to, activation, administration, diagnosis, and assessment of a biological activity or property. Kits optionally include instructions for use.

As used herein, a cellular extract or lysate refers to a preparation or fraction which is made from a lysed or disrupted cell.

As used herein, animal includes any animal, such as, but are not limited to primates including humans, gorillas and monkeys; rodents, such as mice and rats; fowl, such as chickens; ruminants, such as goats, cows, deer, sheep; ovine, such as pigs and other animals. Non-human animals exclude humans as the contemplated animal. The enzymes provided herein are from any source, animal, plant, prokaryotic and fungal. Most enzymes are of animal origin, including mammalian origin.

As used herein, a control refers to a sample that is substantially identical to the test sample, except that it is not treated with a test parameter, or, if it is a plasma sample, it can be from a normal volunteer not affected with the condition of interest. A control also can be an internal control.

5 As used herein, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to a compound, comprising "an extracellular domain"" includes compounds with one or a plurality of extracellular domains.

As used herein, ranges and amounts can be expressed as "about" a particular value or range. About also includes the exact amount. Hence "about 5 bases" means 10 "about 5 bases" and also "5 bases."

As used herein, "optional" or "optionally" means that the subsequently described event or circumstance does or does not occur, and that the description includes instances where said event or circumstance occurs and instances where it 15 does not. For example, an optionally substituted group means that the group is unsubstituted or is substituted.

As used herein, anti-cancer treatments include administration of drugs and other agents for treating cancer, and also treatment protocols, such as radiation.

As used herein, a therapeutic antibody, refers to any antibody use for therapy, 20 and includes, but is not limited to monoclonal antibodies, human antibodies, scFvs, diabodies, Fabs, and other fragments of antibodies.

As used herein, antibody fragment refers to any derivative of an antibody that is less than full length, retaining at least a portion of the full-length antibody's specific binding ability. Examples of antibody fragments include, but are not limited to, Fab, 25 Fab', F(ab)2, single-chain Fvs (scFv), Fv, dsFv, diabody and Fd fragments. The fragment can include multiple chains linked together, such as by disulfide bridges. An antibody fragment generally contains at least about 50 amino acids and typically at least 200 amino acids.

As used herein, an Fv antibody fragment is composed of one variable heavy 30 domain (VH) and one variable light (VL) domain linked by noncovalent interactions.

As used herein, a dsFv refers to an Fv with an engineered intermolecular disulfide bond, which stabilizes the VH-VL pair.

As used herein, an F(ab)₂ fragment is an antibody fragment that results from digestion of an immunoglobulin with pepsin at pH 4.0-4.5; it can be recombinantly
5 produced.

As used herein, an Fab fragment is an antibody fragment that results from digestion of an immunoglobulin with papain; it can be recombinantly produced.

As used herein, scFvs refers to antibody fragments that contain a variable light chain (VL) and variable heavy chain (VH) covalently connected by a polypeptide
10 linker in any order. The linker is of a length such that the two variable domains are bridged without substantial interference. Exemplary linkers are (Gly-Ser)_n residues with some Glu or Lys residues dispersed throughout to increase solubility.

As used herein, hsFv refers to antibody fragments in which the constant domains normally present in an Fab fragment have been substituted with a
15 heterodimeric coiled-coil domain (see, e.g., Arndt et al. (2001) J Mol Biol. 7:312:221-228).

As used herein, diabodies are dimeric scFv; diabodies typically have shorter peptide linkers than scFvs, and they preferentially dimerize.

As used herein, humanized antibodies refer to antibodies that are modified to
20 include "human" sequences of amino acids so that administration to a human does not provoke an immune response. Methods for preparation of such antibodies are known. For example, the hybridoma that expresses the monoclonal antibody is altered by recombinant DNA techniques to express an antibody in which the amino acid composition of the non-variable regions is based on human antibodies.
25 Computer programs have been designed to identify such regions.

As used herein, the abbreviations for any protective groups, amino acids and other compounds, are, unless indicated otherwise, in accord with their common usage, recognized abbreviations, or the IUPAC-IUB Commission on Biochemical Nomenclature (see, (1972) *Biochem. 11*:1726).

30 **B. OVERVIEW OF THE METHODS AND COMPOSITIONS FOR TREATING HYALURONAN-ASSOCIATED CONDITIONS, DISEASES AND DISORDERS**

Provided herein are methods, compositions and combinations for treating hyaluronan associated conditions, diseases and disorders. The methods, compositions and combinations provided herein employ hyaluronan degrading enzymes that can degrade hyaluronic acid (also called hyaluronan), which is an essential component of the extracellular matrix and a major constituent of the interstitial barrier. Many diseases and conditions are associated with accumulated expression or overexpression of a hyaluronidase substrate such as hyaluronan, which contributes to the progression and/or severity of the disease or condition. Hence, administration of such enzymes for the purposes of depolymerizing the substrate can result in the treatment of such diseases and conditions.

Any hyaluronan degrading enzyme, including any variant thereof (e.g. truncated variant), can be used herein provided the enzyme exhibits enzymatic activity. Generally, the hyaluronan degrading enzymes are modified by conjugation to a polymer (e.g. PEG) to increase the half-life of the enzyme. The increased half-life can increase systemic hyaluronidase activity and sustained duration of action for hyaluronan degradation. In addition, the increased half-life also can contribute to the prevention of resynthesis and regeneration of the hyaluronidase substrate associated with the disease or condition. For example, as described herein, a modified hyaluronan degrading enzyme that exhibits an increased half-life by virtue of conjugation to a polymer can prevent hyaluronan regeneration within tumors. The modified hyaluronan degrading enzymes provided herein also can be combined and/or co-formulated with a second agent for treating the hyaluronan associated conditions, diseases and/or disorders.

1. Hyaluronan

Glycosaminoglycans (GAGs) are complex linear polysaccharides of the extracellular matrix (ECM). GAGs are characterized by repeating disaccharide structures of an N-substituted hexosamine and an uronic acid, (e.g. hyaluronan (HA), chondroitin sulfate (CS), chondroitin (C), dermatan sulfate (DS), heparan sulfate (HS), heparin (H)), or a galactose (e.g. keratan sulfate (KS)). Except for HA, all exist covalently bound to core proteins. The GAGs with their core proteins are structurally referred to as proteoglycans (PGs).

HA is a linear, repeating polysaccharide made up of N-acetylglucosamine and glucuronic acid disaccharide units. The metabolism of HA is a dynamic process, with normal turnover in tissues ranging from several weeks to less than a day in skin. HA is synthesized at the plasma membrane by three conserved HA synthases (HAS) and degraded by cell-associated or acid-active hyaluronidases (Culty 1992; Zhou 2000) and exoglycosidase enzymes to monosaccharides following receptor-mediated endocytosis.

HA occurs in the extracellular matrix of many cells, especially in soft connective tissues. Hyaluronan (HA) occurs predominantly in connective tissues, skin, cartilage, and in synovial fluid in mammal. Hyaluronan also is the main constituent of the vitreous of the eye. HA has been assigned various physiological functions, such as in water and plasma protein homeostasis in the intracellular matrix (Laurent TC *et al.* (1992) FASEB J 6: 2397-2404). In connective tissue, the water of hydration associated with hyaluronan creates spaces between tissues, thus creating an environment conducive to cell movement and proliferation. In the body, for example, in tissues of a subject, hyaluronan (hyaluronic acid) is replaced with a half-life of approximately 5 h, and is largely responsible for the resistance to fluid flow through the tissues. Hyaluronan plays a role in biological phenomena associated with cell motility including rapid development, regeneration, repair, embryogenesis, embryological development, wound healing, angiogenesis, and tumorigenesis (see, *e.g.*, Toole 1991 Cell Biol. Extracell. Matrix, Hay (ed), Plenum Press, New York, 1384-1386; Bertrand *et al.*, 1992 Int. J. Cancer 52:1-6; Knudson *et al.*, 1993 FASEB J. 7:1233-1241). HA production increases in proliferating cells and may play a role in mitosis. It also has been implicated in locomotion and cell migration, as well as roles in cell regulation, development, and differentiation (Laurent *et al.*, (1992) FASEB J 6: 2397-2404).

HA has been used in clinical medicine. Its tissue protective and rheological properties have proved useful in ophthalmic surgery to protect the corneal endothelium during cataract surgery. Serum HA is diagnostic of liver disease and various inflammatory conditions, such as rheumatoid arthritis. Interstitial edema caused by accumulation of HA may cause dysfunction in various organs (Laurent *et*

al. (1992) FASEB J 6: 2397-2404). Hyaluronan protein interactions also are involved in the structure of the extracellular matrix or "ground substance."

HA synthesis is increased when oncogenic viruses transform fibroblasts and elevated levels of HA are associated with hyperproliferative and malignant phenotypes in various cancers, such as melanomas and some carcinomas (see, *e.g.*, Itano *et al.* (2002) *Proc. Natl. Acad. Sci. U.S.A.* 99:3609-3614.) In addition, hyaluronan levels correlate with tumor aggressiveness (Ozello *et al.* 1960 *Cancer Res.* 20:600-604; Takeuchi *et al.* 1976, *Cancer Res.* 36:2133-2139; Kimata *et al.* 1983 *Cancer Res.* 43:1347-1354). Local aberrations of HA metabolism have been reported in many solid tumor malignancies, where elevated levels of HA frequently correlate with poor prognosis in tumors such as breast, gastric, colorectal, ovarian, prostate and lung carcinoma. HA accumulation reduces contact inhibition between and among tumor cells ((see, *e.g.*, Itano *et al.* (2002) *Proc. Natl. Acad. Sci. U.S.A.* 99:3609-3614.).

2. Hyaluronan-associated diseases

Many diseases or conditions are associated with overexpressed or accumulated expression of hyaluronidase substrate, for example HA, which can exacerbate or contribute to the severity or prognosis of the disease or condition. The underlying cause for HA accumulation is likely due to one or more of HA synthase overexpression, poor lymphatic drainage, or unbalanced synthesis and degradation of the substrate.

Hence, among the hyaluronan-associated diseases, conditions and disorders that can be treated with the provided compositions, compounds and methods are conditions, diseases and disorders that express or accumulate hyaluron as cause, consequence or symptom of the disease, condition or disorder. Such diseases, conditions and/or disorders include, for example, those associated with increased interstitial fluid pressure, decreased vascular volume, increased water content in a tissue, disc pressure and edema. Hyaluronan-associated diseases, conditions and/or disorders, include, but are not limited to, hyaluronan-rich cancers, for example, tumors, including solid tumors, for example, late-stage cancers, a metastatic cancers, undifferentiated cancers, ovarian cancer, in situ carcinoma (ISC), squamous cell

carcinoma (SCC), prostate cancer, pancreatic cancer, non-small cell lung cancer, breast cancer, colon cancer and other cancers. Also exemplary of hyaluronan-associated diseases and disorders are disc pressure, cancer and edema, for example, edema caused by organ transplant, stroke, brain trauma or other injury.

5 For example, hyaluronan-associated diseases or conditions include solid tumors, including benign and malignant tumors. Exemplary of solid tumor malignancies include, for example, those associated with breast, gastric, colorectal ovarian, prostate and lung carcinoma. Aberrant accumulation of HA levels in tumors, in particular malignant tumors, is an indicator of poor prognosis when associated with
10 stromal or cellular compartments.

 For example, in a study of survival of patients having breast carcinoma by Auvinen (2000), the five-year survival deteriorated as a function of increasing stromal HA levels; for low, moderate and high HA levels, respectively, the five-year overall survival was 45%, 39%, and 26% ($p=0.002$) and the recurrence-free survival was
15 66%, 56% and 40% ($p=0.008$). The presence of HA-positive carcinoma cells correlated significantly with axillary lymph node positivity and poor differentiation. The 5-year overall survival of patients exhibiting HA-positive carcinoma cells was significantly lower compared to the patients without HA-positive carcinoma cells (with 54% versus 81%, respectively, $p=0.01$).

20 In gastric carcinoma, Setälä et al. (1999) examined the HA profile of 215 stage I-IV gastric carcinoma patients. A high proportion of HA positive cells were found and were significantly associated with deep tumor invasion, nodal metastasis, positive lymphatic invasion, poor differentiation grade, as well as with inferior prognosis in univariate survival analysis. Forty-four percent of the tumors evaluated had a HA
25 labeling index of 30-100% HA positive cells.

 In colorectal carcinomas, Ropponen et al. (1998) examined the cellular association of HA to overall survival and recurrence-free survival in 202 colorectal carcinoma samples followed up for a mean of 14 years. Both high HA intensity and labeling indices were frequently found and significantly associated with poorer
30 overall survival, shorter recurrence free survival, and elevated Duke classification for 187 evaluable patients.

Anttila et al. (2000) studied HA levels in 309 epithelial ovarian cancers and 45 matched metastatic lesions. While in 73% (227 of 309) of the cases, the fraction of hyaluronan-positive cancer cells was <10%, high stromal HA levels were significantly correlated with poor differentiation, serious histologic type, advanced stage, and large primary residual tumor.

Hyaluronan-associated diseases can be treated by administration of a composition containing a hyaluronan degrading enzyme, such as a hyaluronidase, for example, a soluble hyaluronidase, either alone or in combination with or in addition to another treatment and/or agent. Treatment of the hyaluronan-associated condition, disease or disorder includes amelioration, reduction, or other beneficial effect on one or more of any associated physical manifestation or symptom, such as increased interstitial fluid pressure (IFP), decreased vascular volume, and/or increased water content in a tissue.

In one example, treatment of the hyaluronan-associated condition, disease or disorder includes amelioration, reduction, or other beneficial effect on one or more of increased interstitial fluid pressure (IFP), decreased vascular volume, and increased water content in a tissue. In another example, treatment can include amelioration or beneficial effect on another symptom, for example, tumor size (e.g. mass/volume), prognosis, including survival and/or recurrence-free survival of a subject.

3. Methods of treatment and compositions

Hence, provided herein are methods and compositions for treating hyaluronan (HA)-associated conditions, diseases and disorders. The methods, such as methods of treatment, use compositions containing a modified hyaluronan degrading enzymes alone or compositions or combinations containing a modified hyaluronan degrading enzyme and further containing one or more additional agents for treating the hyaluronan associated conditions, diseases and/or disorders. In the compositions and combinations employed in the methods herein, the hyaluronan degrading enzymes, such as hyaluronidases, are modified, such as by conjugation to one or more polymer(s), whereby half-life of a hyaluronidase, such as a soluble hyaluronidases, is increased. The modified hyaluronan degrading enzymes provided herein can be used alone to treat hyaluronan associated conditions, diseases and disorders, or can be used

in combination with other agents, such as, for example, therapeutic agents (e.g. chemotherapeutic agents). The compositions containing the hyaluronan degrading enzyme and other agent(s) can be provided separately in the combination or provided in a single composition.

5 In some examples, the modified hyaluronan degrading enzymes are administered alone for treatment of a hyaluronan associated disease or condition. Thus, also provided herein are compositions containing a hyaluronan degrading enzyme, such as a modified soluble hyaluronidase, and methods for administering the modified hyaluronan degrading enzyme for treating a hyaluronan associated disease,
10 i.e. a disease associated with accumulated expression of a hyaluronidase substrate. For example, such treatment can be used for effecting decreased interstitial fluid pressure in a hyaluronan-associated disease, for example, a hyaluronan-rich cancer. As shown herein, treatment with a modified hyaluronan degrading enzyme such as a modified hyaluronidase not only removes hyaluronan and reduces interstitial fluid
15 pressure (IFP), but also, can restore contact inhibition. Thus, administration of a hyaluronan degrading enzyme can effect treatment by virtue of restoration of contact between and among cells. As shown herein, modification of the hyaluronan degrading enzyme, such as by pegylation, improves the effectiveness of the hyaluronan degrading enzyme as a treatment for tumors.

20 In particular, systemic administration of the modified hyaluronan degrading enzyme is effective for treating tumors, including brain tumors. For example, systemic administration of modified hyaluronan degrading enzyme, such as modified soluble hyaluronidase, decreases interstitial fluid pressure (IFP) in hyaluronan-rich tumors, effecting a dose-dependent and sustained reduction in IFP, for example,
25 reduction that persists greater than 1 hour, 2 hours, 4 hours, 8 hours, 10 hours, 12 hours, 16 hours, 24 hours, 48 hours or 72 hours. The hyaluronidase can effect such a reduction in a hyaluronan-specific manner.

 Further, consistent with the ability to reduce hyaluronan expression following administration in a subject with a hyaluronan-associated tumor, systemic admini-
30 stration of the modified hyaluronan degrading enzyme, such as a modified soluble hyaluronidase, can effect sustained reduction in tumor water content, for example, for

at least 1 hour, 2 hours, 4 hours, 8 hours, 10 hours, 12 hours, 16 hours 24 hours, 48 hours or 72 hours, and a decrease in vascular volume in hyaluronan-associated tumors, resulting from vascular decompression of blood vessels in the tumor. Thus, further provided herein are compositions containing a modified hyaluronan degrading enzyme, such as a modified soluble hyaluronidase, and methods for administration to effect a sustained increase in vascular volume and/or a sustained increase in water content in a tissue in the subject with a hyaluronan-associated disease or condition, for example, a hyaluronan-associated cancer.

Unmodified hyaluronan-degrading enzymes typically have a short half-life of enzymatic activity in blood of minutes, generally less than 5 minutes. This means that such enzymes are generally unsuitable for use in intravenous administrations, and other administrations, where their duration of action is short-lived. The hyaluronan degrading enzymes, such as soluble hyaluronidases, used in the composition and methods herein are modified by conjugation to a polymer, for example, a sialation moiety or pegylation moiety (PEG). Typically, the polymer increases the half-life of the hyaluronan degrading enzyme, such as hyaluronidase, following administration to the subject. The plasma half-life of enzymatic activity of modified hyaluronan-degrading enzymes (e.g. via conjugation to a polymer) provided herein are generally or are about 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 10 hours, 11 hours, 12 hours, 13 hours, 14 hours, 15 hours, 20 hours, 25 hours, 30 hours, 35 hours, 40 hours, 45 hours, 50 hours, 55 hours, 60 hours or more. In one example, a soluble hyaluronidase modified by conjugation to a polymer effects more than 100-, 200-, 250-, 300-, 400-, 500-, 600-, 700-, 800-, 900-, 1000-, 1500, 2000-fold or more increase in plasma half-life compared to the unmodified enzyme. In one example, the half-life of the enzyme in plasma is over 24 hours.

Due to the differences in half-life, the duration of activity of a modified hyaluronidase is increased compared to a native (not modified by conjugation to a polymer) hyaluronidase. For example, following administration of native (non-modified) soluble hyaluronidase, for example, hyaluronic acid can be restored within 24 hours, leaving no observable changes. Following administration of a hyaluronan degrading enzyme, such as a hyaluronidase, that has been modified by conjugation to

a polymer (e.g. PEG) that increases the half-life of the hyaluronan degrading enzyme, expression of hyaluronan remains reduced at 24 hours, 48 hours, 72 hours, or more following the administration.

Typically, a modified hyaluronan-degrading enzyme conjugated to a polymer
5 has a reduced specific activity compared to the unmodified hyaluronan-degrading enzyme. The specific activity is generally reduced by about or 2-fold, 3-fold, 4-fold, 5-fold or more. For example, as described elsewhere herein, the specific activity of unmodified PH20 designated rHuPH20 is about 120,000 U/mg. The specific activity of a PEGylated rHuPH20 is about 30,000 U/mg. Nevertheless, due to the increased
10 half-life, modified hyaluronan-degrading enzymes exhibit an increased duration of activity. Since HA is able to be regenerated, an enzyme that has a sustained duration of action can counteract HA resynthesis and deposition in the ECM.

Hence, in the methods, combinations and compositions provided herein, a modified hyaluronan-degrading enzyme, for example a soluble hyaluronidase, is
15 provided in an amount sufficient to sustain a minimal plasma level of the HA of at least 3 U/mL of the enzyme in the plasma. For example, the minimal plasma level of HA is maintained at a level that is or is about 3 U/mL- 12 U/mL or more, for example, from about or at a level of 4 U/mL, 5 U/mL, 6 U/mL, 7 U/mL, 8 U/mL, 9 U/mL, 10 U/mL, 11 U/mL, 12 U/mL, 13 U/mL, 14 U/mL, 15 U/mL, 16 U/mL, 17
20 U/mL, 18 U/mL, 19 U/mL, 20 U/mL, 25 U/mL, 30 U/mL, 35 U/mL, 40 U/mL, 45 U/mL, 50 U/mL or more. By maintaining at least 3 U/mL of the enzyme in the plasma, the enzyme can remove HA associated with a disease or condition, for example, tumoral HA, and also counteract HA resynthesis. Thus, in such treatments, the hyaluronidase substrate is not allowed to accumulate. For diseases or conditions,
25 such as cancers having malignant solid tumors, whose prognosis is associated with HA expression, the condition (e.g. cancerous condition) can be treated and/or ameliorated.

Hence, the provided compositions containing hyaulronan degrading enzymes, such as soluble hyaluronidases, conjugated to polymers can provide prolonged or
30 sustained treatment of diseases or conditions associated with accumulated hyaluronidase substrate, for example, prolonged or sustained amelioration of one or

more symptoms. For example, systemic (e.g. intravenous) administration of a modified soluble hyaluronidase, which is modified by conjugation with a polymer, can effect a sustained (e.g. at least 24, 48, and 72 hours) reduction in hyaluronan expression in per cellular matrices in a tissue of hyaluronan-rich tumors.

5 In order to sustain effect of the modified enzyme in plasma for longer periods of times, cycles of administration can be effected. Hence, the modified enzymes can be administered successively over a dosing regime in order to maintain a constant level of the modified hyaluronidase in the plasma for any desired length of time. The level in the plasma can be monitored during the course of treatment as described
10 herein by measuring the level of the modified hyaluronidase in the plasma. This means that over the course of treatment a minimal level of the hyaluronidase is present in plasma sufficient not only to remove HA associated with a disease or condition, but also to counteract HA resynthesis. Successive administrations can be made periodically whenever the level in the plasma falls below at least or about 3
15 U/mL. It, however, is not necessary to measure the plasma level before each administration. Generally, to maintain levels in plasma of the hyaluronidase of at least 3 U/mL, compositions containing hyaluronan-degrading enzymes, such as soluble hyaluronidases, conjugated to polymers are administered several times a month, generally at least once a week and typically more than once a week. For
20 example, the modified hyaluronan degrading enzymes, for example, modified soluble hyaluronidase, are administered twice a week, three times a week, four times a week, five times a week, six times a week or daily. Typically, such enzymes are administered twice a week.

 As discussed elsewhere herein, the dose of modified hyaluronidase enzyme
25 necessary to maintain a plasma level of the hyaluronidase of at least 3 U/mL can be empirically determined. Generally, as exemplified herein, the dose of a single administration of a modified soluble hyaluronidase to maintain at least 3 U/mL in the plasma over a cycle of administration is or is about 0.02 mg/kg (of the subject), 0.03 mg/kg, 0.04 mg/kg, 0.05 mg/kg, 0.06 mg/kg, 0.07 mg/kg, 0.08 mg/kg, 0.09 mg/kg,
30 0.1 mg/kg, 0.15 mg/kg, 0.2 mg/kg, 0.25 mg/kg, 0.30 mg/kg, 0.35 mg/kg, 0.40 mg/kg, 0.45 mg/kg, 0.5 mg/kg, 0.55 mg.kg, 0.6 mg/kg, 0.7 mg/kg, 0.8 mg/kg, 0.9 mg/kg, 1.0

mg/kg, 1.1 mg/kg, 1.2 mg/kg, 1.3 mg/kg, 1.4 mg/kg, 1.5 mg/kg or more. Typically, the dose is or is about 0.05 mg/kg to at or about 0.8 mg/kg. As discussed below, it is understood that such amounts are administered periodically (e.g. twice weekly) over a cycle of administration to maintain the plasma level for a desired length of time.

- 5 Assuming that the average human is 75 kg, a modified soluble hyaluronidase that has a specific activity of at or about 20,000 U/mg to 60,000 U/mg, generally at or about 35,000 U/mg, is administered at or about 60,000 U; 70,000 U; 80,000 U; 90,000 U; 100,000 U; 200,000 U; 300,000 U; 400,000 U; 500,000 U; 600,000 U; 700,000 U; 800,000 U; 900,000 U; 1,000,000 U; 1,500,000 U; 2,000,000 U; 2,500,000 U; 10 3,000,000 U; 3,500,000 U; 4,000,000 U or more. For example, compositions of a modified hyaluronidase can be administered that contain at or about 2.0-60 mg of a modified hyaluronidase. Also provided herein are such compositions.

The length of time of the cycle of administration can be empirically determined, and is dependent on the disease to be treated, the severity of the disease, the particular patient, and other considerations within the level of skill of the treating physician. The length of time of treatment with a modified hyaluronidase enzyme can be one week, two weeks, one months, several months, one year, several years or more. For example, a modified hyaluronidase enzyme can be administered twice weekly over a period of a year or more. If disease symptoms persist in the 15 absence of discontinued treatment, treatment can be continued for an additional length of time. Over the course of treatment, evidence of disease and/or treatment-related toxicity or side effects can be monitored.

- In addition, the cycle of administration can be tailored to add periods of discontinued treatment in order to provide a rest period from exposure to the enzyme. 25 The length of time for the discontinuation of treatment can be for a predetermined time or can be empirically determined depending on how the patient is responding or depending on observed side effects. For example, the treatment can be discontinued for one week, two weeks, one month or several months. It is understood and expected that during the period of discontinued treatment, the plasma level of the 30 hyaluronidase will fall below 3 U/mL. Generally, the period of discontinued treatment is built into a cycle of dosing regime for a patient. For example, an

exemplary dosing regime is a treatment cycle of 28 days, with the modified enzyme administered for the first 3 weeks, twice weekly, followed by a one week without dosing. In one example, 0.05 mg/kg – 0.8 mg/kg of modified enzyme can be administered twice weekly for 3 weeks, followed by a one week without dosing.

5 Thus, for example, a patient can be dosed with modified enzyme on days 1, 4, 8, 11, 15 and 18, followed by a one-week of discontinued treat, over the course of the 28-day cycle. As noted above, the cycle of administration can be for any desired length of time. Hence, the 28-day cycle of administration can be repeated for any length of time. It is within the level of skill of the treating physician to adopt a cycle of
10 administration and dosing regime that meets the needs of the patient depending on personal considerations specific to the patient and disease to be treated.

4. Combinations and Methods of treatment Thereof

Hyaluronan degrading enzymes such as hyaluronidase temporarily digest the hyaluronic acid, thereby facilitating delivery of agents. Thus, for example, due to the
15 ability of hyaluronan degrading enzymes to open channels in the interstitial space through degradation of glycosaminoglycans, administration of a hyaluronan degrading enzyme, such as a soluble hyaluronidase, permits the diffusion of molecules, thereby improving the bioavailability, pharmacokinetics and/or pharmacodynamic characteristics of co-formulated or co-administered molecules, or
20 molecules administered after administration of the hyaluronidase. In some examples, the bioavailability of the molecules with hyaluronidase is 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of the bioavailability of the molecule without hyaluronidase administration. Typically, the bioavailability is greater than 90%. In addition, as discussed above, hyaluronan degrading enzymes,
25 including modified hyaluronan degrading enzymes, can treat tumors by virtue of other mechanisms.

Thus, in one example, treatment of the hyaluronan-associated condition, disease or disorder includes administration of a composition containing a modified hyaluronan degrading enzyme, such a modified soluble hyaluronidase, and one or
30 more additional agents and/or treatment for treating the disease or disorder, for example, an anti-cancer agent, such as a chemotherapy, antibody, vector or nucleic

acid for treating cancer. In this example, the second treatment or agent can be administered separately or together with the hyaluronidase. For example, the modified hyaluronan degrading enzymes are administered before, after or with an additional agent or treatment. Hence, hyaluronan degrading enzymes, particularly
5 modified hyaluronan degrading enzymes, such as pegylated soluble hyaluronidases, can be administered as therapeutic agents alone or in combination with other therapeutic agents. Hyaluronan degrading enzyme, such as hyaluronidase, administration can facilitate therapeutic agent delivery, for example, via intravenous, subcutaneous and/or intra-tumoral delivery, particularly for delivery of treatments or
10 agents to tissues having a high expression of extracellular hyaluronan, for example, tissues that exhibit HALO (pericellular matrix regions that are rich in proteoglycans, including hyaluronan) formation. By virtue of the ability of the hyaluronan degrading enzyme, such as a hyaluronidase, to break down hyaluronan in the extracellular matrix, hyaluronan degrading enzymes facilitate administration of therapeutic agents
15 to the desired location.

Typically, the second agent and the modified hyaluronan degrading enzyme, for example, a modified hyaluronidase enzyme, are administered separately. For example, the additional agent or treatment can be administered simultaneously, sequentially or intermittently in any order. Typically, the modified hyaluronan
20 degrading enzyme, such as a hyaluronidase, is administered prior to the additional agent and/or treatment, for example, at least 0.5, 1, 5, 15, or 30 minutes, or 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 18, 20, 22, 24, 36, 48, 72 or more hours prior to the additional agent or treatment. In some examples, due to the long-half life of a modified enzyme and its duration of action, a modified enzyme can be administered at
25 least 24 or about 24 hours, at least 48 or about 48 hours, or at least 72 or about 72 hours or more, prior to the administration of the additional agent or treatment.

The frequency of administration of the second agent can be empirically determined. The determination of frequency of administration is within the level of a skilled physician and is dependent on a number of factors including the particular
30 disease or condition being treated, the severity of disease, the patient to be treated, and the cycle of administration of the modified enzyme. Generally, the timing of

administration of the second agent, for example an anti-cancer agent or treatment (e.g. chemotherapeutic), is typically a function of the cycle of administration of the modified enzyme. For example, the second agent can be administered after the first administration of the modified enzyme in a cycle of administration, and/or after any one or more subsequent administrations in the cycle. In other examples, the second agent is administered after each subsequent administration of the modified enzyme in the cycle, after every other subsequent administration of the modified enzyme in the cycle, or is administered once a week, once every two weeks, once every three weeks, or once a month during the cycle of administration of the modified enzyme.

10 In some examples, the second agent is only administered once per cycle of administration of the modified enzyme. In additional example, the second agent is administered intermittently between cycles of administration. For example, the second agent is not administered during the first cycle of administration, but is administered during a second cycle, followed by skipping the third cycle and

15 administered again during a fourth cycle, etc... or any variation thereof.

The following sections describe exemplary compositions and compounds containing modified hyaluronan degrading enzymes, such as modified soluble hyaluronidases and/or other agents, methods of making them, and using them to treat hyaluronan-associated diseases, disorders and conditions.

20 **C. ~~COMPOSITIONS CONTAINING HYALURONAN DEGRADING ENZYMES~~**

Provided herein are compositions containing modified hyaluronan degrading enzymes, in particular soluble hyaluronidases, and methods of using such compositions for administration for the treatment of hyaluronan-associated diseases and conditions. The hyaluronan degrading enzymes contained in the compositions provided herein are modified by conjugation to a polymer (e.g. PEG). Any such modified hyaluronan degrading enzyme can be used herein provided the enzyme exhibits enzymatic activity for hyaluronic acid (e.g. hyaluronidase activity). In some instances, the modified hyaluronan degrading enzymes used in the methods,

25 compositions and combinations herein exhibit increased hyaluronidase activity compared to the unmodified hyaluronan degrading enzyme (e.g. not conjugated to a polymer). Generally, as discussed elsewhere herein, a modified hyaluronan-

30

degrading enzyme exhibits increased half-life compared to an unmodified hyaluronan-degrading enzyme.

Hyaluronan, also called hyaluronic acid or hyaluronate, is a non-sulfated glycosaminoglycan that is widely distributed throughout connective, epithelial, and neural tissues. Hyaluronan is an essential component of the extracellular matrix and a major constituent of the interstitial barrier. By catalyzing the hydrolysis of hyaluronan, hyaluronan degrading enzymes lower the viscosity of hyaluronan, thereby increasing tissue permeability and increasing the absorption rate of fluids administered parenterally. As such, hyaluronan degrading enzymes, such as hyaluronidases, have been used, for example, as spreading or dispersing agents in conjunction with other agents, drugs and proteins to enhance their dispersion and delivery.

Hyaluronan degrading enzymes act to degrade hyaluronan by cleaving hyaluronan polymers, which are composed of repeating disaccharides units, D-glucuronic acid (GlcA) and N-acetyl-D-glucosamine (GlcNAc), linked together via alternating β -1 $\rightarrow$ 4 and β -1 $\rightarrow$ 3 glycosidic bonds. Hyaluronan chains can reach about 25,000 disaccharide repeats or more in length and polymers of hyaluronan can range in size from about 5,000 to 20,000,000 Da *in vivo*. Accordingly, hyaluronan degrading enzymes for the uses and methods provided include any enzyme having the ability to catalyze the cleavage of a hyaluronan disaccharide chain or polymer. In some examples the hyaluronan degrading enzyme cleaves the β -1 $\rightarrow$ 4 glycosidic bond in the hyaluronan chain or polymer. In other examples, the hyaluronan degrading enzyme catalyze the cleavage of the β -1 $\rightarrow$ 3 glycosidic bond in the hyaluronan chain or polymer.

Hence, hyaluronan degrading enzymes, such as hyaluronidases, are a family of enzymes that degrade hyaluronic acid, which is an essential component of the extracellular matrix and a major constituent of the interstitial barrier. By catalyzing the hydrolysis of hyaluronic acid, a major constituent of the interstitial barrier, hyaluronan degrading enzymes lower the viscosity of hyaluronic acid, thereby increasing tissue permeability. As such, hyaluronan degrading enzymes, such as hyaluronidases, have been used, for example, as a spreading or dispersing agent in

conjunction with other agents, drugs and proteins to enhance their dispersion and delivery. Hyaluronan-degrading enzymes also are used as an adjuvant to increase the absorption and dispersion of other injected drugs, for hypodermoclysis (subcutaneous fluid administration), and as an adjunct in subcutaneous urography for improving resorption of radiopaque agents. Hyaluronan-degrading enzymes, for example, hyaluronidase can be used in applications of ophthalmic procedures, for example, peribulbar and sub-Tenon's block in local anesthesia prior to ophthalmic surgery. Hyaluronidase also can be use in other therapeutic and cosmetic uses, for example, by promoting akinesia in cosmetic surgery, such as blepharoplasties and face lifts.

Various forms of hyaluronan degrading enzymes, including hyaluronidases have been prepared and approved for therapeutic use in subjects, including humans. The provided compositions and methods can be used, via these and other therapeutic uses, to treat hyaluronan-associated diseases and conditions. For example, animal-derived hyaluronidase preparations include Vitrase® (ISTA Pharmaceuticals), a purified ovine testicular hyaluronidase, and Amphadase® (Amphastar Pharmaceuticals), a bovine testicular hyaluronidase. Hylenex® (Halozyme Therapeutics) is a human recombinant hyaluronidase produced by genetically engineered Chinese Hamster Ovary (CHO) cells containing nucleic acid encoding for soluble rHuPH20.

~~Exemplary of hyaluronan degrading enzymes in the compositions and~~
methods provided herein are soluble hyaluronidases. Other exemplary hyaluronan degrading enzymes include, but are not limited to particular chondroitinases and lyases that have the ability to cleave hyaluronan.

As described below, hyaluronan-degrading enzymes exist in membrane-bound or soluble form. For purposes herein, soluble hyaluronan-degrading enzymes are provided for use in the methods, uses, compositions or combinations herein. Thus, where hyaluronan-degrading enzymes include a glycosylphosphatidylinositol (GPI) anchor and/or are otherwise membrane-anchored or insoluble, hyaluronan-degrading enzymes are provided herein in soluble form. Thus, hyaluronan-degrading enzymes include truncated variants, e.g. truncated to remove all or a portion of a GPI anchor. Hyaluronan-degrading enzymes provide herein also include allelic or species variants

or other variants, of a soluble hyaluronan-degrading enzyme. For example, hyaluronan degrading enzymes can contain one or more variations in its primary sequence, such as amino acid substitutions, additions and/or deletions. A variant of a hyaluronan-degrading enzyme generally exhibits at least or about 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity compared to the hyaluronan-degrading enzyme not containing the variation. Any variation can be included in the hyaluronan degrading enzyme for the purposes herein provided the enzyme retains hyaluronidase activity, such as at least or about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more of the activity of a hyaluronan degrading enzyme not containing the variation (as measured by *in vitro* and/or *in vivo* assays well known in the art and described herein).

Where the methods and uses provided herein describe the use of a soluble hyaluronidase, accordingly any hyaluronan degrading enzyme, generally a soluble hyaluronan degrading enzyme, can be used.

1. Hyaluronidases

Hyaluronidases are members of a large family of hyaluronan degrading enzymes. There are three general classes of hyaluronidases: mammalian-type hyaluronidases, bacterial hyaluronidases and hyaluronidases from leeches, other parasites and crustaceans. Such enzymes can be used in the compositions, combinations and methods provided.

a. Mammalian-type hyaluronidases

Mammalian-type hyaluronidases (EC 3.2.1.35) are *endo-β-N*-acetyl-hexosaminidases that hydrolyze the β-1→4 glycosidic bond of hyaluronan into various oligosaccharide lengths such as tetrasaccharides and hexasaccharides. These enzymes have both hydrolytic and transglycosidase activities, and can degrade hyaluronan and chondroitin sulfates (CS), generally C4-S and C6-S. Hyaluronidases of this type include, but are not limited to, hyaluronidases from cows (bovine) (SEQ ID NOS:10, 11 and 64 and BH55 (U.S. Pat. Nos. 5,747,027 and 5,827,721)), sheep (ovis aries) (SEQ ID NO: 26, 27, 63 and 64), yellow jacket wasp (SEQ ID NOS:12 and 13), honey bee (SEQ ID NO:14), white-face hornet (SEQ ID NO:15), paper wasp

(SEQ ID NO:16), mouse (SEQ ID NOS:17-19, 31), pig (SEQ ID NOS:20-21), rat (SEQ ID NOS:22-24, 30), rabbit (SEQ ID NO:25), orangutan (SEQ ID NO:28), cynomolgus monkey (SEQ ID NO:29), guinea pig (SEQ ID NO:32), and human hyaluronidases. Exemplary of hyaluronidases in the compositions, combinations and methods provided herein are soluble hyaluronidases.

Mammalian hyaluronidases can be further subdivided into those that are neutral active, predominantly found in testes extracts, and acid active, predominantly found in organs such as the liver. Exemplary neutral active hyaluronidases include PH20, including but not limited to, PH20 derived from different species such as ovine (SEQ ID NO:27), bovine (SEQ ID NO:11) and human (SEQ ID NO:1). Human PH20 (also known as SPAM1 or sperm surface protein PH20), is generally attached to the plasma membrane via a glycosylphosphatidyl inositol (GPI) anchor. It is naturally involved in sperm-egg adhesion and aids penetration by sperm of the layer of cumulus cells by digesting hyaluronic acid.

Besides human PH20 (also termed SPAM1), five hyaluronidase-like genes have been identified in the human genome, HYAL1, HYAL2, HYAL3, HYAL4 and HYALP1. HYALP1 is a pseudogene, and HYAL3 (SEQ ID NO:38) has not been shown to possess enzyme activity toward any known substrates. HYAL4 (precursor polypeptide set forth in SEQ ID NO:39) is a chondroitinase and exhibits little activity towards hyaluronan. HYAL1 (precursor polypeptide set forth in SEQ ID NO:36) is the prototypical acid-active enzyme and PH20 (precursor polypeptide set forth in SEQ ID NO:1) is the prototypical neutral-active enzyme. Acid-active hyaluronidases, such as HYAL1 and HYAL2 (precursor polypeptide set forth in SEQ ID NO:37) generally lack catalytic activity at neutral pH (*i.e.* pH 7). For example, HYAL1 has little catalytic activity *in vitro* over pH 4.5 (Frost *et al.* (1997) *Anal. Biochem.* 251:263-269). HYAL2 is an acid-active enzyme with a very low specific activity *in vitro*. The hyaluronidase-like enzymes also can be characterized by those which are generally attached to the plasma membrane via a glycosylphosphatidyl inositol (GPI) anchor such as human HYAL2 and human PH20 (Danilkovitch-Miagkova, *et al.* (2003) *Proc Natl Acad Sci USA* 100(8):4580-5), and those which are generally soluble such as human HYAL1 (Frost *et al.* (1997) *Biochem Biophys Res Commun.* 236(1):10-5).

PH20

PH20, like other mammalian hyaluronidases, is an endo- β -N-acetyl-hexosaminidase that hydrolyzes the β 1 $\rightarrow$ 4 glycosidic bond of hyaluronic acid into various oligosaccharide lengths such as tetrasaccharides and hexasaccharides. They have both hydrolytic and transglycosidase activities and can degrade hyaluronic acid and chondroitin sulfates, such as C4-S and C6-S. PH20 is naturally involved in sperm-egg adhesion and aids penetration by sperm of the layer of cumulus cells by digesting hyaluronic acid. PH20 is located on the sperm surface, and in the lysosome-derived acrosome, where it is bound to the inner acrosomal membrane. Plasma membrane PH20 has hyaluronidase activity only at neutral pH, while inner acrosomal membrane PH20 has activity at both neutral and acid pH. In addition to being a hyaluronidase, PH20 also appears to be a receptor for HA-induced cell signaling, and a receptor for the zona pellucida surrounding the oocyte.

Exemplary PH20 proteins include, but are not limited to, human (precursor polypeptide set forth in SEQ ID NO:1, mature polypeptide set forth in SEQ ID NO: 2), chimpanzee (SEQ ID NO:101), Rhesus monkey (SEQ ID NO:102) bovine (SEQ ID NOS: 11 and 64), rabbit (SEQ ID NO: 25), ovine PH20 (SEQ ID NOS: 27, 63 and 65), Cynomolgus monkey (SEQ ID NO: 29), guinea pig (SEQ ID NO: 32), rat (SEQ ID NO: 30) and mouse (SEQ ID NO: 31) PH20 polypeptides.

Bovine PH20 is a 553 amino acid precursor polypeptide (SEQ ID NO:11). Alignment of bovine PH20 with the human PH20 shows only weak homology, with multiple gaps existing from amino acid 470 through to the respective carboxy termini due to the absence of a GPI anchor in the bovine polypeptide (see *e.g.*, Frost GI (2007) *Expert Opin. Drug. Deliv.* 4: 427-440). In fact, clear GPI anchors are not predicted in many other PH20 species besides humans. Thus, PH20 polypeptides produced from ovine and bovine naturally exist as soluble forms. Though bovine PH20 exists very loosely attached to the plasma membrane, it is not anchored via a phospholipase sensitive anchor (Lalancette *et al.* (2001) *Biol Reprod.* 65(2):628-36). This unique feature of bovine hyaluronidase has permitted the use of the soluble bovine testes hyaluronidase enzyme as an extract for clinical use (Wydase®, Hyalase®).

The human PH20 mRNA transcript is normally translated to generate a 509 amino acid precursor polypeptide (SEQ ID NO:1) containing a 35 amino acid signal sequence at the N-terminus (amino acid residue positions 1-35) and a 19 amino acid glycosylphosphatidylinositol (GPI) anchor attachment signal sequence at the C-terminus (amino acid residue positions 491-509). The mature PH20 is, therefore, a 474 amino acid polypeptide set forth in SEQ ID NO:2. Following transport of the precursor polypeptide to the ER and removal of the signal peptide, the C-terminal GPI-attachment signal peptide is cleaved to facilitate covalent attachment of a GPI anchor to the newly-formed C-terminal amino acid at the amino acid position corresponding to position 490 of the precursor polypeptide set forth in SEQ ID NO:1. Thus, a 474 amino acid GPI-anchored mature polypeptide with an amino acid sequence set forth in SEQ ID NO:2 is produced.

Human PH20 exhibits hyaluronidase activity at both neutral and acid pH. In one aspect, human PH20 is the prototypical neutral-active hyaluronidase that is generally locked to the plasma membrane via a GPI anchor. In another aspect, PH20 is expressed on the inner acrosomal membrane where it has hyaluronidase activity at both neutral and acid pH. It appears that PH20 contains two catalytic sites at distinct regions of the polypeptide: the Peptide 1 and Peptide 3 regions (Cherr et al., (2001) *Matrix Biology* 20:515-525). Evidence suggests that the Peptide 1 region of PH20, which corresponds to amino acid positions 107-137 of the mature polypeptide set forth in SEQ ID NO:2 and positions 142-172 of the precursor polypeptide set forth in SEQ ID NO:1, is required for enzyme activity at neutral pH. Amino acids at positions 111 and 113 (corresponding to the mature PH20 polypeptide set forth in SEQ ID NO:2) within this region appear to be important for activity, as mutagenesis by amino acid replacement results in PH20 polypeptides with 3% hyaluronidase activity or undetectable hyaluronidase activity, respectively, compared to the wild-type PH20 (Arming et al., (1997) *Eur. J. Biochem.* 247:810-814).

The Peptide 3 region, which corresponds to amino acid positions 242-262 of the mature polypeptide set forth in SEQ ID NO:2, and positions 277-297 of the precursor polypeptide set forth in SEQ ID NO: 1, appears to be important for enzyme activity at acidic pH. Within this region, amino acids at positions 249 and 252 of the

mature PH20 polypeptide appear to be essential for activity, and mutagenesis of either one results in a polypeptide essentially devoid of activity (Arming et al., (1997) Eur. J. Biochem. 247:810-814).

In addition to the catalytic sites, PH20 also contains a hyaluronan-binding site. Experimental evidence suggest that this site is located in the Peptide 2 region, which corresponds to amino acid positions 205-235 of the precursor polypeptide set forth in SEQ ID NO: 1 and positions 170-200 of the mature polypeptide set forth in SEQ ID NO:2. This region is highly conserved among hyaluronidases and is similar to the heparin binding motif. Mutation of the arginine residue at position 176 (corresponding to the mature PH20 polypeptide set forth in SEQ ID NO:2) to a glycine results in a polypeptide with only about 1% of the hyaluronidase activity of the wild type polypeptide (Arming et al., (1997) Eur. J. Biochem. 247:810-814).

There are seven potential N-linked glycosylation sites in human PH20 at N82, N166, N235, N254, N368, N393, N490 of the polypeptide exemplified in SEQ ID NO: 1. Because amino acids 36 to 464 of SEQ ID NO:1 appears to contain the minimally active human PH20 hyaluronidase domain, the N-linked glycosylation site N-490 is not required for proper hyaluronidase activity. There are six disulfide bonds in human PH20. Two disulphide bonds between the cysteine residues C60 and C351 and between C224 and C238 of the polypeptide exemplified in SEQ ID NO: 1 (corresponding to residues C25 and C316, and C189 and C203 of the mature polypeptide set forth in SEQ ID NO:2, respectively). A further four disulphide bonds are formed between between the cysteine residues C376 and C387; between C381 and C435; between C437 and C443; and between C458 and C464 of the polypeptide exemplified in SEQ ID NO: 1 (corresponding to residues C341 and C352; between C346 and C400; between C402 and C408; and between C423 and C429 of the mature polypeptide set forth in SEQ ID NO:2, respectively).

b. Bacterial hyaluronidases

Bacterial hyaluronidases (EC 4.2.2.1 or EC 4.2.99.1) degrade hyaluronan and, to various extents, chondroitin sulfates and dermatan sulfates. Hyaluronan lyases isolated from bacteria differ from hyaluronidases (from other sources, *e.g.*, hyaluronoglucosaminidases, EC 3.2.1.35) by their mode of action. They are endo-β-

N-acetylhexosaminidases that catalyze an elimination reaction, rather than hydrolysis, of the β 1 $\rightarrow$ 4-glycosidic linkage between N-acetyl-beta-D-glucosamine and D-glucuronic acid residues in hyaluronan, yielding 3-(4-deoxy- β -D-gluc-4-enuronosyl)-N-acetyl-D-glucosamine tetra- and hexasaccharides, and disaccharide end products.

- 5 The reaction results in the formation of oligosaccharides with unsaturated hexuronic acid residues at their nonreducing ends.

Exemplary hyaluronidases from bacteria for use in the compositions, combinations and methods provided include, but are not limited to, hyaluronan degrading enzymes in microorganisms, including strains of *Arthrobacter*,

- 10 *Bdellovibrio*, *Clostridium*, *Micrococcus*, *Streptococcus*, *Peptococcus*, *Propionibacterium*, *Bacteroides*, and *Streptomyces*. Particular examples of such enzymes include, but are not limited to *Arthrobacter sp.* (strain FB24) (SEQ ID NO:67), *Bdellovibrio bacteriovorus* (SEQ ID NO:68), *Propionibacterium acnes* (SEQ ID NO:69), *Streptococcus agalactiae* ((SEQ ID NO:70); 18RS21 (SEQ ID NO:71);
15 serotype Ia (SEQ ID NO:72); serotype III (SEQ ID NO:73), *Staphylococcus aureus* (strain COL (SEQ ID NO:74); strain MRSA252 (SEQ ID NOS:75 and 76); strain MSSA476 (SEQ ID NO:77); strain NCTC 8325 (SEQ ID NO:78); strain bovine RF122 (SEQ ID NOS:79 and 80); strain USA300 (SEQ ID NO:81), *Streptococcus pneumoniae* ((SEQ ID NO:82); strain ATCC BAA-255 / R6 (SEQ ID NO:83);
20 serotype 2, strain D39 / NCTC 7466 (SEQ ID NO:84), *Streptococcus pyogenes* (serotype M1) (SEQ ID NO:85); serotype M2, strain MGAS10270 (SEQ ID NO:86); serotype M4, strain MGAS10750 (SEQ ID NO:87); serotype M6 (SEQ ID NO:88); serotype M12, strain MGAS2096 (SEQ ID NOS:89 and 90); serotype M12, strain MGAS9429 (SEQ ID NO:91); serotype M28 (SEQ ID NO:92); *Streptococcus suis*
25 (SEQ ID NOS:93-95); *Vibrio fischeri* (strain ATCC 700601/ ES114 (SEQ ID NO:96)), and the *Streptomyces hyaluronolyticus* hyaluronidase enzyme, which is specific for hyaluronic acid and does not cleave chondroitin or chondroitin sulfate (Ohya, T. and Kaneko, Y. (1970) *Biochim. Biophys. Acta* 198:607).

30 **c. Hyaluronidases from leeches, other parasites and crustaceans**

Hyaluronidases from leeches, other parasites, and crustaceans (EC 3.2.1.36) are endo- β -glucuronidases that generate tetra- and hexasaccharide end-products.

These enzymes catalyze hydrolysis of 1→3-linkages between β-D-glucuronate and N-acetyl-D-glucosamine residues in hyaluronate. Exemplary hyaluronidases from leeches include, but are not limited to, hyaluronidase from Hirudinidae (e.g., *Hirudo medicinalis*), Erpobdellidae (e.g., *Nephelopsis obscura* and *Erpobdella punctata*),
5 Glossiphoniidae (e.g., *Desserobdella picta*, *Helobdella stagnalis*, *Glossiphonia complanata*, *Placobdella ornata* and *Theromyzon* sp.) and Haemopidae (*Haemopis marmorata*) (Hovingh *et al.* (1999) *Comp Biochem Physiol B Biochem Mol Biol.* 124(3):319-26). An exemplary hyaluronidase from bacteria that has the same mechanism of action as the leech hyaluronidase is that from the cyanobacteria,
10 *Synechococcus* sp. (strain RCC307, SEQ ID NO:97).

2. Other hyaluronan degrading enzymes

In addition to the hyaluronidase family, other hyaluronan degrading enzymes can be used in the compositions, combinations and methods provided. For example, enzymes, including particular chondroitinases and lyases, that have the ability to
15 cleave hyaluronan can be employed. Exemplary chondroitinases that can degrade hyaluronan include, but are not limited to, chondroitin ABC lyase (also known as chondroitinase ABC), chondroitin AC lyase (also known as chondroitin sulfate lyase or chondroitin sulfate eliminase) and chondroitin C lyase. Methods for production and purification of such enzymes for use in the compositions, combinations, and
20 methods provided are known in the art (e.g., U.S. Patent No. 6,054,569; Yamagata, *et al.* (1968) *J. Biol. Chem.* 243(7):1523-1535; Yang *et al.* (1985) *J. Biol. Chem.* 160(30):1849-1857).

Chondroitin ABC lyase contains two enzymes, chondroitin-sulfate-ABC endolyase (EC 4.2.2.20) and chondroitin-sulfate-ABC exolyase (EC 4.2.2.21) (Hamai
25 *et al.* (1997) *J Biol Chem.* 272(14):9123-30), which degrade a variety of glycosaminoglycans of the chondroitin-sulfate- and dermatan-sulfate type. Chondroitin sulfate, chondroitin-sulfate proteoglycan and dermatan sulfate are the preferred substrates for chondroitin-sulfate-ABC endolyase, but the enzyme also can act on hyaluronan at a lower rate. Chondroitin-sulfate-ABC endolyase degrades a
30 variety of glycosaminoglycans of the chondroitin-sulfate- and dermatan-sulfate type, producing a mixture of Δ4-unsaturated oligosaccharides of different sizes that are

ultimately degraded to Δ 4-unsaturated tetra- and disaccharides. Chondroitin-sulfate-ABC exolyase has the same substrate specificity but removes disaccharide residues from the non-reducing ends of both polymeric chondroitin sulfates and their oligosaccharide fragments produced by chondroitin-sulfate-ABC endolyase (Hamai, A. *et al.* (1997) *J. Biol. Chem.* 272:9123-9130). A exemplary chondroitin-sulfate-ABC endolyases and chondroitin-sulfate-ABC exolyases include, but are not limited to, those from *Proteus vulgaris* and *Flavobacterium heparinum* (the *Proteus vulgaris* chondroitin-sulfate-ABC endolyase is set forth in SEQ ID NO: 98 (Sato *et al.* (1994) *Appl. Microbiol. Biotechnol.* 41(1):39-46).

10 Chondroitin AC lyase (EC 4.2.2.5) is active on chondroitin sulfates A and C, chondroitin and hyaluronic acid, but is not active on dermatan sulfate (chondroitin sulfate B). Exemplary chondroitinase AC enzymes from the bacteria include, but are not limited to, those from *Flavobacterium heparinum* and *Victivallis vadensis*, set forth in SEQ ID NOS:99 and 100, respectively, and *Arthrobacter aureescens* (Tkalec 15 *et al.* (2000) *Applied and Environmental Microbiology* 66(1):29-35; Ernst *et al.* (1995) *Critical Reviews in Biochemistry and Molecular Biology* 30(5):387-444).

Chondroitinase C cleaves chondroitin sulfate C producing tetrasaccharide plus an unsaturated 6-sulfated disaccharide (Δ Di-6S). It also cleaves hyaluronic acid producing unsaturated non-sulfated disaccharide (Δ Di-OS). Exemplary 20 chondroitinase C enzymes from the bacteria include, but are not limited to, those from *Streptococcus* and *Flavobacterium* (Hibi *et al.* (1989) *FEMS-Microbiol-Lett.* 48(2):121-4; Michelacci *et al.* (1976) *J. Biol. Chem.* 251:1154-8; Tsuda *et al.* (1999) *Eur. J. Biochem.* 262:127-133)

3. Soluble hyaluronan degrading enzymes

25 Provided in the compositions, combinations, uses and methods herein are modified soluble hyaluronan degrading enzymes, including modified soluble hyaluronidases. Soluble hyaluronan degrading enzymes include any hyaluronan degrading enzymes that exist in soluble form, including, but not limited to, soluble hyaluronidases, including non-human soluble hyaluronidases, including non-human 30 animal soluble hyaluronidases, bacterial soluble hyaluronidases and human hyaluronidases, Hyal1, bovine PH20 and ovine PH20, allelic variants thereof and

other variants thereof. For example, included among soluble hyaluronan degrading enzymes are any hyaluronan degrading enzymes that have been modified to be soluble. For example, hyaluronan degrading enzymes that contain a GPI anchor can be made soluble by truncation of and removal of all or a portion of the GPI anchor.

- 5 In one example, the human hyaluronidase PH20, which is normally membrane anchored via a GPI anchor, can be made soluble by truncation of and removal of all or a portion of the GPI anchor at the C-terminus.

Soluble hyaluronan degrading enzymes also include neutral active and acid active hyaluronidases. Depending on factors, such as, but not limited to, the desired
10 level of activity of the enzyme following administration and/or site of administration, neutral active and acid active hyaluronidases can be selected. In a particular example, the hyaluronan degrading enzyme for use in the compositions, combinations and methods herein is a soluble neutral active hyaluronidase.

Exemplary of a soluble hyaluronidase is PH20 from any species, such as any
15 set forth in any of SEQ ID NOS: 1, 2, 11, 25, 27, 30, 31, 63-65 and 101-102, or truncated forms thereof lacking all or a portion of the C-terminal GPI anchor, so long as the hyaluronidase is soluble and retains hyaluronidase activity. Also included among soluble hyaluronidases are allelic variants or other variants of any of SEQ ID NOS:1, 2, 11, 25, 27, 30 31, 63-65 and 101-102, or truncated forms thereof. Allelic
20 variants and other variants are known to one of skill in the art, and include polypeptides having 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or more sequence identity to any of SEQ ID NOS: 1, 2, 11, 25, 27, 30 31, 63-65 and 101-102, or truncated forms thereof. Amino acid variants include conservative and non-conservative mutations. It is understood that residues that are important or
25 otherwise required for the activity of a hyaluronidase, such as any described above or known to skill in the art, are generally invariant and cannot be changed. These include, for example, active site residues. Thus, for example, amino acid residues 111, 113 and 176 (corresponding to residues in the mature PH20 polypeptide set forth in SEQ ID NO:2) of a human PH20 polypeptide, or soluble form thereof, are
30 generally invariant and are not altered. Other residues that confer glycosylation and formation of disulfide bonds required for proper folding also can be invariant.

In some instances, the soluble hyaluronan degrading enzyme is normally GPI-anchored (such as, for example, human PH20) and is rendered soluble by truncation at the C-terminus. Such truncation can remove all of the GPI anchor attachment signal sequence, or can remove only some of the GPI anchor attachment signal sequence.

5 The resulting polypeptide, however, is soluble. In instances where the soluble hyaluronan degrading enzyme retains a portion of the GPI anchor attachment signal sequence, 1, 2, 3, 4, 5, 6, 7 or more amino acid residues in the GPI-anchor attachment signal sequence can be retained, provided the polypeptide is soluble. Polypeptides containing one or more amino acids of the GPI anchor are termed extended soluble
10 hyaluronan degrading enzymes. One of skill in the art can determine whether a polypeptide is GPI-anchored using methods well known in the art. Such methods include, but are not limited to, using known algorithms to predict the presence and location of the GPI-anchor attachment signal sequence and ω -site, and performing solubility analyses before and after digestion with phosphatidylinositol-specific
15 phospholipase C (PI-PLC) or D (PI-PLD).

Extended soluble hyaluronan degrading enzymes can be produced by making C-terminal truncations to any naturally GPI-anchored hyaluronan degrading enzyme such that the resulting polypeptide is soluble and contains one or more amino acid residues from the GPI-anchor attachment signal sequence. Exemplary extended
20 ~~soluble hyaluronan degrading enzymes that are C-terminally truncated but retain a~~ portion of the GPI anchor attachment signal sequence include, but are not limited to, extended soluble PH20 (esPH20) polypeptides of primate origin, such as, for example, human and chimpanzee esPH20 polypeptides. For example, the esPH20 polypeptides can be made by C-terminal truncation of any of the mature or precursor
25 polypeptides set forth in SEQ ID NOS:1, 2 or 101, or allelic or other variation thereof, including active fragment thereof, wherein the resulting polypeptide is soluble and retains one or more amino acid residues from the GPI-anchor attachment signal sequence. Allelic variants and other variants are known to one of skill in the art, and include polypeptides having 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95% or
30 more sequence identity to any of SEQ ID NOS: 1 or 2. The esPH20 polypeptides provided herein can be C-terminally truncated by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more

amino acids compared to the wild type polypeptide, such as a polypeptide with a sequence set forth in SEQ ID NOS: 1, 2 or 101, provided the resulting esPH20 polypeptide is soluble and retains 1 or more amino acid residues from the GPI-anchor attachment signal sequence.

5 Typically, for use in the compositions, combinations and methods herein, a soluble human hyaluronan degrading enzyme, such as a soluble human PH20, is used. Although hyaluronan degrading enzymes, such as PH20, from other animals can be utilized, such preparations are potentially immunogenic, since they are animal proteins. For example, a significant proportion of patients demonstrate prior
10 sensitization secondary to ingested foods, and since these are animal proteins, all patients have a risk of subsequent sensitization. Thus, non-human preparations may not be suitable for chronic use. If non-human preparations are desired, it is contemplated herein that such polypeptides can be prepared to have reduced immunogenicity. Such modifications are within the level of skill in the art and
15 can include, for example, removal and/or replacement of one or more antigenic epitopes on the molecule.

 Hyaluronan degrading enzymes, including hyaluronidases (*e.g.*, PH20), used in the methods herein can be recombinantly produced or can be purified or partially-purified from natural sources, such as, for example, from testes extracts. Methods for
20 production of recombinant proteins, including recombinant hyaluronan degrading enzymes, are provided elsewhere herein and are well known in the art.

a. Soluble Human PH20

 Exemplary of a soluble hyaluronidase is soluble human PH20. Soluble forms of recombinant human PH20 have been produced and can be used in the
25 compositions, combinations and methods described herein. The production of such soluble forms of PH20 is described in U.S. Published Patent Application Nos. US20040268425; US 20050260186 and US20060104968, and in the Examples, below. For example, soluble PH20 polypeptides, include C-terminally truncated variant polypeptides that include a sequence of amino acids in SEQ ID NO:1, or have
30 at least 91%, 92%, 93%, 94%, 95%, 95%, 97%, 98% sequence identity to a sequence of amino acids included in SEQ ID NO:1, retain hyaluronidase activity and are

soluble. Included among these polypeptides are soluble PH20 polypeptides that completely lack all or a portion of the GPI-anchor attachment signal sequence. Also included are extended soluble PH20 (esPH20) polypeptides that contain at least one amino acid of the GPI anchor. Thus, instead of having a GPI-anchor covalently
5 attached to the C-terminus of the protein in the ER and being anchored to the extracellular leaflet of the plasma membrane, these polypeptides are secreted and are soluble. C-terminally truncated PH20 polypeptides can be C-terminally truncated by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 5, 60 or more amino acids compared to the full length wild type polypeptide, such as a
10 full length wild type polypeptide with a sequence set forth in SEQ ID NOS:1 or 2, or allelic or species variants or other variants thereof.

Exemplary C-terminally truncated human PH20 polypeptides provided herein include any having C-terminal truncations to generate polypeptides containing amino acid 1 to amino acid 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477,
15 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, of the sequence of amino acids set forth in SEQ ID NO: 1, or corresponding positions in an allelic or species variant thereof. When expressed in mammalian cells, the 35 amino acid N-terminal signal sequence is cleaved during processing, and the mature form of the protein is secreted. Thus, exemplary mature
20 C-terminally truncated soluble PH20 polypeptides can contain amino acids 36 to 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497 of the sequence of amino acids set forth in SEQ ID NO: 1 or corresponding positions in an allelic or species variant thereof. Table 2 provides non-limiting examples of
25 exemplary C-terminally truncated PH20 polypeptides, including C-terminally truncated soluble PH20 polypeptides. In Table 2 below, the length (in amino acids) of the precursor and mature polypeptides, and the sequence identifier (SEQ ID NO) in which exemplary amino acid sequences of the precursor and mature polypeptides of the C-terminally truncated PH20 proteins are set forth, are provided. The wild-type
30 PH20 polypeptide also is included in Table 2 for comparison.

Table 2. Exemplary C-terminally truncated PH20 polypeptides

Polypeptide	Precursor (amino acids)	Precursor SEQ ID NO	Mature (amino acids)	Mature SEQ ID NO
wildtype	509	1	474	2
SPAM1-FIVS	497	107	462	151
SPAM1-MFIV	496	141	461	185
SPAM1-TMFI	495	108	460	152
SPAM1-ATMF	494	142	459	186
SPAM1-SATM	493	109	458	153
SPAM1-LSAT	492	143	457	187
SPAM1-TLSA	491	110	456	154
SPAM1-PSTL	489	111	454	155
SPAM1-SPST	488	144	453	188
SPAM1-STLS	490	112	455	156
SPAM1-ASPS	487	113	452	157
SPAM1-NASP	486	145	451	189
SPAM1-YNAS	485	114	450	158
SPAM1-FYNA	484	115	449	159
SPAM1-IFYN	483	46	448	48
SPAM1-QIFY	482	3	447	4
SPAM1-PQIF	481	45	446	5
SPAM1-EPQI	480	44	445	6
SPAM1-EEPQ	479	43	444	7
SPAM1-TEEP	478	42	443	8
SPAM1-ETEE	477	41	442	9
SPAM1-METE	476	116	441	160
SPAM1-PMET	475	117	440	161
SPAM1-PPME	474	118	439	162
SPAM1-KPPM	473	119	438	163
SPAM1-LKPP	472	120	437	164
SPAM1-FLKP	471	121	436	165
SPAM1-AFLK	470	122	435	166
SPAM1-DAFL	469	123	434	167
SPAM1-IDAF	468	124	433	168
SPAM1-CIDA	467	40	432	47
SPAM1-VCID	466	125	431	169
SPAM1-GVCI	465	126	430	170

Soluble forms include, but are not limited to, any having C-terminal truncations to generate polypeptides containing amino acids 1 to amino acid 467, 477, 478, 479, 480, 481, 482 and 483 of the sequence of amino acids set forth in SEQ ID NO:1. When expressed in mammalian cells, the 35 amino acid N-terminal signal sequence is cleaved during processing, and the mature form of the protein is secreted.

Thus, the mature soluble polypeptides contain amino acids 36 to 467, 477, 478, 479, 480, 481, 482 and 483 of SEQ ID NO:1. Deletion mutants ending at amino acid position 477 to 483 (corresponding to the precursor polypeptide set forth in SEQ ID NO:1) exhibit higher secreted hyaluronidase activity than the full length GPI-

5 anchored form. Hence, exemplary of soluble hyaluronidases soluble human PH20 polypeptides that are 442, 443, 444, 445, 446 or 447 amino acids in length, such as set forth in any of SEQ ID NOS: 4-9, or allelic or species variants or other variants thereof.

Generally soluble forms of PH20 are produced using protein expression
10 systems that facilitate correct N-glycosylation to ensure the polypeptide retains activity, since glycosylation is important for the catalytic activity and stability of hyaluronidases. Such cells include, for example Chinese Hamster Ovary (CHO) cells (e.g. DG44 CHO cells).

b. rHuPH20

15 Recombinant soluble forms of human PH20 have been generated and can be used in the compositions, combinations and methods provided herein. The generation of such soluble forms of recombinant human PH20 are described in U.S. Published Patent Application Nos. US20040268425; US 20050260186 and US20060104968, and in Examples 2-6, below. Exemplary of such polypeptides are those generated
20 from a nucleic acid molecule encoding amino acids 1-482 (set forth in SEQ ID NO:3).

Such an exemplary nucleic acid molecule is set forth in SEQ ID NO:49. Post translational processing removes the 35 amino acid signal sequence, leaving a 447 amino acid soluble recombinant human PH20 (SEQ ID NO:4). As produced in the culture medium there is heterogeneity at the C-terminus such that the product,
25 designated rHuPH20, includes a mixture of species that can include any one or more of SEQ ID NOS. 4-9 in various abundance. Typically, rHuPH20 is produced in cells that facilitate correct N-glycosylation to retain activity, such as CHO cells (e.g. DG44 CHO cells).

4. Glycosylation of hyaluronan degrading enzymes

30 Glycosylation, including N- and O-linked glycosylation, of some hyaluronan degrading enzymes, including hyaluronidases, can be important for their catalytic

activity and stability. While altering the type of glycan modifying a glycoprotein can have dramatic affects on a protein's antigenicity, structural folding, solubility, and stability, most enzymes are not thought to require glycosylation for optimal enzyme activity. For some hyaluronidases, removal of N-linked glycosylation can result in
5 near complete inactivation of the hyaluronidase activity. Thus, for such hyaluronidases, the presence of N-linked glycans is critical for generating an active enzyme.

N-linked oligosaccharides fall into several major types (oligomannose, complex, hybrid, sulfated), all of which have (Man) 3-GlcNAc-GlcNAc-cores
10 attached via the amide nitrogen of Asn residues that fall within-Asn-Xaa-Thr/Ser-sequences (where Xaa is not Pro). Glycosylation at an-Asn-Xaa-Cys-site has been reported for coagulation protein C. In some instances, a hyaluronan degrading enzyme, such as a hyaluronidase, can contain both N-glycosidic and O-glycosidic linkages. For example, PH20 has O-linked oligosaccharides as well as N-linked
15 oligosaccharides. There are seven potential N-linked glycosylation sites at N82, N166, N235, N254, N368, N393, N490 of human PH20 exemplified in SEQ ID NO: 1. As noted above , N-linked glycosylation at N490 is not required for hyaluronidase activity.

In some examples, the hyaluronan degrading enzymes for use in the
20 compositions, combinations and/or methods provided are glycosylated at one or all of the glycosylation sites. For example, for human PH20, or a soluble form thereof, 2, 3, 4, 5, or 6 of the N-glycosylation sites corresponding to amino acids N82, N166, N235, N254, N368, and N393 of SEQ ID NO: 1 are glycosylated. In some examples the hyaluronan degrading enzymes are glycosylated at one or more native glycosylation
25 sites. In other examples, the hyaluronan degrading enzymes are modified at one or more non-native glycosylation sites to confer glycosylation of the polypeptide at one or more additional site. In such examples, attachment of additional sugar moieties can enhance the pharmacokinetic properties of the molecule, such as improved half-life and/or improved activity.

30 In other examples, the hyaluronan degrading enzymes for use in the compositions, combinations and/or methods provided herein are partially

deglycosylated (or N-partially glycosylated polypeptides). For example, partially deglycosylated soluble PH20 polypeptides that retain all or a portion of the hyaluronidase activity of a fully glycosylated hyaluronidase can be used in the compositions, combinations and/or methods provided herein. Exemplary partially deglycosylated hyaluronidases include soluble forms of a partially deglycosylated PH20 polypeptides from any species, such as any set forth in any of SEQ ID NOS: 1, 2, 11, 25, 27, 29, 30, 31, 32, 63, 65, 101 and 102, or allelic variants, truncated variants, or other variants thereof. Such variants are known to one of skill in the art, and include polypeptides having 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95% or more sequence identity to any of SEQ ID NOS: 1, 2, 11, 25, 27, 29, 30, 31, 32, 63, 65, 101 and 102, or truncated forms thereof. The partially deglycosylated hyaluronidases provided herein also include hybrid, fusion and chimeric partially deglycosylated hyaluronidases, and partially deglycosylated hyaluronidase conjugates.

Glycosidases, or glycoside hydrolases, are enzymes that catalyze the hydrolysis of the glycosidic linkage to generate two smaller sugars. The major types of N-glycans in vertebrates include high mannose glycans, hybrid glycans and complex glycans. There are several glycosidases that result in only partial protein deglycosylation, including: EndoF1, which cleaves high mannose and hybrid type glycans; EndoF2, which cleaves biantennary complex type glycans; EndoF3, which cleaves biantennary and more branched complex glycans; and EndoH, which cleaves high mannose and hybrid type glycans. Treatment of a hyaluronan degrading enzyme, such as a soluble hyaluronidase, such as a soluble PH20, with one or all of these glycosidases can result in only partial deglycosylation and, therefore, retention of hyaluronidase activity.

Partially deglycosylated hyaluronan degrading enzymes, such as partially deglycosylated soluble hyaluronidases, can be produced by digestion with one or more glycosidases, generally a glycosidase that does not remove all N-glycans but only partially deglycosylates the protein. For example, treatment of PH20 (e.g. a recombinant PH20 designated rHuPH20) with one or all of the above glycosidases (e.g. EndoF1, EndoF2 and/or EndoF3) results in partial deglycosylation. These

partially deglycosylated PH20 polypeptides can exhibit hyaluronidase enzymatic activity that is comparable to the fully glycosylated polypeptides. In contrast, treatment of PH20 with PNGaseF, a glycosidase that cleaves all N-glycans, results in complete removal of all N-glycans and thereby renders PH20 enzymatically inactive.

5 Thus, although all N-linked glycosylation sites (such as, for example, those at amino acids N82, N166, N235, N254, N368, and N393 of human PH20, exemplified in SEQ ID NO: 1) can be glycosylated, treatment with one or more glycosidases can render the extent of glycosylation reduced compared to a hyaluronidase that is not digested with one or more glycosidases.

10 The partially deglycosylated hyaluronan degrading enzymes, including partially deglycosylated soluble PH20 polypeptides, can have 10%, 20%, 30%, 40%, 50%, 60%, 70% or 80% of the level of glycosylation of a fully glycosylated polypeptide. Typically, the partially deglycosylated hyaluronan degrading enzymes, including partially deglycosylated soluble PH20 polypeptides, exhibit hyaluronidase
15 activity that is 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 200%, 300%, 400%, 500%, 1000% or more of the hyaluronidase activity exhibited by the fully glycosylated polypeptide.

5. Modified (Polymer-Conjugated) Hyaluronan Degrading Enzymes

In one example, the provided compositions and combinations contain
20 hyaluronan degrading enzymes, in particular soluble hyaluronidases, that have been modified by conjugation to one or more polymeric molecule (polymer), typically to increase the half-life of the hyaluronan degrading enzyme, for example, to promote prolonged/sustained treatment effects in a subject.

Covalent or other stable attachment (conjugation) of polymeric molecules,
25 such as polyethylene glycol (pegylation moiety (PEG)), to the hyaluronan degrading enzymes, such as hyaluronidases, impart beneficial properties to the resulting hyaluronan degrading enzyme-polymer composition. Such properties include improved biocompatibility, extension of protein (and enzymatic activity) half-life in the blood, cells and/or in other tissues within a subject, effective shielding of the
30 protein from proteases and hydrolysis, improved biodistribution, enhanced pharmacokinetics and/or pharmacodynamics, and increased water solubility.

Exemplary polymers that can be conjugated to the hyaluronan degrading enzyme, such as the hyaluronidase, include natural and synthetic homopolymers, such as polyols (i.e. poly-OH), polyamines (i.e. poly-NH₂) and polycarboxyl acids (i.e. poly-COOH), and further heteropolymers i.e. polymers comprising one or more different coupling groups e.g. a hydroxyl group and amine groups. Examples of suitable polymeric molecules include polymeric molecules selected from among polyalkylene oxides (PAO), such as polyalkylene glycols (PAG), including polypropylene glycols (PEG), methoxypolyethylene glycols (mPEG) and polypropylene glycols, PEG-glycidyl ethers (Epox-PEG), PEG-oxycarbonylimidazole (CDI-PEG) branched polyethylene glycols (PEGs), polyvinyl alcohol (PVA), polycarboxylates, polyvinylpyrrolidone, poly-D,L-amino acids, polyethylene-co-maleic acid anhydride, polystyrene-co-maleic acid anhydride, dextrans including carboxymethyl-dextrans, heparin, homologous albumin, celluloses, including methylcellulose, carboxymethylcellulose, ethylcellulose, hydroxyethylcellulose carboxyethylcellulose and hydroxypropylcellulose, hydrolysates of chitosan, starches such as hydroxyethyl-starches and hydroxypropyl-starches, glycogen, agaroses and derivatives thereof, guar gum, pullulan, inulin, xanthan gum, carrageenan, pectin, alginic acid hydrolysates and bio-polymers.

Typically, the polymers are polyalkylene oxides (PAO), such as polyethylene oxides, such as PEG, typically mPEG, which, in comparison to polysaccharides such as dextran and pullulan, have few reactive groups capable of cross-linking. Typically, the polymers are non-toxic polymeric molecules such as (m)polyethylene glycol (mPEG) which can be covalently conjugated to the hyaluronan degrading enzyme, such as the hyaluronidase (e.g. to attachment groups on the protein's surface) using a relatively simple chemistry.

Pegylation of therapeutics has been reported to increase resistance to proteolysis, increase plasma half-life, and decrease antigenicity and immunogenicity. Examples of pegylation methodologies are known in the art (see for example, Lu and Felix, *Int. J. Peptide Protein Res.*, 43: 127-138, 1994; Lu and Felix, *Peptide Res.*, 6: 142-6, 1993; Felix *et al.*, *Int. J. Peptide Res.*, 46 : 253-64, 1995; Benhar *et al.*, *J. Biol. Chem.*, 269: 13398-404, 1994; Brumeanu *et al.*, *J Immunol.*, 154: 3088-95, 1995; see

also, Caliceti *et al.* (2003) *Adv. Drug Deliv. Rev.* 55(10):1261-77 and Molineux (2003) *Pharmacotherapy* 23 (8 Pt 2):3S-8S). Pegylation also can be used in the delivery of nucleic acid molecules *in vivo*. For example, pegylation of adenovirus can increase stability and gene transfer (see, e.g., Cheng *et al.* (2003) *Pharm. Res.* 20(9): 1444-51).

Suitable polymeric molecules for attachment to the hyaluronan degrading enzymes, including hyaluronidases, include, but are not limited to, polyethylene glycol (PEG) and PEG derivatives such as methoxy-polyethylene glycols (mPEG), PEG-glycidyl ethers (Epox-PEG), PEG-oxycarbonylimidazole (CDI-PEG), branched PEGs, and polyethylene oxide (PEO) (see *e.g.* Roberts *et al.*, *Advanced Drug Delivery Review* 2002, 54: 459-476; Harris and Zalipsky, S (eds.) "Poly(ethylene glycol), Chemistry and Biological Applications" ACS Symposium Series 680, 1997; Mehvar *et al.*, *J. Pharm. Pharmaceut. Sci.*, 3(1):125-136, 2000; Harris, *Nature Reviews* 2:215 et seq. (2003); and Tsubery, *J Biol. Chem* 279(37):38118-24, 2004).

The polymeric molecule can be of a molecular weight typically ranging from about 3 kDa to about 60 kDa. In some embodiments the polymeric molecule that is conjugated to a protein, such as rHuPH20, has a molecular weight of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 or more than 60 kDa.

a. PEGylated Soluble hyaluronan degrading enzymes

The hyaluronan degrading enzyme used in the methods, compositions and combinations herein can be a PEGylated hyaluronan degrading enzyme, such as a PEGylated soluble hyaluronan degrading enzyme. In one example, it is a PEGylated soluble hyaluronidase, e.g. PEGylated rHuPH20. Various methods of modifying polypeptides by covalently attaching (conjugating) a PEG or PEG derivative (i.e. "PEGylation") are known in the art (see e.g., U.S. 2006/0104968; U.S. 5,672,662; U.S. 6,737,505; and U.S. 2004/0235734). Techniques for PEGylation include, but are not limited to, specialized linkers and coupling chemistries (see *e.g.*, Harris, *Adv. Drug Deliv. Rev.* 54:459-476, 2002), attachment of multiple PEG moieties to a single conjugation site (such as via use of branched PEGs; see *e.g.*, Veronese *et al.*, *Bioorg. Med. Chem. Lett.* 12:177-180, 2002), site-specific PEGylation and/or mono-PEGylation (see *e.g.*, Chapman *et al.*, *Nature Biotech.* 17:780-783, 1999), and site-

directed enzymatic PEGylation (see *e.g.*, Sato, *Adv. Drug Deliv. Rev.*, 54:487-504, 2002). Methods and techniques described in the art can produce proteins having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more than 10 PEG or PEG derivatives attached to a single protein molecule (see *e.g.*, U.S. 2006/0104968).

5 Numerous reagents for PEGylation have been described in the art. Such reagents include, but are not limited to, N-hydroxysuccinimidyl (NHS) activated PEG, succinimidyl mPEG, mPEG₂-N-hydroxysuccinimide, mPEG succinimidyl alpha-methylbutanoate, mPEG succinimidyl propionate, mPEG succinimidyl butanoate, mPEG carboxymethyl 3-hydroxybutanoic acid succinimidyl ester, 10 homobifunctional PEG-succinimidyl propionate, homobifunctional PEG propionaldehyde, homobifunctional PEG butyraldehyde, PEG maleimide, PEG hydrazide, p-nitrophenyl-carbonate PEG, mPEG-benzotriazole carbonate, propionaldehyde PEG, mPEG butyraldehyde, branched mPEG₂ butyraldehyde, mPEG acetyl, mPEG piperidone, mPEG methylketone, mPEG "linkerless" maleimide, 15 mPEG vinyl sulfone, mPEG thiol, mPEG orthopyridylthioester, mPEG orthopyridyl disulfide, Fmoc-PEG-NHS, Boc-PEG-NHS, vinylsulfone PEG-NHS, acrylate PEG-NHS, fluorescein PEG-NHS, and biotin PEG-NHS (see *e.g.*, Monfardini *et al.*, *Bioconjugate Chem.* 6:62-69, 1995; Veronese *et al.*, *J. Bioactive Compatible Polymers* 12:197-207, 1997; U.S. 5,672,662; U.S. 5,932,462; U.S. 6,495,659; U.S. 20 6,737,505; U.S. 4,002,531; U.S. 4,179,337; U.S. 5,122,614; U.S. 5,183,550; U.S. 5,324, 844; U.S. 5,446,090; U.S. 5,612,460; U.S. 5,643,575; U.S. 5,766,581; U.S. 5,795, 569; U.S. 5,808,096; U.S. 5,900,461; U.S. 5,919,455; U.S. 5,985,263; U.S. 5,990, 237; U.S. 6,113,906; U.S. 6,214,966; U.S. 6,258,351; U.S. 6,340,742; U.S. 6,413,507; U.S. 6,420,339; U.S. 6,437,025; U.S. 6,448,369; U.S. 6,461,802; U.S. 25 6,828,401; U.S. 6,858,736; U.S. 2001/0021763; U.S. 2001/0044526; U.S. 2001/0046481; U.S. 2002/0052430; U.S. 2002/0072573; U.S. 2002/0156047; U.S. 2003/0114647; U.S. 2003/0143596; U.S. 2003/0158333; U.S. 2003/0220447; U.S. 2004/0013637; US 2004/0235734; U.S. 2005/000360; U.S. 2005/0114037; U.S. 2005/0171328; U.S. 2005/0209416; EP 01064951; EP 0822199; WO 00176640; WO 30 0002017; WO 0249673; WO 9428024; and WO 0187925).

**D. METHODS OF PRODUCING NUCLEIC ACIDS ENCODING A
HYALURONAN DEGRADING ENZYME AND POLYPEPTIDES THEREOF**

Polypeptides of a hyaluronan degrading enzyme, such as a soluble hyaluronidase, set forth herein, can be obtained by methods well known in the art for protein purification and recombinant protein expression. Any method known to those of skill in the art for identification of nucleic acids that encode desired genes can be used. Any method available in the art can be used to obtain a full length (*i.e.*, encompassing the entire coding region) cDNA or genomic DNA clone encoding a hyaluronidase, such as from a cell or tissue source. Modified or variant soluble hyaluronidases, can be engineered from a wildtype polypeptide, such as by site-directed mutagenesis.

Polypeptides can be cloned or isolated using any available methods known in the art for cloning and isolating nucleic acid molecules. Such methods include PCR amplification of nucleic acids and screening of libraries, including nucleic acid hybridization screening, antibody-based screening and activity-based screening.

Methods for amplification of nucleic acids can be used to isolate nucleic acid molecules encoding a desired polypeptide, including for example, polymerase chain reaction (PCR) methods. A nucleic acid containing material can be used as a starting material from which a desired polypeptide-encoding nucleic acid molecule can be isolated. For example, DNA and mRNA preparations, cell extracts, tissue extracts, fluid samples (*e.g.* blood, serum, saliva), samples from healthy and/or diseased subjects can be used in amplification methods. Nucleic acid libraries also can be used as a source of starting material. Primers can be designed to amplify a desired polypeptide. For example, primers can be designed based on expressed sequences from which a desired polypeptide is generated. Primers can be designed based on back-translation of a polypeptide amino acid sequence. Nucleic acid molecules generated by amplification can be sequenced and confirmed to encode a desired polypeptide.

Additional nucleotide sequences can be joined to a polypeptide-encoding nucleic acid molecule, including linker sequences containing restriction endonuclease sites for the purpose of cloning the synthetic gene into a vector, for example, a protein expression vector or a vector designed for the amplification of the core protein coding DNA sequences. Furthermore, additional nucleotide sequences specifying functional

DNA elements can be operatively linked to a polypeptide-encoding nucleic acid molecule. Examples of such sequences include, but are not limited to, promoter sequences designed to facilitate intracellular protein expression, and secretion sequences, for example heterologous signal sequences, designed to facilitate protein secretion. Such sequences are known to those of skill in the art. Additional nucleotide residues sequences such as sequences of bases specifying protein binding regions also can be linked to enzyme-encoding nucleic acid molecules. Such regions include, but are not limited to, sequences of residues that facilitate or encode proteins that facilitate uptake of an enzyme into specific target cells, or otherwise alter pharmacokinetics of a product of a synthetic gene. For example, enzymes can be linked to PEG moieties.

In addition, tags or other moieties can be added, for example, to aid in detection or affinity purification of the polypeptide. For example, additional nucleotide residues sequences such as sequences of bases specifying an epitope tag or other detectable marker also can be linked to enzyme-encoding nucleic acid molecules. Exemplary of such sequences include nucleic acid sequences encoding a His tag (e.g., 6xHis, HHHHHH; SEQ ID NO:54) or Flag Tag (DYKDDDDK; SEQ ID NO:55).

The identified and isolated nucleic acids can then be inserted into an appropriate cloning vector. A large number of vector-host systems known in the art can be used. Possible vectors include, but are not limited to, plasmids or modified viruses, but the vector system must be compatible with the host cell used. Such vectors include, but are not limited to, bacteriophages such as lambda derivatives, or plasmids such as pCMV4, pBR322 or pUC plasmid derivatives or the Bluescript vector (Stratagene, La Jolla, CA). Other expression vectors include the HZ24 expression vector exemplified herein. The insertion into a cloning vector can, for example, be accomplished by ligating the DNA fragment into a cloning vector which has complementary cohesive termini. Insertion can be effected using TOPO cloning vectors (INVITROGEN, Carlsbad, CA). If the complementary restriction sites used to fragment the DNA are not present in the cloning vector, the ends of the DNA molecules can be enzymatically modified. Alternatively, any site desired can be

produced by ligating nucleotide sequences (linkers) onto the DNA termini; these ligated linkers can contain specific chemically synthesized oligonucleotides encoding restriction endonuclease recognition sequences. In an alternative method, the cleaved vector and protein gene can be modified by homopolymeric tailing. Recombinant
5 molecules can be introduced into host cells via, for example, transformation, transfection, infection, electroporation and sonoporation, so that many copies of the gene sequence are generated.

In specific embodiments, transformation of host cells with recombinant DNA molecules that incorporate the isolated protein gene, cDNA, or synthesized DNA
10 sequence enables generation of multiple copies of the gene. Thus, the gene can be obtained in large quantities by growing transformants, isolating the recombinant DNA molecules from the transformants and, when necessary, retrieving the inserted gene from the isolated recombinant DNA.

1. Vectors and cells

15 For recombinant expression of one or more of the desired proteins, such as any described herein, the nucleic acid containing all or a portion of the nucleotide sequence encoding the protein can be inserted into an appropriate expression vector, *i.e.*, a vector that contains the necessary elements for the transcription and translation of the inserted protein coding sequence. The necessary transcriptional and
20 translational signals also can be supplied by the native promoter for enzyme genes, and/or their flanking regions.

Also provided are vectors that contain a nucleic acid encoding the enzyme. Cells containing the vectors also are provided. The cells include eukaryotic and prokaryotic cells, and the vectors are any suitable for use therein.

25 Prokaryotic and eukaryotic cells, including endothelial cells, containing the vectors are provided. Such cells include bacterial cells, yeast cells, fungal cells, Archea, plant cells, insect cells and animal cells. The cells are used to produce a protein thereof by growing the above-described cells under conditions whereby the encoded protein is expressed by the cell, and recovering the expressed protein. For
30 purposes herein, for example, the enzyme can be secreted into the medium.

Provided are vectors that contain a sequence of nucleotides that encodes the hyaluronan degrading enzyme polypeptide, in some examples a soluble hyaluronidase polypeptide, coupled to the native or heterologous signal sequence, as well as multiple copies thereof. The vectors can be selected for expression of the enzyme protein in the cell or such that the enzyme protein is expressed as a secreted protein.

A variety of host-vector systems can be used to express the protein coding sequence. These include but are not limited to mammalian cell systems infected with virus (e.g. vaccinia virus, adenovirus and other viruses); insect cell systems infected with virus (e.g. baculovirus); microorganisms such as yeast containing yeast vectors; or bacteria transformed with bacteriophage, DNA, plasmid DNA, or cosmid DNA. The expression elements of vectors vary in their strengths and specificities. Depending on the host-vector system used, any one of a number of suitable transcription and translation elements can be used.

Any methods known to those of skill in the art for the insertion of DNA fragments into a vector can be used to construct expression vectors containing a chimeric gene containing appropriate transcriptional/translational control signals and protein coding sequences. These methods can include *in vitro* recombinant DNA and synthetic techniques and *in vivo* recombinants (genetic recombination). Expression of nucleic acid sequences encoding protein, or domains, derivatives, fragments or homologs thereof, can be regulated by a second nucleic acid sequence so that the genes or fragments thereof are expressed in a host transformed with the recombinant DNA molecule(s). For example, expression of the proteins can be controlled by any promoter/enhancer known in the art. In a specific embodiment, the promoter is not native to the genes for a desired protein. Promoters which can be used include but are not limited to the SV40 early promoter (Bernoist and Chambon, *Nature* 290:304-310 (1981)), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto *et al.* *Cell* 22:787-797 (1980)), the herpes thymidine kinase promoter (Wagner *et al.*, *Proc. Natl. Acad. Sci. USA* 78:1441-1445 (1981)), the regulatory sequences of the metallothionein gene (Brinster *et al.*, *Nature* 296:39-42 (1982)); prokaryotic expression vectors such as the β -lactamase promoter (Jay *et al.*, (1981) *Proc. Natl. Acad. Sci. USA* 78:5543) or the *tac* promoter (DeBoer *et al.*, *Proc. Natl.*

Acad. Sci. USA 80:21-25 (1983)); see also "Useful Proteins from Recombinant Bacteria": in *Scientific American* 242:79-94 (1980)); plant expression vectors containing the nopaline synthetase promoter (Herrera-Estrella *et al.*, *Nature* 303:209-213 (1984)) or the cauliflower mosaic virus 35S RNA promoter (Gardner *et al.*,
5 *Nucleic Acids Res.* 9:2871 (1981)), and the promoter of the photosynthetic enzyme ribulose biphosphate carboxylase (Herrera-Estrella *et al.*, *Nature* 310:115-120 (1984)); promoter elements from yeast and other fungi such as the Gal4 promoter, the alcohol dehydrogenase promoter, the phosphoglycerol kinase promoter, the alkaline phosphatase promoter, and the following animal transcriptional control regions that
10 exhibit tissue specificity and have been used in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift *et al.*, *Cell* 38:639-646 (1984); Ornitz *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* 50:399-409 (1986); MacDonald, *Hepatology* 7:425-515 (1987)); insulin gene control region which is active in pancreatic beta cells (Hanahan *et al.*, *Nature* 315:115-122 (1985)),
15 immunoglobulin gene control region which is active in lymphoid cells (Grosschedl *et al.*, *Cell* 38:647-658 (1984); Adams *et al.*, *Nature* 318:533-538 (1985); Alexander *et al.*, *Mol. Cell Biol.* 7:1436-1444 (1987)), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder *et al.*, *Cell* 45:485-495 (1986)), albumin gene control region which is active in liver (Pinckert *et al.*, *Genes and Devel.* 1:268-276 (1987)), alpha-fetoprotein gene control region which
20 is active in liver (Krumlauf *et al.*, *Mol. Cell. Biol.* 5:1639-1648 (1985); Hammer *et al.*, *Science* 235:53-58 (1987)), alpha-1 antitrypsin gene control region which is active in liver (Kelsey *et al.*, *Genes and Devel.* 1:161-171 (1987)), beta globin gene control region which is active in myeloid cells (Magram *et al.*, *Nature* 315:338-340 (1985);
25 Kollias *et al.*, *Cell* 46:89-94 (1986)), myelin basic protein gene control region which is active in oligodendrocyte cells of the brain (Readhead *et al.*, *Cell* 48:703-712 (1987)), myosin light chain-2 gene control region which is active in skeletal muscle (Shani, *Nature* 314:283-286 (1985)), and gonadotrophic releasing hormone gene control region which is active in gonadotrophs of the hypothalamus (Mason *et al.*,
30 *Science* 234:1372-1378 (1986)).

In a specific embodiment, a vector is used that contains a promoter operably linked to nucleic acids encoding a desired protein, or a domain, fragment, derivative or homolog, thereof, one or more origins of replication, and optionally, one or more selectable markers (*e.g.*, an antibiotic resistance gene). Exemplary plasmid vectors for transformation of *E. coli* cells, include, for example, the pQE expression vectors (available from Qiagen, Valencia, CA; see also literature published by Qiagen describing the system). pQE vectors have a phage T5 promoter (recognized by *E. coli* RNA polymerase) and a double lac operator repression module to provide tightly regulated, high-level expression of recombinant proteins in *E. coli*, a synthetic ribosomal binding site (RBS II) for efficient translation, a 6XHis tag coding sequence, t_0 and T1 transcriptional terminators, ColE1 origin of replication, and a beta-lactamase gene for conferring ampicillin resistance. The pQE vectors enable placement of a 6xHis tag at either the N- or C-terminus of the recombinant protein. Such plasmids include pQE 32, pQE 30, and pQE 31 which provide multiple cloning sites for all three reading frames and provide for the expression of N-terminally 6xHis-tagged proteins. Other exemplary plasmid vectors for transformation of *E. coli* cells, include, for example, the pET expression vectors (see, U.S. patent 4,952,496; available from NOVAGEN, Madison, WI; see, also literature published by Novagen describing the system). Such plasmids include pET 11a, which contains the T7lac promoter, T7 terminator, the inducible *E. coli* lac operator, and the lac repressor gene; pET 12a-c, which contains the T7 promoter, T7 terminator, and the *E. coli* ompT secretion signal; and pET 15b and pET19b (NOVAGEN, Madison, WI), which contain a His-TagTM leader sequence for use in purification with a His column and a thrombin cleavage site that permits cleavage following purification over the column, the T7-lac promoter region and the T7 terminator.

Exemplary of a vector for mammalian cell expression is the HZ24 expression vector. The HZ24 expression vector was derived from the pCI vector backbone (Promega). It contains DNA encoding the Beta-lactamase resistance gene (AmpR), an F1 origin of replication, a Cytomegalovirus immediate-early enhancer/promoter region (CMV), and an SV40 late polyadenylation signal (SV40). The expression

vector also has an internal ribosome entry site (IRES) from the ECMV virus (Clontech) and the mouse dihydrofolate reductase (DHFR) gene.

2. Expression

Hyaluronan degrading enzyme polypeptides, including soluble hyaluronidase polypeptides, can be produced by any method known to those of skill in the art including *in vivo* and *in vitro* methods. Desired proteins can be expressed in any organism suitable to produce the required amounts and forms of the proteins, such as for example, needed for administration and treatment. Expression hosts include prokaryotic and eukaryotic organisms such as *E.coli*, yeast, plants, insect cells, mammalian cells, including human cell lines and transgenic animals. Expression hosts can differ in their protein production levels as well as the types of post-translational modifications that are present on the expressed proteins. The choice of expression host can be made based on these and other factors, such as regulatory and safety considerations, production costs and the need and methods for purification.

Many expression vectors are available and known to those of skill in the art and can be used for expression of proteins. The choice of expression vector will be influenced by the choice of host expression system. In general, expression vectors can include transcriptional promoters and optionally enhancers, translational signals, and transcriptional and translational termination signals. Expression vectors that are used for stable transformation typically have a selectable marker which allows selection and maintenance of the transformed cells. In some cases, an origin of replication can be used to amplify the copy number of the vector.

Hyaluronan degrading enzyme polypeptides, such as soluble hyaluronidase polypeptides, also can be utilized or expressed as protein fusions. For example, an enzyme fusion can be generated to add additional functionality to an enzyme. Examples of enzyme fusion proteins include, but are not limited to, fusions of a signal sequence, a tag such as for localization, *e.g.* a his₆ tag or a myc tag, or a tag for purification, for example, a GST fusion, and a sequence for directing protein secretion and/or membrane association.

30 a. Prokaryotic Cells

Prokaryotes, especially *E. coli*, provide a system for producing large amounts of proteins. Transformation of *E. coli* is simple and rapid technique well known to those of skill in the art. Expression vectors for *E. coli* can contain inducible promoters, such promoters are useful for inducing high levels of protein expression and for expressing proteins that exhibit some toxicity to the host cells. Examples of inducible promoters include the lac promoter, the trp promoter, the hybrid tac promoter, the T7 and SP6 RNA promoters and the temperature regulated λ PL promoter.

Proteins, such as any provided herein, can be expressed in the cytoplasmic environment of *E. coli*. The cytoplasm is a reducing environment and for some molecules, this can result in the formation of insoluble inclusion bodies. Reducing agents such as dithiothreitol and β -mercaptoethanol and denaturants, such as guanidine-HCl and urea can be used to resolubilize the proteins. An alternative approach is the expression of proteins in the periplasmic space of bacteria which provides an oxidizing environment and chaperonin-like and disulfide isomerases and can lead to the production of soluble protein. Typically, a leader sequence is fused to the protein to be expressed which directs the protein to the periplasm. The leader is then removed by signal peptidases inside the periplasm. Examples of periplasmic-targeting leader sequences include the pelB leader from the pectate lyase gene and the leader derived from the alkaline phosphatase gene. In some cases, periplasmic expression allows leakage of the expressed protein into the culture medium. The secretion of proteins allows quick and simple purification from the culture supernatant. Proteins that are not secreted can be obtained from the periplasm by osmotic lysis. Similar to cytoplasmic expression, in some cases proteins can become insoluble and denaturants and reducing agents can be used to facilitate solubilization and refolding. Temperature of induction and growth also can influence expression levels and solubility, typically temperatures between 25°C and 37°C are used. Typically, bacteria produce aglycosylated proteins. Thus, if proteins require glycosylation for function, glycosylation can be added *in vitro* after purification from host cells.

b. Yeast Cells

c. Insect Cells

Insect cells, particularly using baculovirus expression, are useful for expressing polypeptides such as hyaluronidase polypeptides. Insect cells express high levels of protein and are capable of most of the post-translational modifications used by higher eukaryotes. Baculovirus have a restrictive host range which improves the safety and reduces regulatory concerns of eukaryotic expression. Typical expression vectors use a promoter for high level expression such as the polyhedrin promoter of baculovirus. Commonly used baculovirus systems include the baculoviruses such as *Autographa californica* nuclear polyhedrosis virus (AcNPV), and the *Bombyx mori* nuclear polyhedrosis virus (BmNPV) and an insect cell line such as Sf9 derived from *Spodoptera frugiperda*, *Pseudaletia unipuncta* (A7S) and *Danaus plexippus* (DpN1). For high-level expression, the nucleotide sequence of the molecule to be expressed is

fused immediately downstream of the polyhedrin initiation codon of the virus.

Mammalian secretion signals are accurately processed in insect cells and can be used to secrete the expressed protein into the culture medium. In addition, the cell lines *Pseudaletia unipuncta* (A7S) and *Danaus plexippus* (DpN1) produce proteins with glycosylation patterns similar to mammalian cell systems.

An alternative expression system in insect cells is the use of stably transformed cells. Cell lines such as the Schneider 2 (S2) and Kc cells (*Drosophila melanogaster*) and C7 cells (*Aedes albopictus*) can be used for expression. The *Drosophila* metallothionein promoter can be used to induce high levels of expression in the presence of heavy metal induction with cadmium or copper. Expression vectors are typically maintained by the use of selectable markers such as neomycin and hygromycin.

d. Mammalian Cells

Mammalian expression systems can be used to express proteins including hyaluronan degrading enzyme polypeptides, such as soluble hyaluronidase polypeptides. Expression constructs can be transferred to mammalian cells by viral infection such as adenovirus or by direct DNA transfer such as liposomes, calcium phosphate, DEAE-dextran and by physical means such as electroporation and microinjection. Expression vectors for mammalian cells typically include an mRNA cap site, a TATA box, a translational initiation sequence (Kozak consensus sequence) and polyadenylation elements. IRES elements also can be added to permit bicistronic expression with another gene, such as a selectable marker. Such vectors often include transcriptional promoter-enhancers for high-level expression, for example the SV40 promoter-enhancer, the human cytomegalovirus (CMV) promoter and the long terminal repeat of Rous sarcoma virus (RSV). These promoter-enhancers are active in many cell types. Tissue and cell-type promoters and enhancer regions also can be used for expression. Exemplary promoter/enhancer regions include, but are not limited to, those from genes such as elastase I, insulin, immunoglobulin, mouse mammary tumor virus, albumin, alpha fetoprotein, alpha 1 antitrypsin, beta globin, myelin basic protein, myosin light chain 2, and gonadotropic releasing hormone gene control. Selectable markers can be used to select for and maintain cells with the

expression construct. Examples of selectable marker genes include, but are not limited to, hygromycin B phosphotransferase, adenosine deaminase, xanthine-guanine phosphoribosyl transferase, aminoglycoside phosphotransferase, dihydrofolate reductase (DHFR) and thymidine kinase. For example, expression can be performed in the presence of methotrexate to select for only those cells expressing the DHFR gene. Fusion with cell surface signaling molecules such as TCR- ζ and Fc ϵ RI- γ can direct expression of the proteins in an active state on the cell surface.

Many cell lines are available for mammalian expression including mouse, rat human, monkey, chicken and hamster cells. Exemplary cell lines include but are not limited to CHO, Balb/3T3, HeLa, MT2, mouse NS0 (nonsecreting) and other myeloma cell lines, hybridoma and heterohybridoma cell lines, lymphocytes, fibroblasts, Sp2/0, COS, NIH3T3, HEK293, 293S, 2B8, and HKB cells. Cell lines also are available adapted to serum-free media which facilitates purification of secreted proteins from the cell culture media. Examples include CHO-S cells (Invitrogen, Carlsbad, CA, cat # 11619-012) and the serum free EBNA-1 cell line (Pham *et al.*, (2003) *Biotechnol. Bioeng.* 84:332-42.). Cell lines also are available that are adapted to grow in special mediums optimized for maximal expression. For example, DG44 CHO cells are adapted to grow in suspension culture in a chemically defined, animal product-free medium.

e. **Plants**

Transgenic plant cells and plants can be used to express proteins such as any described herein. Expression constructs are typically transferred to plants using direct DNA transfer such as microprojectile bombardment and PEG-mediated transfer into protoplasts, and with agrobacterium-mediated transformation. Expression vectors can include promoter and enhancer sequences, transcriptional termination elements and translational control elements. Expression vectors and transformation techniques are usually divided between dicot hosts, such as *Arabidopsis* and tobacco, and monocot hosts, such as corn and rice. Examples of plant promoters used for expression include the cauliflower mosaic virus promoter, the nopaline synthetase promoter, the ribose biphosphate carboxylase promoter and the ubiquitin and UBQ3 promoters. Selectable markers such as hygromycin, phosphomannose isomerase and neomycin

phosphotransferase are often used to facilitate selection and maintenance of transformed cells. Transformed plant cells can be maintained in culture as cells, aggregates (callus tissue) or regenerated into whole plants. Transgenic plant cells also can include algae engineered to produce hyaluronidase polypeptides. Because plants
5 have different glycosylation patterns than mammalian cells, this can influence the choice of protein produced in these hosts.

3. Purification Techniques

Method for purification of polypeptides, including hyaluronan degrading enzyme polypeptides (e.g. soluble hyaluronidase polypeptides) or other proteins, from
10 host cells will depend on the chosen host cells and expression systems. For secreted molecules, proteins are generally purified from the culture media after removing the cells. For intracellular expression, cells can be lysed and the proteins purified from the extract. When transgenic organisms such as transgenic plants and animals are used for expression, tissues or organs can be used as starting material to make a lysed
15 cell extract. Additionally, transgenic animal production can include the production of polypeptides in milk or eggs, which can be collected, and if necessary, the proteins can be extracted and further purified using standard methods in the art.

Proteins, such as soluble hyaluronidase polypeptides, can be purified using standard protein purification techniques known in the art including but not limited to,
20 SDS-PAGE, size fraction and size exclusion chromatography, ammonium sulfate precipitation and ionic exchange chromatography, such as anion exchange. Affinity purification techniques also can be utilized to improve the efficiency and purity of the preparations. For example, antibodies, receptors and other molecules that bind hyaluronidase enzymes can be used in affinity purification. Expression constructs
25 also can be engineered to add an affinity tag to a protein such as a myc epitope, GST fusion or His₆ and affinity purified with myc antibody, glutathione resin and Ni-resin, respectively. Purity can be assessed by any method known in the art including gel electrophoresis and staining and spectrophotometric techniques. Purified rHuPH20 compositions, as provided herein, typically has a specific activity of about 120,000
30 Units/mg, as determined in Example 2.

4. PEGylation of Hyaluronan Degrading Enzyme Polypeptides

Polyethylene glycol (PEG) has been widely used in biomaterials, biotechnology and medicine primarily because PEG is a biocompatible, nontoxic, nonimmunogenic and water-soluble polymer (Zhao and Harris, ACS Symposium Series 680: 458-72, 1997). In the area of drug delivery, PEG derivatives have been widely used in covalent attachment (i. e., "PEGylation") to proteins to reduce immunogenicity, proteolysis and kidney clearance and to enhance solubility (Zalipsky, Adv. Drug Del. Rev. 16:157-82, 1995). Similarly, PEG has been attached to low molecular weight, relatively hydrophobic drugs to enhance solubility, reduce toxicity and alter biodistribution. Typically, PEGylated drugs are injected as solutions.

A closely related application is synthesis of crosslinked degradable PEG networks or formulations for use in drug delivery since much of the same chemistry used in design of degradable, soluble drug carriers can also be used in design of degradable gels (Sawhney et al., Macromolecules 26: 581-87, 1993). It also is known that intermacromolecular complexes can be formed by mixing solutions of two complementary polymers. Such complexes are generally stabilized by electrostatic interactions (polyanion-polycation) and/or hydrogen bonds (polyacid-polybase) between the polymers involved, and/or by hydrophobic interactions between the polymers in an aqueous surrounding (Krupers et al., Eur. Polym J. 32:785-790, 1996). For example, mixing solutions of polyacrylic acid (PAAc) and polyethylene oxide (PEO) under the proper conditions results in the formation of complexes based mostly on hydrogen bonding. Dissociation of these complexes at physiologic conditions has been used for delivery of free drugs (i.e., non-PEGylated). In addition, complexes of complementary polymers have been formed from both homopolymers and copolymers.

Numerous reagents for PEGylation have been described in the art. Such reagents include, but are not limited to, N-hydroxysuccinimidyl (NHS) activated PEG, succinimidyl mPEG, mPEG₂-N-hydroxysuccinimide, mPEG succinimidyl alpha-methylbutanoate, mPEG succinimidyl propionate, mPEG succinimidyl butanoate, mPEG carboxymethyl 3-hydroxybutanoic acid succinimidyl ester, homobifunctional PEG-succinimidyl propionate, homobifunctional PEG

propionaldehyde, homobifunctional PEG butyraldehyde, PEG maleimide, PEG hydrazide, p-nitrophenyl-carbonate PEG, mPEG-benzotriazole carbonate, propionaldehyde PEG, mPEG butyraldehyde, branched mPEG₂ butyraldehyde, mPEG acetyl, mPEG piperidone, mPEG methylketone, mPEG "linkerless" maleimide, 5 mPEG vinyl sulfone, mPEG thiol, mPEG orthopyridylthioester, mPEG orthopyridyl disulfide, Fmoc-PEG-NHS, Boc-PEG-NHS, vinylsulfone PEG-NHS, acrylate PEG-NHS, fluorescein PEG-NHS, and biotin PEG-NHS (see *e.g.*, Monfardini *et al.*, Bioconjugate Chem. 6:62-69, 1995; Veronese *et al.*, J. Bioactive Compatible Polymers 12:197-207, 1997; U.S. 5,672,662; U.S. 5,932,462; U.S. 6,495,659; U.S. 10 6,737,505; U.S. 4,002,531; U.S. 4,179,337; U.S. 5,122,614; U.S. 5,183,550; U.S. 5,324, 844; U.S. 5,446,090; U.S. 5,612,460; U.S. 5,643,575; U.S. 5,766,581; U.S. 5,795, 569; U.S. 5,808,096; U.S. 5,900,461; U.S. 5,919,455; U.S. 5,985,263; U.S. 5,990, 237; U.S. 6,113,906; U.S. 6,214,966; U.S. 6,258,351; U.S. 6,340,742; U.S. 6,413,507; U.S. 6,420,339; U.S. 6,437,025; U.S. 6,448,369; U.S. 6,461,802; U.S. 15 6,828,401; U.S. 6,858,736; U.S. 2001/0021763; U.S. 2001/0044526; U.S. 2001/0046481; U.S. 2002/0052430; U.S. 2002/0072573; U.S. 2002/0156047; U.S. 2003/0114647; U.S. 2003/0143596; U.S. 2003/0158333; U.S. 2003/0220447; U.S. 2004/0013637; US 2004/0235734; U.S. 2005/000360; U.S. 2005/0114037; U.S. 2005/0171328; U.S. 2005/0209416; EP 01064951; EP 0822199; WO 00176640; WO 20 0002017; WO 0249673; WO 9428024; and WO 0187925).

In one example, the polyethylene glycol has a molecular weight ranging from about 3 kD to about 50 kD, and preferably from about 5 kD to about 30 kD. Covalent attachment of the PEG to the drug (known as "PEGylation") may be accomplished by known chemical synthesis techniques. For example, the PEGylation of protein may be 25 accomplished by reacting NHS-activated PEG with the protein under suitable reaction conditions.

While numerous reactions have been described for PEGylation, those that are most generally applicable confer directionality, utilize mild reaction conditions, and do not necessitate extensive downstream processing to remove toxic catalysts or bi- 30 products. For instance, monomethoxy PEG (mPEG) has only one reactive terminal hydroxyl, and thus its use limits some of the heterogeneity of the resulting PEG-

protein product mixture. Activation of the hydroxyl group at the end of the polymer opposite to the terminal methoxy group is generally necessary to accomplish efficient protein PEGylation, with the aim being to make the derivatised PEG more susceptible to nucleophilic attack. The attacking nucleophile is usually the epsilon-amino group of a lysyl residue, but other amines also can react (e.g. the N-terminal alpha-amine or the ring amines of histidine) if local conditions are favorable. A more directed attachment is possible in proteins containing a single lysine or cysteine. The latter residue can be targeted by PEG-maleimide for thiol-specific modification. Alternatively, PEG hydrazide can be reacted with a periodate oxidized hyaluronan degrading enzyme and reduced in the presence of NaCNBH₃. More specifically, PEGylated CMP sugars can be reacted with a hyaluronan degrading enzyme in the presence of appropriate glycosyl-transferases. One technique is the "PEGylation" technique where a number of polymeric molecules are coupled to the polypeptide in question. When using this technique the immune system has difficulties in recognizing the epitopes on the polypeptide's surface responsible for the formation of antibodies, thereby reducing the immune response. For polypeptides introduced directly into the circulatory system of the human body to give a particular physiological effect (i.e. pharmaceuticals) the typical potential immune response is an IgG and/or IgM response, while polypeptides which are inhaled through the respiratory system (i.e. industrial polypeptide) potentially may cause an IgE response (i.e. allergic response). One of the theories explaining the reduced immune response is that the polymeric molecule(s) shield(s) epitope(s) on the surface of the polypeptide responsible for the immune response leading to antibody formation. Another theory or at least a partial factor is that the heavier the conjugate is, the more reduced immune response is obtained.

Typically, to make the PEGylated hyaluronan degrading enzymes provided herein, including the PEGylated hyaluronidases, PEG moieties are conjugated, via covalent attachment, to the polypeptides. Techniques for PEGylation include, but are not limited to, specialized linkers and coupling chemistries (see *e.g.*, Harris, Adv. Drug Deliv. Rev. 54:459-476, 2002), attachment of multiple PEG moieties to a single conjugation site (such as via use of branched PEGs; see *e.g.*, Veronese *et al.*, Bioorg.

Med. Chem. Lett. 12:177-180, 2002), site-specific PEGylation and/or mono-
PEGylation (see *e.g.*, Chapman *et al.*, Nature Biotech. 17:780-783, 1999), and site-
directed enzymatic PEGylation (see *e.g.*, Sato, Adv. Drug Deliv. Rev., 54:487-504,
2002). Methods and techniques described in the art can produce proteins having 1, 2,
5 3, 4, 5, 6, 7, 8, 9, 10 or more than 10 PEG or PEG derivatives attached to a single
protein molecule (see *e.g.*, U.S. 2006/0104968).

As an exemplary illustration of the pegylation of an illustrative method for
making PEGylated hyaluronan degrading enzymes, such as PEGylated
hyaluronidases, PEG aldehydes, succinimides and carbonates have each been applied
10 to conjugate PEG moieties, typically succinimidyl PEGs, to rHuPH20. For example,
rHuPH20 has been conjugated with exemplary succinimidyl monoPEG (mPEG)
reagents including mPEG-Succinimidyl Propionates (mPEG-SPA), mPEG-
Succinimidyl Butanoates (mPEG-SBA), and (for attaching "branched" PEGs)
mPEG2-N-Hydroxysuccinimide. These pegylated succinimidyl esters contain
15 different length carbon backbones between the PEG group and the activated cross-
linker, and either a single or branched PEG group. These differences can be used, for
example, to provide for different reaction kinetics and to potentially restrict sites
available for PEG attachment to rHuPH20 during the conjugation process.

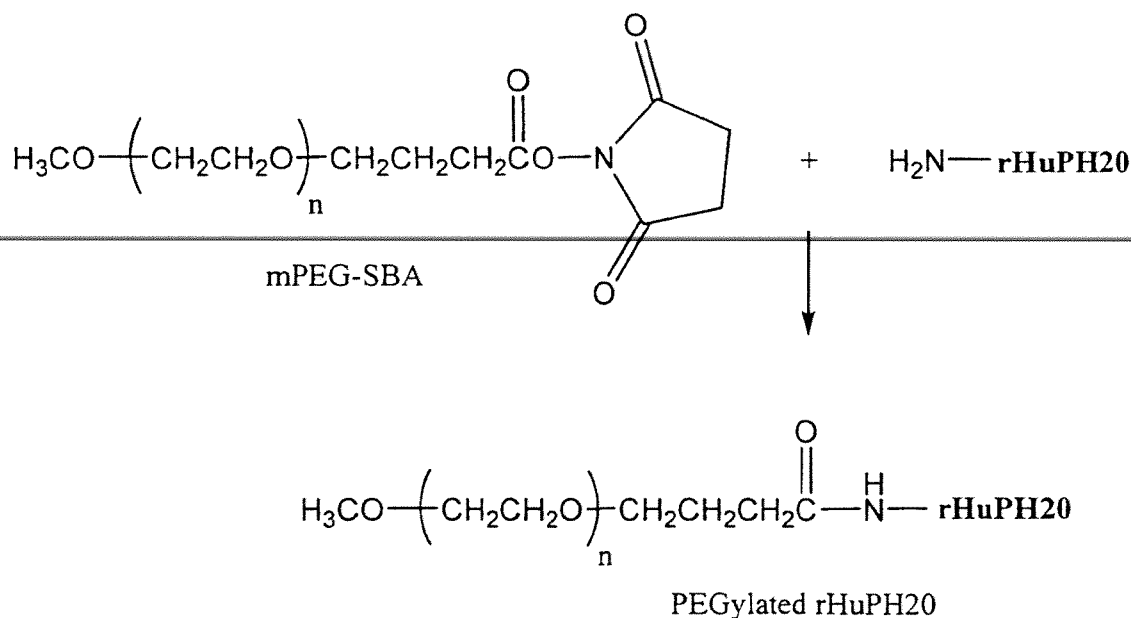
Succinimidyl PEGs (as above) comprising either linear or branched PEGs can
20 be conjugated to rHuPH20. PEGs can be used to generate rHuPH20s reproducibly
comprising a combination of molecules having between about three to six PEG
molecules per hyaluronidase. Such pegylated rHuPH20 compositions can be readily
purified to yield compositions having specific activities of approximately 25,000 or
30,000 Unit/mg protein hyaluronidase activity, and being substantially free of non-
25 PEGylated rHuPH20 (less than 5% non-PEGylated).

Using various PEG reagents, exemplary versions of hyaluronan degrading
enzymes, in particular soluble human recombinant hyaluronidases (*e.g.* rHuPH20),
can be prepared, for example, using mPEG-SBA (30 kD), mPEG-SMB (30 kD), and
branched versions based on mPEG2-NHS (40 kD), mPEG2-NHS (60 kD). PEGylated
30 versions of rHuPH20 have been generated using NHS chemistries, as well as
carbonates, and aldehydes, using each of the following reagents: mPEG2-NHS-40K

branched, mPEG-NHS-10K branched, mPEG-NHS-20K branched, mPEG-NHS-40K branched, mPEG2-NHS-60K branched; mPEG-SBA-5K; mPEG-SBA-20K; mPEG-SBA-30K; mPEG-SMB-20K; mPEG-SMB-30K; mPEG-butyraldehyde-; mPEG-SPA-20K; mPEG-SPA-30K; and PEG-NHS-5K-biotin. PEGylated hyaluronidases have
 5 also been prepared using PEG reagents available from Dowpharma, a division of Dow Chemical Corporation; including hyaluronidases PEGylated with Dowpharma's p-nitrophenyl-carbonate PEG (30 kDa) and with propionaldehyde PEG (30 kDa).

In one example, the PEGylation includes conjugation of mPEG-SBA, for example, mPEG-SBA-30K (having a molecular weight of about 30 Kda) or another
 10 succinimidyl esters of PEG butanoic acid derivative, to a soluble hyaluronidase. Succinimidyl esters of PEG butanoic acid derivatives, such as mPEG-SBA-30K readily couple to amino groups of proteins. For example, covalent conjugation of m-PEG-SBA-30K and rHuPH20 (which is approximately 60 KDa in size) provides stable amide bonds between rHuPH20 and mPEG, as shown in Scheme 1, below.

15 **Scheme 1:**



Typically, the mPEG-SBA-30K or other PEG is added to the hyaluronan degrading enzyme, in some instances a hyaluronidase, at a PEG:polypeptide molar
 20 ratio of 10:1 in a suitable buffer, e.g. 130 mM NaCl /10 mM HEPES at pH 6.8, followed by sterilization, e.g. sterile filtration, and continued conjugation, for

example, with stirring, overnight at 4°C in a cold room. In one example, the conjugated PEG- hyaluronan degrading enzyme is concentrated and buffer-exchanged.

Other methods of coupling succinimidyl esters of PEG butanoic acid derivatives, such as mPEG-SBA-30K are known in the art (see e.g., U.S. 5,672,662; U.S. 6,737,505; and U.S. 2004/0235734). For example, a polypeptide, such as a hyaluronan degrading enzyme (e.g. a hyaluronidase), can be coupled to an NHS activated PEG derivative by reaction in a borate buffer (0.1 M, pH 8.0) for one hour at 4 °C. The resulting PEGylated protein can be purified by ultrafiltration.

Alternatively, PEGylation of a bovine alkaline phosphatase can be accomplished by mixing the phosphatase with mPEG-SBA in a buffer containing 0.2 M sodium phosphate and 0.5 M NaCl (pH 7.5) at 4°C for 30 minutes. Unreacted PEG can be removed by ultrafiltration. Another method reacts polypeptide with mPEG-SBA in deionized water to which triethylamine is added to raise the pH to 7.2-9. The resulting mixture is stirred at room temperature for several hours to complete the PEGylation.

F. PREPARATION, FORMULATION AND ADMINISTRATION OF COMPOSITIONS

Pharmaceutical compositions of modified hyaluronan degrading enzyme, such as modified soluble hyaluronidases, conjugated to a polymer are provided herein.

Also provided are pharmaceutical compositions containing a second agent that is used to treat a disease or disorder associated with a hyaluronan-associated disease or condition. Exemplary of such agents include, but are not limited to, anti-cancer agents including drugs, polypeptides, nucleic acids, antibodies, peptides, small molecules, gene therapy vector, viruses and other therapeutics. Modified hyaluronan degrading enzymes, including modified soluble hyaluronidases, can be co-formulated or co-administered with pharmaceutical formulations of such second agents to enhance their delivery to desired sites or tissues within the body associated with excess or accumulated hyaluronan. For example, tumors are associated with accumulated hyaluronan. Such excess hyaluronan can contribute to impeding hydraulic conductivity. The introduction of hyaluronan degrading enzymes such as soluble hyaluronidases, in particular soluble hyaluronidases conjugated to a polymer

to increase half-life, can counteract the accumulation of hyaluronan in such tissues, thereby improving hydraulic conductivity within the site or tissue, rendering the site or tissue more susceptible to delivery of a second agent or agents either by local or systemic delivery. For example, provided herein is a composition of a pegylated soluble hyaluronidase, such as rHuPH20, that, when administered together or separately (intermittently, simultaneously or sequentially) with a composition containing an anti-cancer agent to a tumor, can render the tumor more susceptible to such anti-tumor agents. As discussed elsewhere herein, a composition of a pegylated soluble hyaluronidase also can be administered alone to treat diseases or conditions associated with accumulated hyaluronidase substrate expressions.

The compounds can be formulated into suitable pharmaceutical preparations such as solutions, suspensions, tablets, dispersible tablets, pills, capsules, powders, sustained release formulations or elixirs, for oral administration, as well as transdermal patch preparation and dry powder inhalers. Typically, the compounds are formulated into pharmaceutical compositions using techniques and procedures well known in the art (see e.g., Ansel *Introduction to Pharmaceutical Dosage Forms*, Fourth Edition, 1985, 126). Generally, the mode of formulation is a function of the route of administration. The compositions can be co-formulated or provided as separate compositions.

Generally, the compositions are formulated in lyophilized or liquid form.

Where the compositions are provided in lyophilized form they can be reconstituted just prior to use by an appropriate buffer, for example, a sterile saline solution. The compositions can be provided together or separately. For purposes herein, such compositions typically are provided separately. The hyaluronan degrading enzyme, such as soluble hyaluronidase, and second agent can be packaged as separate compositions for administration together, sequentially or intermittently. The combinations can be packaged as a kit.

Compositions can be formulated for administration by any route known to those of skill in the art including intramuscular, intravenous, intradermal, intralesional, intraperitoneal injection, subcutaneous, intratumoral, epidural, nasal, oral, vaginal, rectal, topical, local, otic, inhalational, buccal (e.g., sublingual), and

transdermal administration or any route. Administration can be local, topical or systemic depending upon the locus of treatment. Local administration to an area in need of treatment can be achieved by, for example, but not limited to, local infusion during surgery, topical application, *e.g.*, in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant. Compositions also can be administered with other biologically active agents, either sequentially, intermittently or in the same composition. Administration also can include controlled release systems including controlled release formulations and device controlled release, such as by means of a pump.

The most suitable route in any given case depends on a variety of factors, such as the nature of the disease, the progress of the disease, the severity of the disease the particular composition which is used. For purposes herein, it is desired that a hyaluronan degrading enzyme, such as a soluble hyaluronidase, and/or second agent are administered such that a pharmaceutically available amount or level exists in the plasma). For example, compositions are administered systemically, for example, via intravenous administration. In some cases, such compositions are administered such that they reach interstitium of skin or tissues having accumulated hyaluronan. For example, the introduction of soluble hyaluronidases to tumor interstitial would enhance the delivery of locally delivered as well as systemically available anti-cancer agents which can more readily penetrate the tumor when interstitial fluid pressure is reduced and diffusion and/or connective transport increased. Hence, a hyaluronan degrading enzyme, such as a soluble hyaluronidase, and the second agent or agents can be administered by different routes of administration. Thus, in one example, a soluble hyaluronidase is administered locally, for example, intratumorally, to the site or tissue associated with accumulated hyaluronan, and the second agent is administered systemically, for example, by intravenous administration. Other modes of administration also are contemplated. Pharmaceutical compositions can be formulated in dosage forms appropriate for each route of administration.

Administration methods can be employed to decrease the exposure of hyaluronan degrading enzymes, *e.g.* soluble hyaluronidases, and other molecules to degradative processes, such as proteolytic degradation and immunological

intervention via antigenic and immunogenic responses. Examples of such methods include local administration at the site of treatment.

1. Formulations

Pharmaceutically acceptable compositions are prepared in view of approvals
5 for a regulatory agency or other agency prepared in accordance with generally
recognized pharmacopeia for use in animals and in humans. Compositions can take
the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, and
sustained release formulations. A composition can be formulated as a suppository,
with traditional binders and carriers such as triglycerides. Oral formulation can
10 include standard carriers such as pharmaceutical grades of mannitol, lactose, starch,
magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and other
such agents. The formulation should suit the mode of administration.

Pharmaceutical compositions can include carriers such as a diluent, adjuvant,
excipient, or vehicle with which an enzyme or activator is administered. Examples of
15 suitable pharmaceutical carriers are described in "Remington's Pharmaceutical
Sciences" by E. W. Martin. Such compositions will contain a therapeutically effective
amount of the compound, generally in purified form, together with a suitable amount
of carrier so as to provide the form for proper administration to the patient. Such
pharmaceutical carriers can be sterile liquids, such as water and oils, including those
20 of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil,
mineral oil, and sesame oil. Water is a typical carrier when the pharmaceutical
composition is administered intravenously. Saline solutions and aqueous dextrose
and glycerol solutions also can be employed as liquid carriers, particularly for
injectable solutions. Compositions can contain along with an active ingredient: a
25 diluent such as lactose, sucrose, dicalcium phosphate, or carboxymethylcellulose; a
lubricant, such as magnesium stearate, calcium stearate and talc; and a binder such as
starch, natural gums, such as gum acaciagelatin, glucose, molasses,
polyvinylpyrrolidone, celluloses and derivatives thereof, povidone, crospovidones and
other such binders known to those of skill in the art. Suitable pharmaceutical
30 excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk,
silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim

milk, glycerol, propylene, glycol, water, and ethanol. A composition, if desired, also can contain minor amounts of wetting or emulsifying agents, or pH buffering agents, for example, acetate, sodium citrate, cyclodextrine derivatives, sorbitan monolaurate, triethanolamine sodium acetate, triethanolamine oleate, and other such agents.

5 In one example, pharmaceutical preparation can be in liquid form, for example, solutions, syrups or suspensions. Such liquid preparations can be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond
10 oil, oily esters, or fractionated vegetable oils); and preservatives (*e.g.*, methyl or propyl-p-hydroxybenzoates or sorbic acid). In another example, pharmaceutical preparations can be presented in lyophilized form for reconstitution with water or other suitable vehicle before use.

 Pharmaceutically therapeutically active compounds and derivatives thereof are
15 typically formulated and administered in unit dosage forms or multiple dosage forms. Each unit dose contains a predetermined quantity of therapeutically active compound sufficient to produce the desired therapeutic effect, in association with the required pharmaceutical carrier, vehicle or diluent. Unit dosage forms, include, but are not limited to, tablets, capsules, pills, powders, granules, sterile parenteral solutions or
20 suspensions, and oral solutions or suspensions, and oil water emulsions containing suitable quantities of the compounds or pharmaceutically acceptable derivatives thereof. Unit dose forms can be contained ampoules and syringes or individually packaged tablets or capsules. Unit dose forms can be administered in fractions or multiples thereof. A multiple dose form is a plurality of identical unit dosage forms
25 packaged in a single container to be administered in segregated unit dose form. Examples of multiple dose forms include vials, bottles of tablets or capsules or bottles of pints or gallons. Hence, multiple dose form is a multiple of unit doses that are not segregated in packaging. Generally, dosage forms or compositions containing active ingredient in the range of 0.005 % to 100 % with the balance made up from non-toxic
30 carrier can be prepared.

Pharmaceutical composition can be formulated in dosage forms appropriate for each route of administration.

a. Injectables, solutions and emulsions

Parenteral administration, generally characterized by injection, either
5 subcutaneously, intramuscularly, intratumorally, intravenously or intradermally is contemplated herein. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Suitable excipients are, for example, water, saline, dextrose, glycerol or ethanol. In addition, if desired, the pharmaceutical
10 compositions to be administered may also contain an activator in the form of a solvent such as pH buffering agents, metal ion salts, or other such buffers. The pharmaceutical compositions also may contain other minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents, stabilizers, solubility enhancers, and other such agents, such as for example, sodium
15 acetate, sorbitan monolaurate, triethanolamine oleate and cyclodextrins. Implantation of a slow-release or sustained-release system, such that a constant level of dosage is maintained (see, e. g., U. S. Patent No. 3,710,795) also is contemplated herein. The percentage of active compound contained in such parenteral compositions is highly dependent on the specific nature thereof, as well as the activity of the compound and
20 the needs of the subject.

For example, a standard stabilized formulation of a modified soluble hyaluronidase as provided herein is formulated with one or more of EDTA, NaCl, CaCl₂, histidine, lactose, albumin, Pluronic® F68, TWEEN® and/or other detergent or other similar agents. For example, compositions provided herein can contain one
25 or more pH buffers (such as, for example, histidine, phosphate, or other buffers), or acidic buffer (such as acetate, citrate, pyruvate, Gly-HCl, succinate, lactate, maleate or other buffers), tonicity modifier (such as, for example, an amino acid, polyalcohol, NaCl, trehalose, other salts and/or sugars), stabilizer, chelating agent, such as ethylenediaminetetraacetic acid, ethylenediaminetetraacetate or calcium EDTA,
30 oxygen scavenger, such as methionine, ascorbic acid/ascorbate, citric acid/citrate, or albumin, and/or a preservative, such as preservative containing an aromatic ring (e.g.

phenol or cresol). Exemplary stabilizers that are useful for compositions containing a hyaluronan degrading enzyme include detergents, such as polysorbates and proteins such as human serum albumin. Exemplary concentrations of serum albumin that are useful in the compositions herein include 0.1 mg/mL, 0.2 mg/mL, 0.3 mg/mL, 0.4
5 mg/mL, 0.5 mg/mL, 0.6 mg/mL, 0.7 mg/mL, 0.8 mg/mL, 0.9 mg/mL or 1 mg/mL, but can be more or less. Polysorbates also can be present in the compositions at, for example, concentrations of or about 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.007%, 0.008%, 0.009%, 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09% or 0.1%. A metal chelating agent, such as calcium EDTA
10 (CaEDTA), also can be present, such as for example, at concentrations of between approximately 0.02 mM to 20 mM, such as 0.02 mM, 0.04 mM, 0.06 mM, 0.08 mM, 0.1 mM, 0.2 mM, 0.3 mM, 0.4 mM, 0.5 mM, 0.6 mM, 0.7 mM, 0.8 mM, 0.9 mM, 1 mM, 5 mM, 10 mM, 15 mM, 20 mM or more. The pH and the osmolarity of the compositions can be adjusted by one of skill in the art to optimize the conditions for
15 the desired activity and stability of the composition. In some examples, the compositions provided herein have an osmolarity of at or about 100 mOsm/kg, 120 mOsm/kg, 140 mOsm/kg, 160 mOsm/kg, 180 mOsm/kg, 200 mOsm/kg, 220 mOsm/kg, 240 mOsm/kg, 260 mOsm/kg, 280 mOsm/kg, 300 mOsm/kg, 320 mOsm/kg, 340 mOsm/kg, 360 mOsm/kg, 380 mOsm/kg, 400 mOsm/kg, 420
20 mOsm/kg, 440 mOsm/kg, 460 mOsm/kg, 500 or more mOsm/kg, and a pH of at or about 6, 6.2, 6.4, 6.6, 6.8, 7, 7.2, 7.4, 7.6, 7.8 or 8.

Generally NaCl is provided in formulations herein, for example, in an amount that is or is about 100 mM- 150 mM or more. For example, an exemplary formulation can contain at or about 10 mM histidine and/or at or about 130 mM NaCl. Other
25 formulations can contain in addition or alternatively lactose, for example, at or about 13 mg/ml. Additionally, an anti-bacterial or anti-fungal agent, including, but not limited to thiomersal, can be present in the formulation. Formulations can further contain Albumin, Pluronic® F68, TWEEN® and/or other detergent. The formulations are provided at a pH that is or is about 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6,
30 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3 or 7.4, generally that is or is about pH 6.5. Concentrated formulations of a modified soluble hyaluronidase for use herein are

generally diluted in a saline solution or other salt buffered solution prior administration to maintain the appropriate salt concentration.

Injectables are designed for local and systemic administration. For purposes herein, local administration is desired for direct administration to the affected interstitium associated with accumulated or excess hyaluronan. Preparations for parenteral administration include sterile solutions ready for injection, sterile dry soluble products, such as lyophilized powders, ready to be combined with a solvent just prior to use, including hypodermic tablets, sterile suspensions ready for injection, sterile dry insoluble products ready to be combined with a vehicle just prior to use and sterile emulsions. The solutions may be either aqueous or nonaqueous. If administered intravenously, suitable carriers include physiological saline or phosphate buffered saline (PBS), and solutions containing thickening and solubilizing agents, such as glucose, polyethylene glycol, and polypropylene glycol and mixtures thereof.

Pharmaceutically acceptable carriers used in parenteral preparations include aqueous vehicles, nonaqueous vehicles, antimicrobial agents, isotonic agents, buffers, antioxidants, local anesthetics, suspending and dispersing agents, emulsifying agents, sequestering or chelating agents and other pharmaceutically acceptable substances. Examples of aqueous vehicles include Sodium Chloride Injection, Ringers Injection, Isotonic Dextrose Injection, Sterile Water Injection, Dextrose and Lactated Ringers Injection. Nonaqueous parenteral vehicles include fixed oils of vegetable origin, cottonseed oil, corn oil, sesame oil and peanut oil. Antimicrobial agents in bacteriostatic or fungistatic concentrations can be added to parenteral preparations packaged in multiple-dose containers, which include phenols or cresols, mercurials, benzyl alcohol, chlorobutanol, methyl and propyl p-hydroxybenzoic acid esters, thimerosal, benzalkonium chloride and benzethonium chloride. Isotonic agents include sodium chloride and dextrose. Buffers include phosphate and citrate. Antioxidants include sodium bisulfate. Local anesthetics include procaine hydrochloride. Suspending and dispersing agents include sodium carboxymethylcellulose, hydroxypropyl methylcellulose and polyvinylpyrrolidone. Emulsifying agents include Polysorbate 80 (TWEENS 80). A sequestering or chelating agent of metal ions include EDTA. Pharmaceutical carriers also include

ethyl alcohol, polyethylene glycol and propylene glycol for water miscible vehicles and sodium hydroxide, hydrochloric acid, citric acid or lactic acid for pH adjustment.

If administered intravenously, suitable carriers include physiological saline or phosphate buffered saline (PBS), and solutions containing thickening and solubilizing agents, such as glucose, polyethylene glycol, and polypropylene glycol and mixtures thereof.

The concentration of the pharmaceutically active compound is adjusted so that an injection provides an effective amount to produce the desired pharmacological effect. As discussed elsewhere herein, the modified soluble hyaluronidase is provided in a sufficient amount to maintain at or about 3 U/mL of the hyaluronidase in the plasma. The exact dose depends on the age, weight and condition of the patient or animal as is known in the art. The unit-dose parenteral preparations are packaged in an ampoule, a vial or a syringe with a needle. The volume of liquid solution or reconstituted powder preparation, containing the pharmaceutically active compound, is a function of the disease to be treated and the particular article of manufacture chosen for package. All preparations for parenteral administration must be sterile, as is known and practiced in the art.

b. Lyophilized powders

Of interest herein are lyophilized powders, which can be reconstituted for administration as solutions, emulsions and other mixtures. They may also be reconstituted and formulated as solids or gels.

The sterile, lyophilized powder is prepared by dissolving a compound of a soluble hyaluronidase and/or second agent in a buffer solution. The buffer solution may contain an excipient which improves the stability or other pharmacological component of the powder or reconstituted solution, prepared from the powder. Subsequent sterile filtration of the solution followed by lyophilization under standard conditions known to those of skill in the art provides the desired formulation. Briefly, the lyophilized powder is prepared by dissolving an excipient, such as dextrose, sorbital, fructose, corn syrup, xylitol, glycerin, glucose, sucrose or other suitable agent, in a suitable buffer, such as citrate, sodium or potassium phosphate or other such buffer known to those of skill in the art. Then, a selected enzyme is added to the

resulting mixture, and stirred until it dissolves. The resulting mixture is sterile filtered or treated to remove particulates and to insure sterility, and apportioned into vials for lyophilization. Each vial will contain a single dosage (1 mg – 1 g, generally 1-100 mg, such as 1-5 mg) or multiple dosages of the compound. The lyophilized powder can be stored under appropriate conditions, such as at about 4 °C to room temperature.

Reconstitution of this lyophilized powder with a buffer solution provides a formulation for use in parenteral administration. The precise amount depends upon the indication treated and selected compound. Such amount can be empirically determined.

c. Topical administration

Topical mixtures are prepared as described for the local and systemic administration. The resulting mixture may be a solution, suspension, emulsions or the like and are formulated as creams, gels, ointments, emulsions, solutions, elixirs, lotions, suspensions, tinctures, pastes, foams, aerosols, irrigations, sprays, suppositories, bandages, dermal patches or any other formulations suitable for topical administration.

The compounds or pharmaceutically acceptable derivatives thereof may be formulated as aerosols for topical application, such as by inhalation (see, e. g., U. S. Patent Nos. 4,044,126, 4,414,209, and 4,364,923, which describe aerosols for delivery of a steroid useful for treatment inflammatory diseases, particularly asthma). These formulations for administration to the respiratory tract can be in the form of an aerosol or solution for a nebulizer, or as a microfine powder for insufflation, alone or in combination with an inert carrier such as lactose. In such a case, the particles of the formulation will typically diameters of less than 50 microns, preferably less than 10 microns.

The compounds may be formulated for local or topical application, such as for topical application to the skin and mucous membranes, such as in the eye, in the form of gels, creams, and lotions and for application to the eye or for intracisternal or intraspinal application. Topical administration is contemplated for transdermal delivery and also for administration to the eyes or mucosa, or for inhalation therapies.

Nasal solutions of the active compound alone or in combination with other pharmaceutically acceptable excipients also can be administered.

Formulations suitable for transdermal administration are provided. They can be provided in any suitable format, such as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Such patches contain the active compound in optionally buffered aqueous solution of, for example, 0.1 to 0.2M concentration with respect to the active compound. Formulations suitable for transdermal administration also can be delivered by iontophoresis (see, *e.g.*, *Pharmaceutical Research* 3(6), 318 (1986)) and typically take the form of an optionally buffered aqueous solution of the active compound.

d. Compositions for other routes of administration

Depending upon the condition treated other routes of administration, such as topical application, transdermal patches, oral and rectal administration also are contemplated herein. For example, pharmaceutical dosage forms for rectal administration are rectal suppositories, capsules and tablets for systemic effect. Rectal suppositories include solid bodies for insertion into the rectum which melt or soften at body temperature releasing one or more pharmacologically or therapeutically active ingredients. Pharmaceutically acceptable substances utilized in rectal suppositories are bases or vehicles and agents to raise the melting point. Examples of bases include cocoa butter (theobroma oil), glycerin-gelatin, carbowax (polyoxyethylene glycol) and appropriate mixtures of mono-, di- and triglycerides of fatty acids. Combinations of the various bases may be used. Agents to raise the melting point of suppositories include spermaceti and wax. Rectal suppositories may be prepared either by the compressed method or by molding. The typical weight of a rectal suppository is about 2 to 3 gm. Tablets and capsules for rectal administration are manufactured using the same pharmaceutically acceptable substance and by the same methods as for formulations for oral administration.

Formulations suitable for rectal administration can be provided as unit dose suppositories. These can be prepared by admixing the active compound with one or more conventional solid carriers, for example, cocoa butter, and then shaping the resulting mixture.

For oral administration, pharmaceutical compositions can take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.*, pregelatinized maize starch, polyvinyl pyrrolidone or hydroxypropyl methylcellulose); fillers (*e.g.*, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (*e.g.*, magnesium stearate, talc or silica); disintegrants (*e.g.*, potato starch or sodium starch glycolate); or wetting agents (*e.g.*, sodium lauryl sulphate). The tablets can be coated by methods well-known in the art.

Formulations suitable for buccal (sublingual) administration include, for example, lozenges containing the active compound in a flavored base, usually sucrose and acacia or tragacanth; and pastilles containing the compound in an inert base such as gelatin and glycerin or sucrose and acacia.

Pharmaceutical compositions also can be administered by controlled release formulations and/or delivery devices (see, *e.g.*, in U.S. Patent Nos. 3,536,809; 3,598,123; 3,630,200; 3,845,770; 3,847,770; 3,916,899; 4,008,719; 4,687,610; 4,769,027; 5,059,595; 5,073,543; 5,120,548; 5,354,566; 5,591,767; 5,639,476; 5,674,533 and 5,733,566).

Various delivery systems are known and can be used to administer selected compositions, such as but not limited to, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor mediated endocytosis, and delivery of nucleic acid molecules encoding a soluble hyaluronidase or other agent such as retrovirus delivery systems.

Hence, in certain embodiments, liposomes and/or nanoparticles also can be employed with administration of compositions herein. Liposomes are formed from phospholipids that are dispersed in an aqueous medium and spontaneously form multilamellar concentric bilayer vesicles (also termed multilamellar vesicles (MLVs)). MLVs generally have diameters of from 25 nm to 4 μ m. Sonication of MLVs results in the formation of small unilamellar vesicles (SUVs) with diameters in the range of 200 to 500 angstroms containing an aqueous solution in the core.

Phospholipids can form a variety of structures other than liposomes when dispersed in water, depending on the molar ratio of lipid to water. At low ratios, the

liposomes form. Physical characteristics of liposomes depend on pH, ionic strength and the presence of divalent cations. Liposomes can show low permeability to ionic and polar substances, but at elevated temperatures undergo a phase transition which markedly alters their permeability. The phase transition involves a change from a
5 closely packed, ordered structure, known as the gel state, to a loosely packed, less-ordered structure, known as the fluid state. This occurs at a characteristic phase-transition temperature and results in an increase in permeability to ions, sugars and drugs.

Liposomes interact with cells via different mechanisms: endocytosis by
10 phagocytic cells of the reticuloendothelial system such as macrophages and neutrophils; adsorption to the cell surface, either by nonspecific weak hydrophobic or electrostatic forces, or by specific interactions with cell-surface components; fusion with the plasma cell membrane by insertion of the lipid bilayer of the liposome into the plasma membrane, with simultaneous release of liposomal contents into the
15 cytoplasm; and by transfer of liposomal lipids to cellular or subcellular membranes, or vice versa, without any association of the liposome contents. Varying the liposome formulation can alter which mechanism is operative, although more than one can operate at the same time. Nanocapsules can generally entrap compounds in a stable and reproducible way. To avoid side effects due to intracellular polymeric
20 overloading, such ultrafine particles (sized around 0.1 μm) should be designed using polymers able to be degraded *in vivo*. Biodegradable polyalkyl-cyanoacrylate nanoparticles that meet these requirements are contemplated for use herein, and such particles can be easily made.

2. Dosage and Administration

25 Active agents, for example a hyaluronan degrading enzyme, such as a hyaluronidase, and/or second agent, are included in an amount sufficient that exert a therapeutically useful effect in the absence of undesirable side effects on the patient treated. For example, as described elsewhere herein, the modified soluble hyaluronidase is formulated for systemic administration in a sufficient amount to
30 maintain at least or about 3 U/mL in the plasma, generally 3 U/mL- 12 U/mL or more, for example, from about or at a level of 4 U/mL, 5 U/mL, 6 U/mL, 7 U/mL, 8 U/mL,

9 U/mL, 10 U/mL, 11 U/mL, 12 U/mL, 13 U/mL, 14 U/mL, 15 U/mL, 16 U/mL, 17 U/mL, 18 U/mL, 19 U/mL, 20 U/mL, 25 U/mL, 30 U/mL, 35 U/mL, 40 U/mL, 45 U/mL, 50 U/mL or more.

It is within the level of one of skill in the art to determine the amounts of modified hyaluron-degrading enzyme, for example, modified soluble hyaluronidase to maintain at least 3 U/mL of the hyaluronidase in the blood. The level of hyaluronidase in the blood can be monitored over time in order to ensure that a sufficient amount of the hyaluronidase is present in the blood. Any assay known to one of skill in the art to measure the hyaluronidase in the plasma can be performed. For example, a microturbidity assay or enzymatic assay described in the Examples herein can be performed on protein in plasma. It is understood that plasma normally contains hyaluronidase enzymes. Such plasma hyaluronidase enzymes typically have activity at an acidic pH (U.S. Patent No. 7,105,330). Hence, before treatment of with a modified enzyme, the plasma levels of hyaluronidase should be determined and used as a baseline. Subsequent measurements of plasma hyaluronidase levels after treatment can be compared to the levels before treatments. Alternatively, the assay can be performed under pH conditions that suppress endogenous lysosomal hyaluronidase activity in plasma, which normally exhibits activity at acidic pH. Thus, where the modified soluble hyaluronidase is active at neutral pH (e.g. human PH20), only the level of the modified neutral-active soluble hyaluronidase is measured.

The composition containing the active agent can include a pharmaceutically acceptable carrier. Therapeutically effective concentration can be determined empirically by testing the compounds in known *in vitro* and *in vivo* systems, such as the assays provided herein. The concentration of a hyaluronan degrading enzyme such as a soluble hyaluronidase or second agent in the composition depends on absorption, inactivation and excretion rates of the complex, the physicochemical characteristics of the complex, the dosage schedule, and amount administered as well as other factors known to those of skill in the art. For example, it is understood that the precise dosage and duration of treatment is a function of the tissue being treated and may be determined empirically using known testing protocols or by extrapolation from *in vivo* or *in vitro* test data and/or can be determined from known dosing regimes

of the particular agent. For example, agents and treatments for treatment of hyaluronan-associated diseases and conditions, such as anti-cancer agents, are well known in the art. Thus, dosages of second agents in a composition can be chosen based on standard dosing regimes for that agent under a given route of administration.

5 It is to be noted that concentrations and dosage values may also vary with the age of the individual treated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the formulations, and that the concentration ranges
10 set forth herein are exemplary only and are not intended to limit the scope thereof. The compositions can be administered hourly, daily, weekly, monthly, yearly or once. Generally, dosage regimens are chosen to limit toxicity. It should be noted that the attending physician would know how to and when to terminate, interrupt or adjust therapy to lower dosage due to toxicity, or bone marrow, liver or kidney or other
15 tissue dysfunctions. Conversely, the attending physician would also know how to and when to adjust treatment to higher levels if the clinical response is not adequate (precluding toxic side effects).

 The amount of a hyaluronan degrading enzyme, such as a soluble hyaluronidase, to be administered for the treatment of a disease or condition, for
20 ~~example a hyaluronan-associated disease or condition such as an HA-rich tumor, can~~
be determined by standard clinical techniques. In addition, *in vitro* assays and animal models can be employed to help identify optimal dosage ranges. The precise dosage, which can be determined empirically, can depend on the particular enzyme, the route of administration, the type of disease to be treated and the seriousness of the disease.
25 Exemplary dosage range is at or about 50 Units to 50,000,000 Units of a soluble hyaluronidase conjugated to a polymer, or a functionally equivalent amount of another hyaluronan degrading enzyme conjugated to a polymer. It is understood herein that a unit of activity is normalized to a standard activity, for example, an activity as measured in a microturbidity assay assaying hyaluronidase activity.

30 A soluble hyaluronidase conjugated to a polymer, for example a pegylated soluble hyaluronidase, can exhibit lower activity per mg of total protein, i.e. exhibits a

lower specific activity, compared to a native soluble hyaluronidase not so conjugated. For example, as described elsewhere herein, an exemplary rHuPH20 preparation exhibits a specific activity of 120,000 Units/mg, while a pegylated form of rHuPH20 exhibits a specific activity of 30,000 Units/mg. Typically, a PEGylated form of
5 rHuPH20 exhibits a specific activity within the range of between at or about 26,000 and at or about 38,000 U/mg. Hence, to achieve an equal unit of activity, a greater of amount of total protein is required. For example, a hyaluronan degrading enzyme, such as a soluble hyaluronidase, conjugated to a polymer requires 1.5 time, 3 times, 4 times, 5 times, 6 times, 7 times, 8 times, 9 times, 10 times or more total protein (in
10 mg) to achieve the same units of activity of a hyaluronan degrading enzyme, such as a soluble hyaluronidase, that is not so conjugated. For purposes herein, however, dosages are with reference to Units.

Thus, for example, a soluble hyaluronidase provided herein conjugated to polymer, for example, a PEG, can be administered at or about 10 to 50,000,000 Units, 15 10 to 40,000,000 Units, 10 to 36,000,000 Units, 10 to 12,000,000 Units, 10 to 1,200,000 Units, 10 to 1,000,000 Units, 10 to 500,000 Units, 100 to 100,000 Units, 500 to 50,000 Units, 1000 to 10,000 Units, 5000 to 7500 Units, 5000 Units to 50,000 Units, or 1,000 to 10,000 Units. In instances where a hyaluronan degrading enzyme that is not a hyaluronidase is conjugated to a polymer, it can be administered at an
20 ~~amount that is functionally equivalent to at or about 10 to 50,000,000 Units, 10 to 40,000,000 Units, 10 to 36,000,000 Units, 10 to 12,000,000 Units, 10 to 1,200,000 Units, 10 to 1,000,000 Units, 10 to 500,000 Units, 100 to 100,000 Units, 500 to 50,000 Units, 1000 to 10,000 Units, 5000 to 7500 Units, 5000 Units to 50,000 Units, or 1,000 to 10,000 Units.~~

25 Generally, for purposes herein to maintain at least 3 U/mL of the hyaluronidase in plasma, at or about 0.02 mg/kg (of the subject), 0.03 mg/kg, 0.04 mg/kg, 0.05 mg/kg, 0.06 mg/kg, 0.07 mg/kg, 0.08 mg/kg, 0.09 mg/kg, 0.1 mg/kg, 0.15 mg/kg, 0.2 mg/kg, 0.25 mg/kg, 0.30 mg/kg, 0.35 mg/kg, 0.40 mg/kg, 0.45 mg/kg, 0.5 mg/kg, 0.55 mg/kg, 0.6 mg/kg, 0.7 mg/kg, 0.8 mg/kg, 0.9 mg/kg, 1.0
30 mg/kg, 1.1 mg/kg, 1.2 mg/kg, 1.3 mg/kg, 1.4 mg/kg, 1.5 mg/kg or more is administered. Generally, where the specific activity of the modified hyaluronidase is

or is about 20,000 U/mg to 60,000 U/mg, generally at or about 35,000 U/mg, 60,000 U; 70,000 U; 80,000 U; 90,000 U; 100,000 U; 200,000 U; 300,000 U; 400,000 U; 500,000 U; 600,000 U; 700,000 U; 800,000 U; 900,000 U; 1,000,000 U; 1,500,000 U; 2,000,000 U; 2,500,000 U; 3,000,000 U; 3,500,000 U; 4,000,000 U or more is
5 administered.

Typically, volumes of injections or infusions of hyaluronidase contemplated herein are from at or about 0.5 ml, 1 ml, 2 ml, 3 ml, 4 ml, 5 ml, 6 ml, 7 ml, 8 ml, 9 ml, 10 ml, 15 ml, 20 ml, 30 ml, 40 ml, 50 ml or more. The hyaluronan degrading enzyme, such as a hyaluronidase can be provided as a stock solution at or about 50
10 U/ml, 100 U/ml, 150 U/ml, 200 U/ml, 400 U/ml or 500 U/ml (or a functionally equivalent amount) or can be provided in a more concentrated form, for example at or about 1000 U/ml, 1500 Units/ml, 2000 U/ml, 4000 U/ml or 5000 U/ml for use directly or for dilution to the effective concentration prior to use. The volume of hyaluronan degrading enzyme, such as soluble hyaluronidase, administered is a function of the
15 dosage required, but can be varied depending on the concentration of a hyaluronan degrading enzyme, such as soluble hyaluronidase, stock formulation available. For example, it is contemplated herein that hyaluronan degrading enzyme, such as soluble hyaluronidase, is not administered in volumes greater than about 50 ml, and typically is administered in a volume of 5-30 ml, generally in a volume that is not greater than
20 about 10 mL. A syringe pump can be used for the higher volumes, at the discretion of the physician. The timing of administration can also be adjusted by the treating physician. For example, when administered intravenously, a syringe pump can be used to administer the composition over a time period that is 1 minute, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, or more minutes, generally at or about 15
25 minutes. The hyaluronan degrading enzyme, such as soluble hyaluronidase, can be provided as a liquid or lyophilized formulation. Lyophilized formulations are ideal for storage of large unit doses of hyaluronan degrading enzyme.

In one example, the hyaluronan degrading enzyme, such as soluble hyaluronidase, is administered as part of a combination therapy, by administering the
30 hyaluronan-degrading enzyme and a second agent or treatment for treating the disease or condition. In one example, the hyaluronan-degrading enzyme and second agent or

treatment can be co-formulated and administered together. In another example, the hyaluronan degrading enzyme, such as soluble hyaluronidase, is administered subsequently, intermittently or simultaneously with the second agent or treatment preparation. Generally, the hyaluronan degrading enzyme is administered prior to administration of the second agent or treatment preparation to permit the hyaluronan degrading enzyme to degrade the hyaluronic acid in a cell, tissue or fluid of the subject, such as, for example, the interstitial space, extracellular matrix, tumor tissue, blood or other tissue. For example, the hyaluronan degrading enzyme, such as soluble hyaluronidase, can be administered 0.5 minutes, 1 minute, 2 minute, 3 minute, 4 minute, 5 minute, 6 minute, 7 minutes, 8 minutes, 9 minutes, 10 minutes, 20 minutes, 30 minutes, 1 hour or more prior to administration of the second agent preparation. In some examples, the hyaluronan degrading enzyme is administered together with the second agent preparation. As will be appreciated by those of skill in the art, the desired proximity of co-administration depends in significant part in the effect half lives of the agents in the particular tissue setting, and the particular disease being treated, and can be readily optimized by testing the effects of administering the agents at varying times in suitable models, such as in suitable animal models. In some situations, the optimal timing of administration of the hyaluronan degrading enzyme, such as a hyaluronidase, will exceed 60 minutes.

20 ~~A preparation of a second agent or agents or treatment or treatments can be~~
administered at once, or can be divided into a number of smaller doses to be administered at intervals of time. Selected agent / treatment preparations can be administered in one or more doses over the course of a treatment time for example over several hours, days, weeks, or months. In some cases, continuous administration is useful. It is understood that the precise dosage and course of administration depends on the indication and patient's tolerability. Generally, dosing regimes for second agents/treatments herein are known to one of skill in the art.

3. **Combination Therapies**

Any of the compositions or combinations described herein can be further co-formulated or co-administered together with, prior to, intermittently with, or subsequent to, other therapeutic or pharmacologic agents or treatments, such as procedures, for example, agents or treatments to treat hyaluronan-associated cancers.

Such agents include, but are not limited to, other biologics, small molecule compounds, dispersing agents, anesthetics, vasoconstrictors and surgery, and combinations thereof. Such other agents and treatments that are available for the treatment of a disease or condition, including all those exemplified herein, are known to one of skill in the art or can be empirically determined. In another example, a local anesthetic, for example, lidocaine can be administered to provide pain relief. In some examples, the anesthetic can be provided in combination with a vasoconstrictor to increase the duration of the anesthetic effects.

Thus, in one example, compositions provided herein can be co-formulated or co-administered with a local anesthesia. Anesthetics include short-acting and long-lasting local anesthetic drug formulations. Short-acting local anesthetic drug formulations contain lidocaine or a related local anesthetic drug dissolved in saline or other suitable injection vehicle. Typically, local anesthesia with short-acting local anesthetics last approximately 20-30 minutes. Exemplary anesthetics include, for example, non-inhalation local anesthetics such as ambucaines; amoxecaines; amylocaines; aptocaines; articaines; benoxinates; benzyl alcohols; benzocaines; betoxycaines; biphenamines; bucricaines; bumecaines; bupivacaines; butacaines; butambens; butanilicaines; carbizocaines; chloroprocaine; clibucaines; clodacaines; cocaines; dexivacaines; diamocaines; dibucaines; dyclonines; elucaines; etidocaines; euprocins; fexicaines; fomocaines; heptacaines; hexylcaines; hydroxyprocaines; hydroxytetracaines; isobutambens; ketocaines; leucinocaines; lidocaines; mepivacaines; meprylcaines; octocaines; orthocaines; oxethacaines; oxybuprocaines; phenacaines; pinolcaines; piperocaines; piridocaines; polidocanols; pramocaines; prilocaines; procaines; propanocaines; propipocaines; propoxycaines; proxymetacaines; pyrrocaines; quatacaines; quinisocaines; risocaines; rodocaines; ropivacaines; salicyl alcohols; suicaines; tetracaines; trapencaines; and trimecaines; as well as various other non-inhalation anesthetics such as alfaxalones; amolanones; etoxadrols; fentanyl; ketamines; levoxadrols; methitural; methohexitals; midazolams; minaxolones; propanidids; propoxates; pramoxines; propofols; remifentanyl; sufentanyl; tiletamines; and zolamine. The effective amount in the formulation will vary depending on the particular patient, disease to be treated, route

of administration and other considerations. Such dosages can be determined empirically.

Due to the short half-life of local anesthetics, it is often desirable to co-administer or co-formulate such anesthetics with a vasoconstrictor. Examples of vasoconstrictors include alpha adrenergic receptor agonists including catecholamines and catecholamine derivatives. Particular examples include, but are not limited to, levonordefrin, epinephrine and norepinephrine. For example, a local anesthetic formulation, such as lidocaine, can be formulated to contain low concentrations of epinephrine or another adrenergic receptor agonist such as levonordefrin. Combining local anesthetics with adrenergic receptor agonists is common in pharmaceutical preparations (see e.g., U.S. Patent No. 7,261,889 and 5,976,556). The vasoconstrictor is necessary to increase the half-life of anesthetics. The vasoconstrictor, such as epinephrine, stimulates alpha-adrenergic receptors on the blood vessels in the injected tissue. This has the effect of constricting the blood vessels in the tissue. The blood vessel constriction causes the local anesthetic to stay in the tissue much longer, resulting in a large increase in the duration of the anesthetic effect.

Generally, a vasoconstrictor is used herein in combination with an anesthetic. The anesthetic agent and vasoconstrictor can be administered together as part of a single pharmaceutical composition or as part of separate pharmaceutical compositions so long as the vasoconstrictor acts to constrict the blood vessels in the vicinity of whether the anesthetic agent has been administered to result in a prolonging of anesthesia. In one example, the anesthetic agent and vasoconstrictor are administered together in solution. In addition, the anesthetic agent and vasoconstrictor can be formulated together or separate from the compositions provided herein. Single formulations are preferred. The anesthetic agent and vasoconstrictor can be administered by injection, by infiltration or by topical administration, e.g., as part of a gel or paste. Typically, the anesthetic agent and vasoconstrictor are administered by injection directly into the site to be anesthetized, for example, by subcutaneous administration. The effective amount in the formulation will vary depending on the particular patient, disease to be treated, route of administration and other considerations. Such dosages can be determined empirically. For example,

exemplary amounts of lidocaine is or is about 10 mg to 1000 mg, 100 mg to 500 mg, 200 mg to 400 mg, 20 mg to 60 mg, or 30 mg to 50 mg. The dosage of lidocaine administered will vary depending on the individual and the route of administration. Epinephrine can be administered in amounts such as, for example, 10 µg to 5 mg, 50 µg to 1 mg, 50 µg to 500 µg, 50 µg to 250 µg, 100 µg to 500 µg, 200 µg to 400 µg, 1 mg to 5 mg or 2 mg to 4 mg. Typically, epinephrine can be combined with lidocaine in a 1:100,000 to 1:200,000 dilution, which means that 100 ml of anesthetic contains 0.5 to 1 mg of epinephrine. Volumes administered can be adjusted depending on the disease to be treated and the route of administration. Exemplary of volumes include 1 -100 ml, 1 -50 ml, 10- 50 ml, 10-30 ml, 1-20 ml, or 1-10 ml, typically 10-50 ml of an anesthetic/vasoconstrictor formulation. The administration can be subsequently, simultaneously or intermittently with administration of compositions of soluble hyaluronidases and other agents provided herein..

4. Packaging and Articles of Manufacture

Also provided are articles of manufacture containing packaging materials, a pharmaceutical composition that is effective for treating a hyaluronan-associated disease or condition, and a label that indicates that the composition and combinations are to be used for treating a hyaluronan-associated disease or condition. In one example, the pharmaceutical composition contains the hyaluronan-degrading enzyme, and no second agent or treatment. In another example, the article of manufacture contains the hyaluronan-degrading enzyme and a second agent or agents or treatment or treatments. In this example, the pharmaceutical compositions of a second agent and a hyaluronan degrading enzyme, such as a soluble hyaluronidase, can be provided together or separately, for packaging as articles of manufacture. Exemplary of articles of manufacture are containers including single chamber and dual chamber containers. The containers include, but are not limited to, tubes, bottles and syringes. The containers can further include a needle for subcutaneous administration.

The articles of manufacture provided herein contain packaging materials. Packaging materials for use in packaging pharmaceutical products are well known to those of skill in the art. See, for example, U.S. Patent Nos. 5,323,907, 5,033,252 and 5,052,558, each of which is incorporated herein in its entirety. Examples of pharmaceutical packaging materials include, but are not limited to, blister packs,

bottles, tubes, inhalers, pumps, bags, vials, containers, syringes, bottles, and any packaging material suitable for a selected formulation and intended mode of administration and treatment. A wide array of formulations of the compounds and compositions provided herein are contemplated as are a variety of treatments for any
5 hyaluronan-associated disease or condition.

Compositions effective for treating a hyaluronan-associated disease or condition (e.g. composition(s) containing a hyaluronan degrading enzyme, such as a soluble hyaluronidase, and/or a second agent or treatment, provided together or separately), also can be provided as kits. Kits can include a pharmaceutical
10 composition described herein and an item for administration. For example compositions can be supplied with a device for administration, such as a syringe, an inhaler, a dosage cup, a dropper, or an applicator. The kit can, optionally, include instructions for application including dosages, dosing regimens and instructions for modes of administration. Kits also can include a pharmaceutical composition
15 described herein and an item for diagnosis. For example, such kits can include an item for measuring the concentration, amount or activity of hyaluronan.

G. METHODS OF ASSESSING ACTIVITY, BIOAVAILABILITY AND PHARMACOKINETICS

Assays can be used to assess whether a subject has a markers that are
20 ~~associated with hyaluronan-associated diseases, conditions or disorders and is~~
therefore amendable to treatment using the methods provided herein. Such assays can include measuring the amount of hyaluronan, measuring interstitial fluid pressure, vascular volume and water content. Assays also can be used to assess the *in vitro* and *in vivo* activities of a hyaluronan degrading enzyme, including a soluble
25 hyaluronidase, as well as the *in vitro* and *in vivo* activities of other agents, such as, for example, chemotherapeutic agents, in the presence and/or absence of a hyaluronan degrading enzyme, such as a soluble modified hyaluronidase. Included among such assays are those that assess the pharmacokinetic properties of an agent that is co-administered with a modified hyaluronan degrading enzyme, such as a PEGylated
30 hyaluronan degrading enzyme (e.g. PEGylated soluble hyaluronidase) including bioavailability, and tolerability. Such assays can be used, for example, to determine

appropriate dosages of modified hyaluronan degrading enzyme and, optionally, a co-administered agent, such as a chemotherapeutic, and the frequency of dosing, for treatment.

1. Assays to assess the activity of hyaluronan degrading enzymes

5 The activity of a hyaluronan degrading enzyme can be assessed using methods well known in the art. For example, the USP XXII assay for hyaluronidase determines activity indirectly by measuring the amount of undegraded hyaluronic acid, or hyaluronan, (HA) substrate remaining after the enzyme is allowed to react with the HA for 30 min at 37°C (USP XXII-NF XVII (1990) 644-645 United States
10 Pharmacopeia Convention, Inc, Rockville, MD). A Hyaluronidase Reference Standard (USP) or National Formulary (NF) Standard Hyaluronidase solution can be used in an assay to ascertain the activity, in units, of any hyaluronidase. In one example, activity is measured using a microturbidity assay. This is based on the formation of an insoluble precipitate when hyaluronic acid binds with serum albumin.
15 The activity is measured by incubating hyaluronidase with sodium hyaluronate (hyaluronic acid) for a set period of time (e.g. 10 minutes) and then precipitating the undigested sodium hyaluronate with the addition of acidified serum albumin. The turbidity of the resulting sample is measured at 640 nm after an additional development period. The decrease in turbidity resulting from hyaluronidase activity
20 ~~on the sodium hyaluronate substrate is a measure of hyaluronidase enzymatic activity.~~
In another example, hyaluronidase activity is measured using a microtiter assay in which residual biotinylated hyaluronic acid is measured following incubation with hyaluronidase (see e.g. Frost and Stern (1997) Anal. Biochem. 251:263-269, U.S. Patent Publication No. 20050260186). The free carboxyl groups on the glucuronic
25 acid residues of hyaluronic acid are biotinylated, and the biotinylated hyaluronic acid substrate is covalently coupled to a microtiter plate. Following incubation with hyaluronidase, the residual biotinylated hyaluronic acid substrate is detected using an avidin-peroxidase reaction, and compared to that obtained following reaction with hyaluronidase standards of known activity. Other assays to measure hyaluronidase
30 activity also are known in the art and can be used in the methods herein (see e.g.

Delpach et al., (1995) Anal. Biochem. 229:35-41; Takahashi et al., (2003) Anal. Biochem. 322:257-263).

The ability of a hyaluronan degrading enzyme, such as a modified soluble hyaluronidase (eg PEGylated rHuPH20) to act as a spreading or diffusing agent also
5 can be assessed. For example, trypan blue dye can be injected, such as subcutaneously or intradermally, with or without a hyaluronan degrading enzyme into the lateral skin on each side of nude mice. The dye area is then measured, such as with a microcaliper, to determine the ability of the hyaluronan degrading enzyme to act as a spreading agent (see e.g. U.S. Published Patent No. 20060104968). The
10 effect of co-administration of a hyaluronan degrading enzyme, such as a hyaluronidase, with another agent, such as a chemotherapeutic, on the pharmacokinetic and pharmacodynamic properties of that agent also can be assessed *in vivo* using animal model and/or human subjects, such as in the setting of a clinical trial, as discussed above and demonstrated in Example 1, below. The functional
15 activity of a hyaluronan degrading enzyme that is not a hyaluronidase can be compared to a hyaluronidase using any of these assays. This can be done to determine what a functionally equivalent amount of a hyaluronan degrading enzyme is. For example, the ability of a hyaluronan degrading enzyme to act as a spreading or diffusing agent can be assessed by injecting it (e.g. subcutaneously or intradermally)
20 ~~into the lateral skin of mice with trypan blue, and the amount required to achieve the~~
same amount of diffusion as, for example, 100 units of a Hyaluronidase Reference Standard, can be determined. The amount of hyaluronan degrading enzyme required is, therefore, functionally equivalent to 100 hyaluronidase units. The hydraulic conductivity (K), such as in a tumor, before and after treatment with a modified
25 hyaluronan degrading enzyme, such as a modified hyaluronidase, also can be measured to assess the activity of a modified hyaluronan degrading enzyme preparation.

The ability of a modified hyaluronan degrading enzyme, such as a modified hyaluronidase, including pegylated hyaluronidase, to affect any one or more of the
30 markers associated with hyaluronan-associated diseases and disorders described above, or any other associated markers or phenotypes, can be assessed using any one

ore more of the assays described above. For example, the ability of a modified hyaluronan degrading enzyme, such as a modified hyaluronidase, to reduce hyaluronan levels or content, formation or size of halos, interstitial fluid pressure, water content and/or vascular volume can be assessed using any one or more of the assays above *in vitro*, *ex vivo* and/or *in vivo*. In one example, a modified hyaluronidase can be administered to a subject with a tumor or an appropriate animal model and the effect on hyaluronan levels, formation or size of halos, interstitial fluid pressure, water content and/or vascular volume assessed and compared to subjects or animal models not administered modified hyaluronidase. In some examples, the modified hyaluronidase can be administered with another agent, such as a chemotherapeutic agent.

2. Pharmacokinetics and tolerability

Pharmacokinetic and tolerability studies can be performed using animal models or can be performed during clinical studies with patients to assess the effect of co-administration with a modified hyaluronan degrading enzyme, such as a modified hyaluronidase, on the properties of an agent, such as a chemotherapeutic agent. Animal models include, but are not limited to, mice, rats, rabbits, dogs, guinea pigs and non-human primate models, such as cynomolgus monkeys or rhesus macaques. In some instances, pharmacokinetic and tolerability studies are performed using healthy animals. In other examples, the studies are performed using animal models of a disease for which therapy with hyaluronan is considered, such as animal models of any with hyaluronan-associated diseases and disorders.

The pharmacokinetic properties of, for example, a chemotherapeutic agent co-administered with modified hyaluronan degrading enzyme, such as a modified hyaluronidase, can be assessed by measuring such parameters as the maximum (peak) chemotherapeutic agent concentration ($C_{\max}$), the peak time (i.e. when maximum chemotherapeutic agent concentration occurs; $T_{\max}$), the minimum chemotherapeutic agent concentration (i.e. the minimum concentration between doses of chemotherapeutic agent; $C_{\min}$), the elimination half-life ($T_{1/2}$) and area under the curve (i.e. the area under the curve generated by plotting time versus concentration; AUC), following administration. In instances where the chemotherapeutic agent is

administered subcutaneously, the absolute bioavailability of the agent is determined by comparing the area under the curve of chemotherapeutic agent following subcutaneous delivery (AUC_{sc}) with the AUC of chemotherapeutic agent following intravenous delivery (AUC_{iv}). Absolute bioavailability (F), can be calculated using the formula: $F = ([AUC]_{sc} \times dose_{sc}) / ([AUC]_{iv} \times dose_{iv})$. The concentration of chemotherapeutic agent in the plasma following subcutaneous administration can be measured using any method known in the art suitable for assessing concentrations of chemotherapeutic agent in samples of blood.

A range of doses and different dosing frequency of dosing can be administered in the pharmacokinetic studies to assess the effect of increasing or decreasing concentrations of the co-administered agent, such as chemotherapeutic agent and/or modified hyaluronan degrading enzyme (e.g. PEGylated rHuPH20) in the dose. Pharmacokinetic properties of subcutaneously administered chemotherapeutic, such as bioavailability, also can be assessed with or without co-administration of modified hyaluronidase. For example, dogs, such as beagles, can be administered a chemotherapeutic in combination with modified hyaluronan degrading enzyme, such as a modified hyaluronidase, or alone, using one or more routes of administration. Such studies can be performed to assess the effect of co-administration with hyaluronidase on pharmacokinetic properties, such as bioavailability, of chemotherapeutic agents.

Studies to assess safety and tolerability also are known in the art and can be used herein. Following administration of a co-administered agent, such as a chemotherapeutic agent, with or without co-administration of modified hyaluronan degrading enzyme, such as a modified hyaluronidase, the development of any adverse reactions can be monitored. Adverse reactions can include, but are not limited to, injection site reactions, such as edema or swelling, headache, fever, fatigue, chills, flushing, dizziness, urticaria, wheezing or chest tightness, nausea, vomiting, rigors, back pain, chest pain, muscle cramps, seizures or convulsions, changes in blood pressure and anaphylactic or severe hypersensitivity responses. Typically, a range of doses and different dosing frequencies are administered in the safety and tolerability studies to assess the effect of increasing or decreasing concentrations of

chemotherapeutic agent and/or modified hyaluronan degrading enzyme (e.g a modified hyaluronidase) in the dose.

3. Animal models

Animal models of hyaluronan-associated diseases, disorders or conditions can be utilized to assess the *in vivo* affect of administration of modified hyaluronan degrading enzymes, such as modified hyaluronidases, with or without co-administration of another agent, such as a chemotherapeutic agent. Exemplary hyaluronan-associated diseases for which an appropriate animal model can be utilized include solid tumors, for example, late-stage cancers, a metastatic cancers, undifferentiated cancers, ovarian cancer, in situ carcinoma (ISC), squamous cell carcinoma (SCC), prostate cancer, pancreatic cancer, non-small cell lung cancer, breast cancer, colon cancer and other cancers. Also exemplary of hyaluronan-associated diseases and disorders are disc pressure, cancer and edema, for example, edema caused by organ transplant, stroke, brain trauma or other injury.

Animal models can include, but are not limited to, mice, rats, rabbits, dogs, guinea pigs and non-human primate models, such as cynomolgus monkeys or rhesus macaques. In some examples, immunodeficient mice, such as nude mice or SCID mice, are transplanted with a tumor cell line from a hyaluronan-associated cancer to establish an animal model of that cancer. Exemplary cell lines from hyaluronan-associated cancers include, but are not limited to, PC3 prostate carcinoma cells, BxPC-3 pancreatic adenocarcinoma cells, MDA-MB-231 breast carcinoma cells, MCF-7 breast tumor cells, BT474 breast tumor cells, Tramp C2 prostate tumor cells and Mat-LyLu prostate cancer cells, and other cell lines described herein that are hyaluronan associated, e.g. contain elevated levels of hyaluronan. Modified hyaluronidase can then be administered to the mice to assess the effect on, for example, hyaluronan levels or content, formation or size of halos, interstitial fluid pressure, water content and/or vascular volume. In some examples, a chemotherapeutic is co-administered with the modified hyaluronan degrading enzyme, such as a modified soluble hyaluronidase, and the effect on, for example, pharmacokinetics, tumor size or morbidity is assessed.

H. USE OF HYALURONAN DEGRADING ENZYMES IN TREATING HYALURONAN-ASSOCIATED CONDITIONS, DISEASES AND DISORDERS

5 The methods described herein include methods for therapeutic uses of hyaluronan degrading enzymes. The therapeutic uses described below are exemplary and do not limit the applications of the methods described herein.

The provided methods include methods for use of the hyaluronan-degrading enzymes to treat any hyaluronan-associated disease or condition, including, but not limited to, hyaluronan-rich cancers, and other diseases associated with hyaluronan, 10 such as other diseases associated with elevated interstitial fluid pressure, such as diseases associated with disc pressure, and edema, for example, edema caused by organ transplant, stroke, brain trauma or other injury or other edemas and other hyaluronan associated diseases and conditions.

The hyaluronan-rich cancers include cancers associated with elevated 15 interstitial fluid pressure, solid tumors, late-stage cancers, metastatic cancers, undifferentiated cancers, such as, but not limited to, ovarian cancer, in situ carcinoma (ISC), squamous cell carcinoma (SCC), prostate cancer, pancreatic cancer, non-small cell lung cancer, breast cancer, colon cancer, brain cancer and other cancers;.

The therapeutic uses include administration of the compositions, alone or in 20 combination with other treatments or agents, for treatment of a hyaluronan-associated disease or condition, including cancer treatment, reduction in tumor volume, increased sensitivity to chemotherapy or other cancer treatment, enhancing bioavailability or delivery of a cancer treating or other treating agent, decreasing interstitial fluid pressure, increasing vascular volume, decreasing water content in a 25 tissue in the subject, and other treatments.

The methods also include selection of subjects for treatment, e.g. prior to treatment of the subject, for example to determine whether the subject has a hyaluronan-associated disease or condition, for example, by using a method for assaying the expression of hyaluronan or associated molecule, for example, using the 30 methods described in Example 3(a), below, such as determining the expression of HA compared to a control, such as a control tissue, cell, fluid or other sample, for example, tissues, cells, fluids from normal subjects (e.g. subjects without a

hyaluronan-associated disease), a sample (e.g. cell line) known to express low levels of hyaluronan, such as exemplary tumor cell lines described herein, for example, the HCT 116 cell line, the HT29 cell line, the NCI H460 cell line, the DU145 cell line, the Capan-1 cell line, and tumors from tumor models generated using such cell lines (see, e.g. Example 17A).

In this example, expression of one or more markers, for example, hyaluronan, or HAS2, is measured in a sample from the subject and, optionally, compared to another sample or a standard. Following the measurement, it is determined whether the disease or condition is a hyaluronan-associated disease or condition. In one example, the method further includes treatment, for example, administration of a soluble hyaluronidase-containing composition alone or in combination with one or more other treatments. The methods further include assessment of treatment, such as by measuring the levels of hyaluronan in a cell, tissue or fluid of a subject,

1. Hyaluronan-associated conditions and diseases

Provided herein are methods for treating hyaluronan-associated diseases and conditions by administration of a composition containing a hyaluronan degrading enzyme, such as a hyaluronidase, for example, a soluble hyaluronidase, typically a soluble hyaluronidase that is modified by conjugation to a polymer (for example, to increase half-life), either alone or in combination with or in addition to another treatment and/or agent.

Hyaluronan-associated conditions and diseases are diseases and conditions in which hyaluronan levels are elevated as cause, consequence or otherwise observed in the disease or condition, and can be treated by administration of a composition containing a hyaluronan degrading enzyme, such as a hyaluronidase, for example, a soluble hyaluronidase, either alone or in combination with or in addition to another treatment and/or agent.

Typically, hyaluronan-associated diseases and conditions are associated with elevated hyaluronan expression in a tissue, cell, or body fluid (e.g. tumor tissue or tumor-associated tissue, blood, or interstitial space) compared to a control, e.g. another tissue, cell or body fluid. The elevated hyaluron expression can be elevated compared to a normal tissue, cell or body fluid, for example, a tissue, cell or body

fluid that is analogous to the sample being tested, but isolated from a different subject, such as a subject that is normal (i.e. does not have a disease or condition, or does not have the type of disease or condition that the subject being tested has), for example, a subject that does not have a hyaluronan-associated disease or condition. The elevated
5 hyaluronan expression can be elevated compared to an analogous tissue from another subject that has a similar disease or condition, but whose disease is not as severe and/or is not hyaluronan-associated or expresses relatively less hyaluronan and thus is hyaluronan-associated to a lesser degree. For example, the subject being tested can be a subject with a hyaluronan-associated cancer, where the HA amounts in the tissue,
10 cell or fluid are relatively elevated compared to a subject having a less severe cancer, such as an early stage, differentiated or other type of cancer. In another example, the cell, tissue or fluid contains elevated levels of hyaluronan compared to a control sample, such as a fluid, tissue, extract (e.g. cellular or nuclear extract), nucleic acid or peptide preparation, cell line, biopsy, standard or other sample, with a known amount
15 or relative amount of HA, such as a sample, for example a tumor cell line, known to express relatively low levels of HA, such as exemplary tumor cell lines described herein that express low levels of HA, for example, the HCT 116 cell line, the HT29 cell line, the NCI H460 cell line, the DU145 cell line, the Capan-1 cell line, and tumors from tumor models generated using such cell lines (see, e.g. Example 17A).

20 In some cases, hyaluronan-associated diseases and conditions are associated with increased interstitial fluid pressure, decreased vascular volume, and/or increased water content in a tissue, such as a tumor. In one example, treatment with the compositions and compounds provided herein ameliorates one or more of these symptoms or other symptoms associated with the disease or condition, for example,
25 improves survival or quality of life of the subject over time, or inhibits tumor growth.

Exemplary hyaluronan-associated diseases and conditions that can be treated using the provided enzymes, compositions and methods, include, but are not limited to, hyaluronan-rich cancers, for example, tumors, including solid tumors such as late-stage cancers, a metastatic cancers, undifferentiated cancers, ovarian cancer, in situ
30 carcinoma (ISC), squamous cell carcinoma (SCC), prostate cancer, pancreatic cancer, non-small cell lung cancer, breast cancer, colon cancer and other cancers.

Also exemplary of hyaluronan-associated diseases and conditions are diseases that are associated with elevated interstitial fluid pressure, such as diseases associated with disc pressure, and edema, for example, edema caused by organ transplant, stroke, brain trauma or other injury. Exemplary hyaluronan-associated diseases and
5 conditions include diseases and conditions associated with elevated interstitial fluid pressure, decreased vascular volume, and/or increased water content in a tissue, including cancers, disc pressure and edema. In one example, treatment of the hyaluronan-associated condition, disease or disorder includes amelioration, reduction, or other beneficial effect on one or more of increased interstitial fluid pressure (IFP),
10 decreased vascular volume, and increased water content in a tissue.

Typically, the hyaluronan-associated disease or condition is associated with increased HA expression, for example, in a diseased tissue, for example, a tumor. In one example, HALOs (pericellular matrix regions that are rich in proteoglycans, including hyaluronan) form in a tissue of the subject, for example, in a diseased
15 tissue. In another example, the presence of HALOs is detected in an in vitro culture of cells from a tissue of the subject, for example, a diseased tissue.

In one example, the hyaluronan-associated condition, disease or disorder is associated with increased interstitial fluid pressure, decreased vascular volume, or increased water content in a tissue. In one example, treatment of the hyaluronan-
20 associated condition, disease or disorder includes amelioration, reduction, or other beneficial effect on one or more of increased interstitial fluid pressure (IFP), decreased vascular volume, and increased water content in a tissue.

Cancers, including hyaluronan-rich cancers

Hyaluronan plays a role in processes associated with cell motility, including
25 development, regeneration, repair, embryogenesis, embryological development, wound healing, angiogenesis, and tumorigenesis (Toole 1991 Cell Biol. Extracell. Matrix, Hay (ed.), Plenum Press, New York, 1384-1386; Bertrand et al. 1992 Int. J. Cancer 52:1-6; Knudson et al, 1993 FASEB J. 7:1233-1241). In addition, hyaluronan levels correlate with tumor aggressiveness (Ozello et al. 1960 Cancer Res. 20:600-
30 604; Takeuchi et al. 1976, Cancer Res. 36:2133-2139; Kimata et al. 1983 Cancer Res. 43:1347-1354); hyaluronan promotes several cancer processes, including, but not

limited to, tumor growth, survival, metastasis and interstitial fluid pressure.

Exemplary of the hyaluronan-associated diseases and conditions that can be treated using the provided compositions containing soluble hyaluronidase and methods are cancers, particularly hyaluronan-rich cancers, for example, hyaluronan-rich cancers that are associated with elevated interstitial fluid pressure.

Hyaluronan-associated cancers are cancers associated with hyaluronan-expression, typically elevated hyaluronan expression, which can be determined, for example, prior to treatment, as described in the sections below.

For example, the hyaluronan-rich cancer can be a cancer in which the cancer cells produce HALOs in an *in vitro* particle exclusion assay, as described in Example 6A, cancers that have elevated expression of hyaluronan (as determined by immunostaining, e.g. histological staining of sections from the tumor), cancers that have elevated HAS2 (Hyaluronan synthase 2), cancers that do not produce hyaluronidase (HYAL1) *in vitro*, as determined, for example, using the enzymatic assay described in Example 2. Hyaluronan-rich cancers can be identified by any method for assessing hyaluronan expression, for example, assays as provided in Section 3(a), below, and other known methods for assaying protein/mRNA expression.

Several hyaluronan-rich cancers have been identified. In some cases, hyaluronan expression correlates with poor prognosis, for example, decreased survival rate and/or recurrence-free survival rate, metastases, angiogenesis, cancer cell invasion into other tissues/areas, and other indicators of poor prognosis. Such correlation has been observed, for example, in hyaluronan-rich tumors including ovarian cancer, SCC, ISC, prostate cancer, lung cancer, including non-small-cell lung cancer (NSCLC), breast cancer, colon cancer and pancreatic cancer (see, for example, Maarit et al., *Cancer Research*, 60:150-155 (2000); Karvinen et al., *British Journal of Dermatology*, 148:86-94 (2003); Lipponen et al., *Eur. Journal of Cancer*, 849-856 (2001); Pirinen et al., *Int. J. Cancer*: 95: 12-17 (2001); Auvinen et al., *American Journal of Pathology*, 156(2):529-536 (2000); Ropponen et al., *Cancer Research*, 58: 342-347 (1998)). Thus, hyaluronan-rich cancers can be treated by administration of a hyaluronan degrading enzyme, such as a hyaluronidase, to treat

one or more symptoms of the cancer. Hyaluronan-rich tumors include, but are not limited to, prostate, breast, colon, ovarian, stomach, head and neck and other tumors and cancers.

For example, hyaluronidase has direct anticarcinogenic effects when injected
5 into tumors. Hyaluronidase prevents growth of tumors transplanted into mice (De Maeyer et al., (1992) Int. J. Cancer 51:657-660) and inhibits tumor formation upon exposure to carcinogens (Pawlowski et al. (1979) Int. J. Cancer 23:105-109) Hyaluronidase is effective as the sole therapeutic agent in the treatment of brain cancer (gliomas) (WO 198802261).

10 Thus, provided are enzymes, compositions and combinations containing hyaluronan-degrading enzymes, such as modified soluble hyaluronidases, for treating cancers, typically hyaluronan-associated cancers.

2. Uses in treating hyaluronan-associated diseases and conditions

Provided are methods for treating hyaluronan-associated diseases and
15 conditions. The methods include administration of the compositions, alone or in combination with other treatments or agents, for treatment of a hyaluronan-associated disease or condition, including cancer treatment, reduction in tumor volume, increased sensitivity to chemotherapy or other cancer treatment, enhancing bioavailability or delivery of a cancer treating or other treating agent, decreasing
20 interstitial fluid pressure, increasing vascular volume, decreasing water content in a tissue in the subject, and other treatments.

The methods also include selection of subjects for treatment, e.g. prior to treatment of the subject, for example to determine whether the subject has a hyaluronan-associated disease or condition, for example, by using a method for
25 assaying the expression of hyaluronan or associated molecule, for example, using the methods described in Example b, below. In this example, expression of one or more markers, for example, hyaluronan, or HAS2, is measured in a sample from the subject and, optionally, compared to another sample or a standard. Following the measurement, it is determined whether the disease or condition is a hyaluronan-
30 associated disease or condition. In one example, the method further includes

treatment, for example, administration of a soluble hyaluronidase-containing composition alone or in combination with one or more other treatments.

Among the provided methods are methods for decreasing interstitial fluid pressure in a tissue of a subject with a hyaluronan-associated disease or disorder, for example, a hyaluronan-associated cancer, by administration of the compositions containing the soluble hyaluronidase. Typically, the reduction of the IFP is sustained, for example, for 24, 48, or 72 hours. Also provided are methods for increasing vascular volume in a tissue of the subject by administering the compositions, typically for sustained decrease of the vascular volume, for example, for at least 24, 48 or 72 or more hours. Also provided are methods for decreasing water content in a tissue of the subject using the compositions, where the effects typically are sustained, for example, for at least 24, 48, or 72 or more hours. Also provided are methods for reducing hyaluronan expression in the subject using the compositions, for example, to reduce pericellular matrix HALOs in a tissue of the subject. Typically, the reduction is sustained, for example, for at least 24, 48, 72 or more hours, following administration.

**a. Detection of hyaluronan-associated disease markers
(selection of subjects for treatment and assessing treatment effects)**

The methods include steps for selecting subjects for treatment with hyaluronan degrading enzymes and for assessing treatment effects, such as efficacy of treatment. Such methods include methods for detecting hyaluronan-associated disease markers, which include any indication that a subject has a hyaluronan-associated disease, that the subject is likely to respond to treatment by hyaluronan degrading enzyme, and/or that a sample from the subject, such as a tissue, cell or fluid, contains elevated hyaluronan expression. Exemplary assays for detecting markers are described below, and include assays for measuring HA expression and/or relative HA expression in a sample from a subject, assays for analyzing effects of hyaluronan-degrading enzymes on a sample from the subject, and assays for measuring readouts typically associated with certain hyaluronan-associated diseases/conditions, such as low hyaluronidase expression or activity, high interstitial fluid pressure, vascular volume and water content. In general, any known assay for detection of proteins or nucleic acids in

samples from subjects, or for assessing the effects of treatment on cells / tissues in vitro can be used.

Subjects selected for treatment in the methods provided herein include subjects having elevated, aberrant or accumulated expression of hyaluronan compared to subjects not having the disease or condition or compared to normal tissues or samples that do not have elevated, aberrant or accumulated expression of HA. Any sample or tissue from a subject can be tested and compared to a normal sample or tissue. Hyaluronan levels can be measured from any source such as from a tissue (e.g. by biopsy), tumor, cells, or from blood, serum, urine or other body fluids. For example, as described elsewhere herein, profiles of HA deposition in solid tumors have generally been categorized as pericellular or stromal. Elevated plasma levels of HA have been observed most notably in patients with Wilm's tumor, mesothelioma and liver metastases. Thus, depending on the disease or condition, a different sample can be measured for hyaluronan levels. The choice of sample is within the level of one of skill in the art.

The assay used to measure hyaluronidase substrate levels is a function of the disease or condition and can be chosen based on the particular disease or condition. One of skill in the art is familiar with methods of detecting hyaluronan, which include, but are not limited to, immunohistochemistry methods, ELISA methods, as described in section (i) below.

In one example, the step for detecting markers is performed prior to treating a subject, for example, to determine whether the subject has a hyaluronan-associated condition or disease that will be amenable to treatment with a hyaluronan-degrading enzyme. In this example, if the marker is detected (e.g. if it is determined that a cell, tissue or fluid from the patient contains elevated hyaluronan expression or is responsive to hyaluronan degrading enzyme), a treatment step is performed, where a hyaluronan-degrading enzyme is administered to the subject. In one example, when the marker is not detected (e.g. if it is determined that a cell, tissue or fluid from the patient contains normal or non-elevated hyaluronan expression or is not responsive to hyaluronan degrading enzyme) another treatment option may be selected.

In another example, the step for detecting markers is performed after treating a subject, or during the course of treatment of the subject, (e.g. treatment with the hyaluronan-degrading enzyme (e.g. soluble modified hyaluronidase) (with or without a co-administered agent)), for example, to determine whether the treatment with the
5 hyaluronan degrading enzyme is having an effect on treating the disease or condition. In one such example, the marker is not detected or is detected at an amount or relative level that is decreased compared to the amount/level prior to treatment, or compared to another sample, treatment is continued, another round of treatment is performed, or another treatment, such as a combination therapy, is initiated. In another such
10 example, if the marker is detected at the same level as prior to treatment or another sample, another treatment option may be selected.

i. Assays for detection of hyaluronan-associated disease markers

The assays to detect markers of hyaluronan-associated diseases and conditions
15 include assays to measure amount (e.g. relative amount) of hyaluronan and/or hyaluronidase expression in a tissue, cell and/or body fluid of a subject, for example, a tumor. Included amongst such assays are those that can detect HA expression, Hyaluronan synthase 2 (HAS2) expression, the presence of HALOs (pericellular matrix regions that are rich in proteoglycans, including hyaluronan), and the presence
20 of hyaluronan-degrading enzymes, such as hyaluronidases, for example, in samples from the subject.

Assays to detect protein and nucleic acid levels are well known in the art and can be used in the methods herein to measure hyaluronan, hyaluronan synthase or other protein and/or nucleic acid expression. Such assays include, but are not limited to, ELISA, SDS-PAGE, Western Blot, PCR, RT-PCR, immunohistochemistry,
25 histology and flow cytometry. For example, a sample from a subject, such as a tissue sample (e.g. a biopsy of a tumor from a patient or animal model, a stromal sample), a fluid (e.g. blood, urine, plasma, saliva or other sample), a cell or cellular sample, or extract, or other sample, can be stained with anti-HA antibodies, for example, using
30 histological staining, such as immunohistochemistry (IHC) of fixed or frozen tissue sections, to determine the presence and extent of hyaluronan in the tissue or sample

(see, e.g. Example 8.B.2.), or immunofluorescent cellular staining, pull-down assays, and flow cytometry. In another example, the sample, e.g. biopsy, can be assayed by RT-PCR to assess the amount of HA mRNA.

Known methods for detection of hyaluronan-expression in cancer include, but
5 are not limited to, the ELISA-like assay described in Lokeshwar et al., *Cancer Res.*
57: 773-777 (1997), for measuring HA levels in urine or bladder tissue extracts of
subjects having bladder cancer. For the assay, urine or extracts are coated on
microwell plates (umbilical cord HA used as a standard also is coated), followed by
incubation (e.g. 16 hours, room temperature) with a labeled (e.g. biotinylated) HA
10 binding protein, such as those described herein, washed and the HA-binding protein
bound to the wells quantified using an avidin-biotin detection agent substrate. Such
methods are well known in the art. In one example, the urine from a subject with an
HA-associated bladder cancer contained HA levels that were elevated 2-9 fold
compared to urine/extracts from normal patients (healthy subjects or subjects with
15 other gastrourinary diseases or conditions); thus the marker would be detected if the
HA levels in the urine was elevated compared to normal subjects, e.g. elevated from
between at or about 2-fold and at or about 9-fold, e.g. at or about 2, 3, 4, 5, 6, 7, 8 or
9-fold elevation compared to normal subject.

In a further example, hyaluronan expression and production in tumor cells *in*
20 *vitro* can be assessed using any one of the methods described above. Similarly,
Hyaluronan synthase 2 (HAS2) production and/or expression by cells *in vitro*, *ex vivo*
or *in vivo* also can be assayed by, for example, ELISA, SDS-PAGE, Western Blot,
PCR, RT-PCR, immunohistochemistry, histology or flow cytometry.

In another example, the amount of hyaluronidase activity in a sample from the
25 subject is determined, such as in the blood or plasma, for example, as described in
Example 2, such as with a turbidity assay.

In another example, a cell or other tissue from a patient is isolated, e.g. a
tumor cell, and used in a study to determine whether the cell or tissue is responsive to
treatment with the hyaluronan degrading enzyme *in vitro*, for example, using a
30 clonogenic assay or any other assay for measuring growth, proliferation and/or
survival of cells or tissues, such as tumor cells, in response to treatment. In one

example, a study as described in Example 7D, below, by seeding cancer cells from a subject on surface, such as an extracellular matrix or protein mixture, such as the mixture sold under the trade name Matrigel® (BD Biosciences). In this example, the hyaluronan-associated marker is the sensitivity of the cell or tissue to administration of hyaluronan degrading enzyme. In this example, if any property, such as proliferation, growth or survival of the cells, is inhibited or blocked by addition of hyaluronan degrading enzyme, it is determined that the subject may be amenable to treatment with hyaluronan degrading enzyme containing compositions.

In addition to assays for determining hyaluronan expression levels, other assays can be used to select a subject for treatment, and/or to assess treatment efficacy and/or duration. Interstitial fluid pressure (IFP) can be measured using an appropriate probe or instrument. For example, a transducer-tipped catheter can be used to measure the IFP in cancer tissues or other tissues of interest. The catheter is passed through the inner bore of a surgical needle, which is then inserted into the center of the tumor. The needle is withdrawn while the catheter is held in position. The IFP (mmHg) can then be measured using an appropriate data acquisition unit (see e.g. Example 6B, Ozerdem et al. (2005) Microvasc. Res. 70:116-120). Other methods to measure IFP include the wick-in-needle method (Fadnes et al (1977) Microvasc. Res. 14:27-36).

Vascular volume can be measured by, for example, ultrasound imaging, such as described in Example 10, below. This method employs hyper-echoic microbubbles to provide the strong ultrasound wave reflections that are detected. The microbubbles, when injected, such as intravenously, into a subject or animal model, become trapped in the vascular space due to their size. Assays to assess tissue water content, such as tumor tissue water content, also are known in the art. For example, samples from a tumor can be harvested, blotted, weighed and snap frozen before being lyophilized. The water weight is then reported as the tissue wet weight to dry (i.e. lyophilized) weight ratio.

The ability of a tumor cell to form pericellular matrices (halos) *in vitro* can be assessed using a particle exclusion assay (see e.g. Example 6). Small particles (formalin-fixed red blood cells) can be added to low-density cultures of tumor cells in the presence of, for example, aggrecan, which is a large aggregating chondroitin

sulfate proteoglycan. After the particles settle, the cultures can be viewed at 400x magnification to determine whether any halos were formed by the tumor cells. This can be visualized as areas around the cells from which the particles are excluded.

ii. Detection of hyaluronan-associated markers relative to control samples

For any of the detection methods, the marker (e.g. HA expression, responsiveness to hyaluronan degrading enzyme, HA-synthase expression or hyaluronidase activity) typically is compared to a control sample, such that detection of the marker typically includes determining that the readout is elevated or reduced compared to the control sample.

For example, the control sample can be another tissue, cell or body fluid, such as a normal tissue, cell or body fluid, for example, a tissue, cell or body fluid that is analogous to the sample being tested, but isolated from a different subject, such as a subject that is normal (i.e. does not have a disease or condition, or does not have the type of disease or condition that the subject being tested has), for example, a subject that does not have a hyaluronan-associated disease or condition, or an analogous tissue from another subject that has a similar disease or condition, but whose disease is not as severe and/or is not hyaluronan-associated or expresses relatively less hyaluronan and thus is hyaluronan-associated to a lesser degree. For example, when the cell, tissue or fluid being tested is a subject having a cancer, it can be compared to a tissue, cell or fluid from a subject having a less severe cancer, such as an early stage, differentiated or other type of cancer. In another example, control sample is a fluid, tissue, extract (e.g. cellular or nuclear extract), nucleic acid or peptide preparation, cell line, biopsy, standard or other sample, with a known amount or relative amount of HA, such as a sample, for example a tumor cell line, known to express relatively low levels of HA, such as exemplary tumor cell lines described herein that express low levels of HA, for example, the HCT 116 cell line, the HT29 cell line, the NCI H460 cell line, the DU145 cell line, the Capan-1 cell line, and tumors from tumor models generated using such cell lines (see, e.g. Example 17A).

It is understood that the particular change, e.g. increase in or decrease in HA, is dependent on the assay used. For example, in an ELISA, the fold increase or

decrease in absorbance at a particular wavelength or in quantity of protein (e.g. as determined by using a standard curve) can be expressed relative to a control. In a PCR assay, such as RT-PCR, can be compared to control expression levels (e.g. expressed as fold change) using methods known to those in the art, such as using standards.

For example, when the amount of hyaluronan in a sample from a subject is being tested, detection of the marker can be determining that the amount of HA in the sample (e.g. cancerous cell, tissue or fluid) from the subject is elevated compared to a control sample, such as a control sample described in the previous paragraph. In one example, the cancer is determined to be a hyaluronan-rich cancer if the amount of HA in the tissue, cell or fluid is elevated at or about 0.5-fold, 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 11-fold, 12-fold, 13-fold, 14-fold, 15-fold, 20-fold, or more, compared to the control sample, which can be, for example, but not limited to, a fluid, tissue, extract (e.g. cellular or nuclear extract), nucleic acid or peptide preparation, cell line, biopsy, standard or other sample, with a known amount or relative amount of HA, such as a sample, for example a tumor cell line, known to express relatively low levels of HA, such as exemplary tumor cell lines described herein that express low levels of HA, for example, the HCT 116 cell line, the HT29 cell line, the NCI H460 cell line, the DU145 cell line, the Capan-1 cell line, and tumors from tumor models generated using such cell lines (see, e.g. Example 17A).

For example, for purposes herein, patients having hyaluronan-rich tumors can be selected for treatment with a hyaluronidase alone or in combination with a second agent. In such examples, the tumor can be directly biopsied and stained for expression of HA. In other examples, a sample, such as a blood or urine sample or other bodily fluid sample associated with the particular tumor can be assayed for HA. The type of assay will vary depending on the tumor-type, although it is contemplated that more than one assay can be used to detect HA. References herein to such assays for particular tumors are for illustration only. For example, for bladder cancers, urine samples can be assayed for hyaluronan by standard ELISA procedures. For purposes herein, subjects that exhibit 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-

fold, 9-fold, 10-fold or more HA compared to urine from normal patient controls (see e.g., Lokeshwar et al. (2000) J. Urol., 163:348-56), can be selected. In another example, tumor cells can be biopsied and stained for HA, such as by immunohistochemistry (see e.g., Anttila et al. (2000) Cancer Research, 60:150-156; Karvinen et al. (2003) British J of Dermatology, 148:86-94; Lipponen et al. (2001) Euro J Can. 37: 849-856); Auvinen et al. (2000) American J of Pathology, 156:529;). Generally, in such examples, a tumor sample or tumor cell is considered positive for HA if any cancer-cell associated HA signal is observed. As a negative control for background staining, cells can be predigested with a hyaluronidase to cleave all cell-associated HA. Samples also can be compared to a normal cell or tissue from the same subject. In addition, in such methods, the level of cell-associated hyaluronan can be scored as low, moderate or high. For example, HA expression is considered high or moderate if 30%, 35%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90% or more of the tumoral area showed persistent HA signal. Typically, treatment of subjects with moderate to high HA is contemplated herein.

b. Use in treating cancers

As noted above, hyaluronan plays a role in processes associated with cancer and hyaluronan levels correlate with tumor aggressiveness, and various markers for tumor aggressiveness and poor prognosis. Thus, provided are methods for treating hyaluronan-associated cancers with hyaluronan degrading enzymes and compositions and combinations containing hyaluronan degrading enzymes, including hyaluronidases. The cancers include hyaluronan-rich cancers and cancers that are associated with elevated interstitial fluid pressure.

Hyaluronan-associated cancers are cancers associated with hyaluronan-expression, typically elevated hyaluronan expression. In one example, the expression of hyaluronan is elevated in a sample, e.g. a cellular sample, a tissue, tumor stromal, plasma, blood or other sample, from a subject with the cancer, for example, elevated expression compared to a sample from a non-diseased tissue, cell, stroma, plasma, blood, or other sample, or from a less severe cancer, for example, an early stage, differentiated or other type of cancer. The level of hyaluronan can be determined prior to treating the subject, for example, as described above, for example, in

Section3(a), above, by histological or other known methods for assessing expression of polypeptide or mRNA.

In one example, the hyaluronan degrading enzyme (e.g. a hyaluronidase) is administered systemically, for example, intravenously (IV), intramuscularly, or by
5 any another systemic route. In another example, the hyaluronan degrading enzyme composition is administered locally, for example, intra-tumorally.

The methods of treatment include repeated administration of the hyaluronan degrading enzyme, for example, administration hourly, every several hours, three times daily, twice daily, once daily, every other day, every third day, every week,
10 every other week, every third week, monthly, or other repeated administration, and also include methods for continuous administration over a period of time.

In one example, treatment of the hyaluronan-associated cancer includes lowering of interstitial fluid pressure in a hyaluronan dependent manner. For example, intravenous administration of a hyaluronan degrading enzyme, such as a
15 hyaluronidase, particularly administration of a modified soluble hyaluronidase (e.g. PEGylated rHuPH20) reduces elevated interstitial fluid pressure in a hyaluronan-rich (PC3) but not a hyaluronan deficient (NCI H460) human carcinoma xenograft model (see Examples 6 and 8-9 below). As shown in the Examples below, interstitial fluid pressure correlates with tumor size in a hyaluronan-rich animal model. Thus,
~~20 provided herein are methods for treating cancer, e.g. by administering a hyaluronan~~
degrading enzyme, such as a soluble hyaluronidase (e.g. by systemic administration, including intravenous administration), typically a polymer-conjugated hyaluronidase, thereby reducing elevated interstitial fluid pressure, in subjects with hyaluronan associated cancers.

25 Treatment with the hyaluronan degrading enzyme, such as a hyaluronidase, also can include increasing vascular volume and/or decreasing water content in the tumor, for example, by intravenous administration of the hyaluronidase,

In addition to treatment of the disease with the hyaluronan degrading enzyme alone, such as a hyaluronidase alone, the compositions and methods provided herein
30 also can be used to treat hyaluronan-associated cancers by administration of the hyaluronan degrading enzyme in combination with, for example, simultaneously or

prior to, a chemotherapeutic or other anti-cancer agent or treatment. In this example, the hyaluronan degrading enzyme, such as a hyaluronidase, typically enhances penetration of chemotherapeutic or other anti-cancer agents into solid tumors, thereby treating the disease. The hyaluronan degrading enzyme, such as a hyaluronidase, can be injected intratumorally with anti-cancer agents or intravenously for disseminated cancers or hard to reach tumors.

Anti-Cancer Agents and Other Treatments

The anticancer agent can be a chemotherapeutic, an antibody, a peptide, or a gene therapy vector, virus or DNA. Additionally, hyaluronan degrading enzymes, such as a hyaluronidase, can be used to recruit tumor cells into the cycling pool for sensitization in previously chemorefractory tumors that have acquired multiple drug resistance (St Croix et al., (1998) Cancer Lett September 131(1): 35-44). Hyaluronan degrading enzymes, including hyaluronidases, such as, for example, rHuPH20, also can enhance delivery of biologics such as monoclonal antibodies, cytokines and other drugs to tumors that accumulate glycosaminoglycans.

Exemplary anti-cancer agents that can be administered after, coincident with or before administration of the hyaluronan degrading enzyme, such as a hyaluronidase, include, but are not limited to Acivicins; Aclarubicins; Acodazoles; Acronines; Adozelesins; Aldesleukins; Alemtuzumabs; Alitretinoins (9-Cis-Retinoic Acids); Allopurinols; Altretamines; Alvocidibs; Ambazones; Ambomycins; Ametantrones; Amifostines; Aminoglutethimides; Amsacrine; Anastrozoles; Anaxirones; Ancitabines; Anthramycins; Apaziquones; Argimesnas; Arsenic Trioxides; Asparaginases; Asperlins; Atrimustines; Azacitidines; Azetepas; Azotomycins; Banoxantrones; Batabulins; Batimastats; BCG Live; Benaxibines; Bendamustines; Benzodepas; Bexarotenes; Bevacizumab; Bicalutamides; Bietaserpines; Biricodars; Bisantrones; Bisantrones; Bisnafide Dimesylates; Bizelesins; Bleomycins; Bortezomibs; Brequinars; Bropirimines; Budotitanes; Busulfans; Cactinomycins; Calusterones; Canertinibs; Capecitabines; Caracemides; Carbetimers; Carboplatins; Carboquones; Carmofurs; Carmustines with Polifeprosans; Carmustines; Carubicins; Carzelesins; Cedefingols; Celecoxibs; Cemadotins; Chlorambucils; Cioteronels; Cirolemycins; Cisplatins; Cladribines;

- Clanfenurs; Clofarabines; Crisnatols; Cyclophosphamides; Cytarabine liposomals;
Cytarabines; Dacarbazines; Dactinomycins; Darbepoetin Alfas; Daunorubicin
liposomals; Daunorubicins/Daunomycins; Daunorubicins; Decitabines; Denileukin
Difitoxes; Dexniguldipines; Dexonnaplatins; Dexrazoxanes; Dezaguanines;
- 5 Diaziquones; Dibrospidium; Dienogests; Dinalins; Disermolides; Docetaxels;
Dofequidars; Doxifluridines; Doxorubicin liposomals; Doxorubicin HCL;
Docorubicin HCL liposome injection; Doxorubicins; Droloxifenes; Dromostanolone
Propionates; Duazomycins; Ecomustines; Edatrexates; Edotecarins; Eflornithines;
Elacridars; Elinafides; Elliott's B Solutions; Elsamitrucins; Emitefurs; Enloplatins;
- 10 Enpromates; Enzastaurins; Epiropidines; Epirubicins; Epoetin alfas; Eptaloprosts;
Erbulozoles; Esorubicins; Estramustines; Etanidazoles; Etoglucids; Etoposide
phosphates; Etoposide VP-16s; Etoposides; Etoprines; Exemestanes; Exisulinds;
Fadrozoles; Fazarabines; Fenretinides; Filgrastims; Floxuridines; Fludarabines;
Fluorouracils; 5-fluorouracils; Fluoxymesterones; Flurocitabines; Fosquidones;
- 15 Fostriecins; Fostriecins; Fotretamines; Fulvestrants; Galarubicins; Galocitabines;
Gemcitabines; Gemtuzumabs/Ozogamicins; Geroquinols; Gimatécans; Gimeracils;
Gloxazones; Glufosfamides; Goserelin acetates; Hydroxyureas;
Ibritumomabs/Tiuxetans; Idarubicins; Ifosfamides; Ilmofofines; Ilomastats; Imatinib
mesylates; Imexons; Improsulfans; Indisulams; Inproquones; Interferon alfa-2as;
-
- 20 Interferon alfa-2bs; Interferon Alfas; Interferon Betas; Interferon Gammas;
Interferons; Interleukin-2s and other Interleukins (including recombinant
Interleukins); Intoplicines; Iobenguanes [131-I]; Iproplatins; Irinotecans; Irsogladines;
Ixabepilones; Ketotrexates; L-Alanosines; Lanreotides; Lapatinibs; Ledoxantrones;
Letrozoles; Leucovorins; Leuprolides; Leuprorelins (Leuprorelides); Levamisoles;
- 25 Lexacalcitols; Liarozoles; Lobaplatins; Lometrexols; Lomustines/CCNUs;
Lomustines; Lonafarnibs; Losoxantrones; Lurtotecans; Mafosfamides; Mannosulfans;
Marimastats; Masoprocals; Maytansines; Mechlorethamines;
Mechlorethamines/Nitrogen mustards; Megestrol acetates; Megestrols; Melengestrols;
Melfhalans; Melfhalans/L-PAMs; Menogarils; Mepitiostanes; Mercaptopurines; 6-
- 30 Mecaptopurine; Mesnas; Metesinds; Methotrexates; Methoxsalens; Metomidates;
Metoprines; Meturedepas; Miboplatins; Miproxifenes; Misonidazoles; Mitindomides;

- Mitocarcins; Mitocromins; Mitoflaxones; Mitogillins; Mitoguazones; Mitomalcins;
Mitomycin Cs; Mitomycins; Mitonafides; Mitoquidones; Mitospers; Mitotanes;
Mitoxantrones; Mitozolomides; Mivobulins; Mizoribines; Mofarotenes; Mopidamols;
Mubritinibs; Mycophenolic Acids; Nandrolone Phenpropionates; Nedaplatins;
5 Nelzarabines; Nemorubicins; Nitracrine; Nocodazoles; Nofetumomabs;
Nogalamycins; Nolatrexeds; Nortopixantrones; Octreotides; Oprelvekins;
Ormaplatins; Ortataxels; Oteracils; Oxaliplatin; Oxisurans; Oxophenarsines;
Paclitaxels; Pamidronates; Patubilones; Pegademases; Pegaspargases; Pegfilgrastims;
Peldesines; Peliomycins; Pelitrexols; Pemetrexeds; Pentamustines; Pentostatins;
10 Peplomycins; Perfosfamides; Perifosines; Picoplatins; Pinafides; Pipobromans;
Piposulfans; Pirfenidones; Piroxantrones; Pixantrones; Plevitrexeds; Plicamycin
Mithramycins; Plicamycins; Plomestanes; Plomestanes; Porfimer sodiums; Porfimers;
Porfirimycins; Prednimustines; Procarbazines; Propamidines; Prospidium;
Pumitepas; Puromycins; Pyrazofurins; Quinacrine; Ranimustines; Rasburicases;
15 Riboprines; Ritrosulfans; Rituximabs; Rogletimides; Roquinimex;
Rufocromomycins; Sabarubicins; Safingols; Sargramostims; Satraplatins;
Sebriplatin; Semustines; Simtrazenes; Sizofirans; Sobuzoxanes; Sorafenibs;
Sparfosates; Sparfosic Acids; Sparsomycins; Spirogermaniums; Spiromustines;
Spiroplatin; Spiroplatin; Squalamines; Streptonigrins; Streptovarycins;
20 ~~Streptozocins; Sufosfamides; Sulofenurs; Sunitinib Malate; 6-thioguanine (6-TG);~~
Tacedinalines; Talcs; Talisomycins; Tallimustines; Tamoxifens; Tariquidars;
Tauromustines; Tecogalans; Tegafurs; Teloxantrones; Temoporfin; Temozolomides;
Teniposides/VM-26s; Teniposides; Teroxirones; Testolactones; Thiamiprines;
Thioguanines; Thiotepa; Tiamiprines; Tiazofurins; Tilomisoles; Tilorones;
25 Timcodars; Timonacics; Tirapazamines; Topixantrones; Topotecans; Toremifenes;
Tositumomabs; Trabectedins (Ecteinascidin 743); Trastuzumabs; Trestolones;
Tretinoin/ATRA; Triciribines; Trilostanes; Trimetrexates; Triplatin Tetranitrates;
Triptorelins; Trofosfamides; Tubulozoles; Ubenimex; Uracil Mustards; Uredepas;
Valrubicins; Valspodars; Vapreotides; Verteporfin; Vinblastines; Vincristines;
30 Vindesines; Vinepidines; Vinflunines; Vinformides; Vinglycinates; Vinleucinols;
Vinleurosines; Vinorelbines; Vinrosidines; Vintriptols; Vinzolidines; Vorozoles;

Xanthomycin As (Guamecyclines); Zeniplatins; Zilascorbs [2-H]; Zinostatins; Zoledronate; Zorubicins; and Zosuquidars, for example:

- Aldesleukins (e.g. PROLEUKIN®); Alemtuzumabs (e.g. CAMPATH®); Alitretinoin (e.g. PANRETIN®); Allopurinols (e.g. ZYLOPRIM®); Altretamines (e.g. HEXALEN®); Amifostines (e.g. ETHYOL®); Anastrozoles (e.g. ARIMIDEX®); Arsenic Trioxides (e.g. TRISENOX®); Asparaginases (e.g. ELSPAR®); BCG Live (e.g. TICE® BCG); Bexarotenes (e.g. TARGRETIN®); Bevacizumab (AVASTIN®); Bleomycins (e.g. BLENOXANE®); Busulfan intravenous (e.g. BUSULFEX®); Busulfan orals (e.g. MYLERAN®); Calusterones (e.g. METHOSARB®); Capecitabines (e.g. XELODA®); Carboplatins (e.g. PARAPLATIN®); Carmustines (e.g. BCNU®, BiCNU®); Carmustines with Polifeprosans (e.g. GLIADEL® Wafer); Celecoxibs (e.g. CELEBREX®); Chlorambucils (e.g. LEUKERAN®); Cisplatin (e.g. PLATINOL®); Cladribines (e.g. LEUSTATIN®, 2-CdA®); Cyclophosphamides (e.g. CYTOXAN®, NEOSAR®); Cytarabines (e.g. CYTOSAR-U®); Cytarabine liposomals (e.g. DepoCyt®); Dacarbazines (e.g. DTIC-Dome); Dactinomycins (e.g. COSMEGEN®); Darbepoetin Alfa (e.g. ARANESP®); Daunorubicin liposomals (e.g. DANUOXOME®); Daunorubicins/Daunomycins (e.g. CERUBIDINE®); Denileukin Diftitoxes (e.g. ONTAK®); Dexrazoxanes (e.g. ZINECARD®); Docetaxels (e.g. TAXOTERE®); Doxorubicins (e.g. ADRIAMYCIN®, RUBEX®); Doxorubicin liposomals, including Doxorubicin HCL liposome injections (e.g. DOXIL®); Dromostanolone propionates (e.g. DROMOSTANOLONE® and MASTERONE® Injection); Elliott's B Solutions (e.g. Elliott's B Solution®); Epirubicins (e.g. ELLENCE®); Epoetin alfa (e.g. EPOGEN®); Estramustines (e.g. EMCYT®); Etoposide phosphates (e.g. ETOPOPHOS®); Etoposide VP-16s (e.g. VEPESID®); Exemestanes (e.g. AROMASIN®); Filgrastims (e.g. NEUPOGEN®); Floxuridines (e.g. FUDR®); Fludarabines (e.g. FLUDARA®); Fluorouracils incl. 5-FUs (e.g. ADRUCIL®); Fulvestrants (e.g. FASLODEX®); Gemcitabines (e.g. GEMZAR®); Gemtuzumabs/Ozogamicins (e.g. MYLOTARG®); Goserelin acetates (e.g. ZOLADEX®); Hydroxyureas (e.g. HYDREA®); Ibritumomabs/Tiuxetans (e.g. ZEVALIN®); Idarubicins (e.g. IDAMYCIN®); Ifosfamides (e.g. IFEX®); Imatinib

- mesylates (e.g. GLEEVEC®); Interferon alfa-2as (e.g. ROFERON-A®); Interferon alfa-2bs (e.g. INTRON A®); Irinotecans (e.g. CAMPTOSAR®); Letrozoles (e.g. FEMARA®); Leucovorins (e.g. WELLCOVORIN®, LEUCOVORIN®); Levamisoles (e.g. ERGAMISOL®); Lomustines/CCNUs (e.g. CeeBU®);
- 5 Meclorethamines/Nitrogen mustards (e.g. MUSTARGEN®); Megestrol acetates (e.g. MEGACE®); Melphalans/L-PAMs (e.g. ALKERAN®); Mercaptopurine, including 6-mercaptopurines (6-MPs; e.g. PURINETHOL®); Mesnas (e.g. MESNEX®); Methotrexates; Methoxsalens (e.g. UVADEX®); Mitomycin Cs (e.g. MUTAMYCIN®, MITOZYTREX®); Mitotanes (e.g. LYSODREN®);
- 10 Mitoxantrones (e.g. NOVANTRONE®); Nandrolone Phenpropionates (e.g. DURABOLIN-50®); Nofetumomabs (e.g. VERLUMA®); Oprelvekins (e.g. NEUMEGA®); Oxaliplatins (e.g. ELOXATIN®); Paclitaxels (e.g. PAXENE®, TAXOL®); Pamidronates (e.g. AREDIA®); Pegademases (e.g. ADAGEN®); Pegaspargases (e.g. ONCASPAR®); Pegfilgrastims (e.g. NEULASTA®);
- 15 Pentostatins (e.g. NIPENT®); Pipobromans (e.g. VERCYTE®); Plicamycin/Mithramycins (e.g. MITHRACIN®); Porfimer sodiums (e.g. PHOTOFRIN®); Procarbazines (e.g. MATULANE®); Quinacrine (e.g. ATABRINE®); Rasburicases (e.g. ELITEK®); Rituximabs (e.g. RITUXAN®); Sargramostims (e.g. PROKINE®); Streptozocins (e.g. ZANOSAR®); Sunitinib
-
- 20 Malates (e.g. SUTENT®); Talcs (e.g. SCLEROSOL®); Tamoxifens (e.g. NOLVADEX®); Temozolomides (e.g. TEMODAR®); Teniposides/VM-26s (e.g. VUMON®); Testolactones (e.g. TESLAC®); Thioguanines including, 6-thioguanine (6-TG); Thiotepas (e.g. THIOPLEX®); Topotecans (e.g. HYCAMTIN®); Toremifenes (e.g. FARESTON®); Tositumomabs (e.g. BEXXAR®); Trastuzumabs
- 25 (e.g. HERCEPTIN®); Tretinoin/ATRA (e.g. VESANOID®); Uracil Mustards; Valrubicins (e.g. VALSTAR®); Vinblastines (e.g. VELBAN®); Vincristines (e.g. ONCOVIN®); Vinorelbines (e.g. NAVELBINE®); and Zoledronates (e.g. ZOMETA®).

- In one example, a hyaluronan degrading enzyme, such as a modified
- 30 hyaluronidase, for example, PEGylated rHuPH20, is administered to a subject after, coincident with or before administration of one or more of docetaxel (e.g.

TAXOTERE®), Doxorubicin liposomal (e.g. DOXIL®), Sunitinib Malate (e.g. SUTENT®) or Bevacizumab (AVASTIN®).

Hyaluronan degrading enzymes, including hyaluronidases, can also be used to increase the sensitivity of tumors that are resistant to conventional chemotherapy. For example, hyaluronan degrading enzymes, including hyaluronidases, such as rHuPH20, can be administered to a patient having a tumor associated with a HYAL1 defect in an amount effective to increase diffusion around the tumor site (e.g., to facilitate circulation and/or concentrations of chemotherapeutic agents in and around the tumor site), inhibit tumor cell motility, such as by hyaluronic acid degradation, and/or to lower the tumor cell apoptosis threshold. This can bring the tumor cell(s) to a state of anoikis, which renders the tumor cell more susceptible to the action of chemotherapeutic agents. Administration of a hyaluronan degrading enzyme, such as a hyaluronidase, can induce responsiveness of previously chemotherapy-resistant tumors of the pancreas, stomach, colon, ovaries, and breast (Baumgartner et al. (1988) Reg. Cancer Treat. 1:55-58; Zanker et al. (1986) Proc. Amer. Assoc. Cancer Res. 27:390).

In one example, hyaluronan degrading enzymes, in particular, hyaluronidases, are used in the treatment of metastatic and non-metastatic cancers, including those that have decreased endogenous hyaluronidase activity relative to non-cancerous cells. Hyaluronan degrading enzymes such as hyaluronidases can be used as a chemotherapeutic agent alone or in combination with other chemotherapeutics. Exemplary cancers include, but are not limited to, small lung cell carcinoma, squamous lung cell carcinoma, and cancers of the breast, ovaries, head and neck, or any other cancer associated with depressed levels of hyaluronidase activity or decreased hyaluronic acid catabolism.

c. Use in treating other diseases associated with elevated interstitial fluid pressure

The provided compositions and methods also can be used to treat other hyaluronan-associated diseases associated with high interstitial fluid pressure, including, but not limited to disc pressure and edema, including edema caused by organ transplant, stroke, brain trauma or other conditions described herein.

3. Use as a spreading agent

In one example, the hyaluronan degrading enzyme, such as a hyaluronidase, for example rHuPH20 produced using the methods described herein, is administered to treat a hyaluronan-associated disease or condition by promoting or enhancing the delivery of agent(s) and/or molecules to any of a variety of mammalian tissues *in vivo*. The hyaluronan degrading enzymes, including hyaluronidases can be used to facilitate the diffusion and, therefore, promote the delivery, of small molecule pharmacologic agents as well as larger molecule pharmacologic agents, such as proteins, nucleic acids and ribonucleic acids, and macromolecular compositions that can contain a combination of components including, but not limited to, nucleic acids, proteins, carbohydrates, lipids, lipid-based molecules and drugs. For example, molecules and macromolecular complexes ranging from about 10 nm to about 500 nm in diameter, can exhibit dramatic improvements in delivery through interstitial spaces when the interstitial space has been previously, or is coincidentally, exposed to hyaluronidase (see e.g. U.S. Patent Application Nos. 10/795,095, 11/065,716 and 11/238,171).

Examples of pharmaceutical, therapeutic, treatment and cosmetic agents, molecules and treatments that can be administered with or following administration of the hyaluronan degrading enzyme, such as a soluble hyaluronidase, include, but are not limited to, anesthetics; anti-metabolites, anti-neoplastics, chemotherapeutics, and other anti-cancer agents and anti-cancer treatments; anti-virals; anti-infectives, including anti-bacterials and other antibiotics, anti-fungals and other anti-infectives; immunomodulatory agents; steroidal and non-steroidal anti-inflammatories; beta blockers; sympathomimetics; ducosanoids, prostaglandins and prostaglandin analogs; miotics, cholinergics and anti-cholinesterases; anti-allergenics and decongestants; hormonal agents; growth factors; immunosuppressants; vaccines and toxoids; immune sera; antibodies; analgesic agents, anti-inflammatory agents, antimicrobial agents, amoebicidal agents, trichomonocidal agents, anti-parkinson's disease agents, anti-malarial agents, anticonvulsant agents, anti-depressant agents, antiarthritis agent, anti-fungal agent, antihypertensive agent, antipyretic agent, anti-parasitic agent, antihistamine agent, alpha-adrenergic agonist agent, alpha blocker agent, anesthetic

agent, bronchial dilator agent, biocide agent, bactericide agent, bacteriostatic agent, beta adrenergic blocker agent, calcium channel blocker agent, cardiovascular drug agent, contraceptive agent, cosmetic or esthetic agent, decongestant agent, diuretic agent, depressant agent, a diagnostic agent, an electrolyte agent, a hypnotic agent, a hormone agent, hyperglycemic agent, muscle relaxant agent, muscle contractant agent, ophthalmic agent, parasympathomimetic agent, psychic energizer agent, sedative agent, sleep inducer, sympathomimetic agent, tranquilizer agent, urinary agent, vaginal agent, viricide agent, vitamin agent, non-steroidal anti-inflammatory agent, and angiotensin converting enzyme inhibitor agent and combinations thereof.

10 4. **Use in hypodermoclysis**

Hypodermoclysis, the infusion of fluids and electrolytes into the hypodermis of the skin, is a useful and simple hydration technique suitable for mildly to moderately dehydrated adult patients, especially the elderly. Although considered safe and effective, the most frequent adverse effect is mild subcutaneous edema that can be treated by local massage or systemic diuretics. Approximately 3 L can be given in a 24-hour period at two separate sites. Common infusion sites include the chest, abdomen, thighs and upper arms. Solutions used in hypodermoclysis include, for example, normal saline, half-normal saline, glucose with saline and 5% glucose. Potassium chloride also can be added to the solution. The addition of one or more

20 hyaluronan degrading enzymes, such as a hyaluronidase, to the solution can enhance fluid absorption and increase the overall rate of administration. Thus, the provided compositions and methods can be used to treat hypodermoclysis by administration to a subject.

25 5. **Application on vitrectomy and ophthalmic disorders and conditions**

The provided compositions containing hyaluronan degrading enzymes, such as soluble hyaluronidases, can be used to minimize the detachment or tearing of the retina during vitrectomy. Such tearing can cause, for example, the vitreous body to become uncoupled or "disinserted" from the retina, prior to removal of the vitreous

30 body. Such disinsertion or uncoupling of the vitreous body can minimize the likelihood that further tearing or detachment of the retina will occur as the vitreous body is removed.

The provided hyaluronan degrading enzyme compositions, including hyaluronidase compositions, and methods can be used for various ophthalmic applications, including the vitrectomy adjunct application described in U.S. Pat. No. 5,292,509. The use of a highly purified hyaluronan degrading enzyme, such as a
5 hyaluronidase, such as, for example, rHuPH20 produced and purified by the methods described herein, is preferable for intraocular procedures to minimize immunogenicity and toxicity. In some examples, a pegylated hyaluronidase can be used to prolong residence within the vitreous and prevent localized uptake.

Hyaluronan degrading enzymes, including hyaluronidases, including the
10 provided compositions containing soluble hyaluronidases, can be used to treat and/or prevent ophthalmic disorders by, for example, preventing neovascularization and increasing the rate of clearance from the vitreous of materials toxic to the retina. Hyaluronan degrading enzymes, including hyaluronidases, can be administered in an amount effective to liquefy the vitreous humor of the eye without causing toxic
15 damage to the eye. Liquefaction of the vitreous humor increases the rate of liquid exchange from the vitreal chamber. This increase in exchange removes the contaminating materials whose presence can cause ophthalmologic and retinal damage.

The provided hyaluronan degrading enzyme compositions, including the
20 ~~hyaluronidase compositions, and methods also can be used to reduce postoperative~~
pressure. Hyaluronic acid has been used in eye primarily as a spacer during cataract and intraocular lens surgical procedures. It also is used in other ocular surgical procedures such as glaucoma, vitreous and retina surgery and in corneal transplantation. A common side effect occurring in postoperative cataract patients is a
25 significant early, and occasionally prolonged, rise in intraocular pressure. Such a condition is sometimes serious, especially in patients with glaucomatous optic disc changes. Hyaluronan degrading enzymes such as hyaluronidases can be co-administered with hyaluronic acid to the eye prior to surgery to reduce postoperative pressure in the eye. For example, a hyaluronidase can be administered in an amount
30 effective to reduce the intraocular pressure to pre-operative levels by breaking down

the hyaluronic acid without decreasing its effectiveness during surgery nor causing side effects in the patient (U.S. Patent No. 6,745,776).

Hyaluronan degrading enzymes such as hyaluronidases also can be administered to patients with glaucoma to remove glycosaminoglycans from the orbicular meshwork and reduce intraocular pressure, and can be applied to the vitreous to promote the resolution of vitreous hemorrhages (i.e. extravasations of blood into the vitreous), which can occur in connection with conditions such as diabetic retinopathy, retinal neovascularization, retinal vein occlusion, posterior vitreous detachment, retinal tears and ocular traumas. The presence of vitreous hemorrhages, which are typically slow to resolve, can delay, complicate or prevent procedures that require the retina to be visualized through the vitreous for diagnosis and/or for treatment procedures, such as, but are not limited to, laser photocoagulation, which are often primary treatments for conditions such as proliferative diabetic retinopathy.

6. Gene therapy applications

The efficacy of most gene delivery vehicles *in vivo* does not correspond to the efficacy found observed *in vitro*. Glycosaminoglycans can hinder the transfer and diffusion of DNA and viral vectors into many cell types. The levels such extracellular matrix material can hinder the process considerably. Administration of hyaluronan degrading enzymes such as hyaluronidases can open channels in the extracellular matrix, thus enhancing delivery of gene therapy. For example, a hyaluronan degrading enzyme such as a hyaluronidase can be administered with collagenase to facilitate transduction of DNA *in vivo* (Dubinsky et al. (1984) Proc Natl Acad Sci USA 81(23):7529-33). Hyaluronan degrading enzymes, including hyaluronidases, also can enhance gene therapy using adeno-associated virus (Favre et al, (2000) Gene Therapy 7(16):1417-20). The channels opened following administration of, for example, hyaluronidase, are of a size that typically enhance diffusion of smaller molecules such as retroviruses, adenoviruses, adeno-associated viruses and DNA complexes (as well as other therapeutic and pharmacological agents of interest). The pores are not so large, however, as to promote the dislocation and movement of cells.

In some examples, viruses can be engineered to express one or more hyaluronan degrading enzymes, such as a hyaluronidase, to facilitate their replication and spread within a target tissue. The target tissue can be, for example, a cancerous tissue whereby the virus is capable of selective replication within the tumor. The virus also can be a non-lytic virus wherein the virus selectively replicates under a tissue specific promoter. As the viruses replicate, the co-expression of the hyaluronan degrading enzyme, such as a hyaluronidase, with viral genes can facilitate the spread of the virus *in vivo*.

7. Cosmetic applications

Hyaluronan degrading enzymes, including hyaluronidases, can be administered to remove glycosaminoglycans involved in the accumulation of cellulite and to promote lymphatic flow. In some examples, hyaluronan degrading enzymes, including human hyaluronidases, such as for example, rHuPH20, are used for the treatment of cellulite. The hyaluronan degrading enzyme, such as a hyaluronidase, can be administered through repeated subcutaneous injections, through transdermal delivery in the form of ointments or creams or through the use of injectable slow release formulations to promote the continual degradation of glycosaminoglycans and prevent their return.

Hyaluronan degrading enzymes, including hyaluronidases also can be used to treat conditions such as "pigskin" edema or "orange peel" edema. Hyaluronan degrading enzymes such as hyaluronidases can effect depolymerization of the long mucopolysaccharide chains that can accumulate in the dermis and which are responsible for the retention of bound water and of the slowing, by capillary compression, of the diffusion of organic liquids, which eliminate metabolic wastes. Such retention of water and wastes associated with fat overloading of the lipocytes, constitutes classical "pigskin" edema or "orange peel" edema. Depolymerization can cut the long chains of mucopolysaccharides into shorter chains, resulting in the elimination of the bound water and wastes and restoration of the venous and lymphatic circulation, culminating in the disappearance of local edema.

8. Use in organ transplantation

The content of hyaluronic acid in an organ can increase with inflammation. An increased concentration of hyaluronic acid has been observed in tissue from different organs characterized by inflammatory-immunological injury such as alveolitis (Nettelbladt et al. (1991) *Am. Rev. Resp. Dis.* 139: 759-762) and myocardial infarction (Waldenstrom et al. (1991) *J. Clin. Invest.* 88(5): 1622-1628). Other examples include allograft rejection after a renal (Haellgren et al. (1990) *J. Exp. Med.* 171: 2063-2076; Wells et al. (1990) *Transplantation* 50: 240-243), small bowel (Wallander et al. (1993) *Transplant. Int.* 6: 133-137) or cardiac (Haellgren et al. (1990) *J Clin Invest* 185:668-673) transplantation; or a myocardial inflammation of viral origin (Waldenstrom et al. (1993) *Eur. J. Clin. Invest.* 23: 277-282). The occurrence of interstitial edemas in connection with the grafting of an organ constitutes a severe problem in the field of transplantation surgery. Grafts with interstitial edemas can swell to such a degree that the function is temporarily be lost. In some instances, the swelling can cause disruption of the kidney, resulting in a massive hemorrhage. Hyaluronan degrading enzymes, including hyaluronidases can be used to degrade accumulated glycosaminoglycans in an organ transplant. Removal of such glycosaminoglycans promotes removal of water from the graft and thus enhances organ function.

9. Use in treatment of glycosaminoglycan accumulation in the brain

Hyaluronic acid levels are elevated in a number of cerebrospinal pathologic conditions. Levels of cerebrospinal hyaluronic acid are normally less than 200 µg/L in adults (Laurent et al. (1996) *Acta Neurol Scand* September 94(3):194-206), but can elevate to levels of over 8000 µg/L in diseases such as meningitis, spinal stenosis, head injury and cerebral infarction. Hyaluronan degrading enzymes, including hyaluronidases, such as, for example, rHuPH20, can be utilized to degrade critically elevated levels of substrate.

The lack of effective lymphatics in the brain also can lead to life threatening edema following head trauma. Hyaluronic acid accumulation is a result of increased synthesis by hyaluronic acid synthases and decreased degradation. Accumulation of hyaluronic acid can initially serve the beneficial purpose of increasing water content in the damaged tissue to facilitate leukocyte extravasation, but continued

accumulation can be lethal. Administration of a hyaluronan degrading enzyme, such as a hyaluronidase, such as intrathecally or intravenously, to a patient suffering from head trauma can serve to remove tissue hyaluronic acid accumulation and the water associated with it.

- 5 Hyaluronan degrading enzymes such as hyaluronidases also can be used in the treatment of edema associated with brain tumors, particularly that associated with glioblastoma multiform. The edema associated with brain tumors results from the accumulation of hyaluronic acid in the non-cancerous portions of the brain adjacent the tumor. Administration of a hyaluronan degrading enzyme, such as a
- 10 hyaluronidase, to the sites of hyaluronic acid accumulation (e.g., by intravenous injection or via a shunt) can relieve the edema associated with such malignancies by degrading the excess hyaluronic acid at these sites.

10. Use in treatment of glycosaminoglycan accumulation in cardiovascular disease

- 15 Hyaluronan degrading enzymes, including hyaluronidases, can be used in the treatment of some cardiovascular disease. Administration of a hyaluronan degrading enzyme, such as a hyaluronidase, in animal models following experimental myocardial infarct can reduce infarct size (Maclean, et al (1976) Science 194(4261):199-200). One proposed mechanism by which this can occur is by
- 20 ~~reducing hyaluronic acid accumulation that occurs following ischemia reperfusion.~~
- Reduction of infarct size is believed to occur from increased lymph drainage and increased tissue oxygenation and reduction of myocardial water content.

- Hyaluronan degrading enzymes, including hyaluronidases, also can be used to limit coronary plaques from arteriosclerosis. Such plaques accumulate
- 25 glycosaminoglycans and mediate macrophage and foam cell adhesion (Kolodgie et al. (2002) Arterioscler Thromb Vasc Biol. 22(10):1642-8).

11. Use in pulmonary disease

- Levels of hyaluronic acid in bronchoalveolar lavages (BAL) from normal individuals are generally below 15 ng/ml. However, hyaluronic acid levels in BAL
- 30 rise dramatically in conditions of respiratory distress (Bjermer et al.(1987) Br Med J (Clin Res Ed) 295(6602):803-6). The increased hyaluronic acid in the lung can

prevent oxygen diffusion and gas exchange as well as activating neutrophil and macrophage responses. Purified preparations of hyaluronan degrading enzymes, such as, for example, rHuPH20, such as those produced using the methods provided herein, can be delivered by either pulmonary or intravenous delivery to patients presenting
5 with such conditions to reduce hyaluronan levels. Hyaluronan degrading enzymes, including hyaluronidases, also can be administered to patients suffering from other pulmonary complications that are associated with elevated glycosaminoglycans or to enhance the delivery of other co delivered molecules to the lung.

12. Other uses

10 In further examples of its therapeutic use, hyaluronan degrading enzymes such as hyaluronidases can be used for such purposes as an antidote to local necrosis from paravenous injection of necrotic substances such as vinka alkaloids (Few et al. (1987) Amer. J. Matern. Child Nurs. 12, 23-26), treatment of ganglion cysts (Paul et al. (1997) J Hand Surg. 22 (2): 219-21) and treatment of tissue necrosis due to venous
15 insufficiency (Elder et al. (1980) Lancet 648-649). Hyaluronan degrading enzymes, including hyaluronidases, also can be used to treat ganglion cysts (also known as a wrist cyst, Bible cyst, or dorsal tendon cyst), which are the most common soft tissue mass of the hand and are fluid filled sacs that can be felt below the skin.

Hyaluronan degrading enzymes such as hyaluronidases can be used in the
20 ~~treatment of spinal cord injury by degrading chondroitin sulfate proteoglycans~~
(CSPGs). Following spinal cord injury, glial scars containing CSPGs are produced by astrocytes. CSPGs play a crucial role in the inhibition of axon growth. In addition, the expression of CSPG has been shown to increase following injury of the central nervous system (CNS). Hyaluronan degrading enzymes also can be utilized for the
25 treatment of herniated disks in a process known as chemonucleolysis. Chondroitinase ABC, an enzyme cleaving similar substrates as hyaluronidase, can induce the reduction of intradiscal pressure in the lumbar spine. There are three types of disk injuries. A protruded disk is one that is intact but bulging. In an extruded disk, the fibrous wrapper has torn and the nucleus pulposus (NP) has oozed out, but is still
30 connected to the disk. In a sequestered disk, a fragment of the NP has broken loose

from the disk and is free in the spinal canal. Chemonucleolysis is typically effective on protruded and extruded disks, but not on sequestered disk injuries.

I. EXAMPLES

The following examples are included for illustrative purposes only and are not
5 intended to limit the scope of the invention.

Example 1.

Generation of a soluble rHuPH20-expressing cell line

The HZ24 plasmid (set forth in SEQ ID NO:52) was used to transfect Chinese Hamster Ovary (CHO cells) (see e.g. application Nos. 10,795,095, 11/065,716 and
10 11/238,171). The HZ24 plasmid vector for expression of soluble rHuPH20 contains a pCI vector backbone (Promega), DNA encoding amino acids 1-482 of human PH20 hyaluronidase (SEQ ID NO:49), an internal ribosomal entry site (IRES) from the ECMV virus (Clontech), and the mouse dihydrofolate reductase (DHFR) gene. The pCI vector backbone also includes DNA encoding the Beta-lactamase resistance gene
15 (AmpR), an fl origin of replication, a Cytomegalovirus immediate-early enhancer/promoter region (CMV), a chimeric intron, and an SV40 late polyadenylation signal (SV40). The DNA encoding the soluble rHuPH20 construct contains an NheI site and a Kozak consensus sequence prior to the DNA encoding the methionine at amino acid position 1 of the native 35 amino acid signal sequence of
20 human PH20, and a stop codon following the DNA encoding the tyrosine corresponding to amino acid position 482 of the human PH20 hyaluronidase set forth in SEQ ID NO:1), followed by a BamHI restriction site. The construct pCI-PH20-IRES-DHFR-SV40pa (HZ24), therefore, results in a single mRNA species driven by the CMV promoter that encodes amino acids 1-482 of human PH20 (set forth in SEQ
25 ID NO:3) and amino acids 1-186 of mouse dihydrofolate reductase (set forth in SEQ ID NO:53), separated by the internal ribosomal entry site (IRES).

Non-transfected DG44 CHO cells growing in GIBCO Modified CD-CHO media for DHFR(-) cells, supplemented with 4 mM Glutamine and 18 ml/L Plurionic F68/L (Gibco), were seeded at 0.5×10^6 cells/ml in a shaker flask in preparation for
30 transfection. Cells were grown at 37° C in 5% CO₂ in a humidified incubator, shaking at 120 rpm. Exponentially growing non-transfected DG44 CHO cells were tested for

viability prior to transfection.

Sixty million viable cells of the non-transfected DG44 CHO cell culture were pelleted and resuspended to a density of 2×10^7 cells in 0.7 mL of 2x transfection buffer (2x HeBS: 40 mM Hepes, pH 7.0, 274 mM NaCl, 10 mM KCl, 1.4 mM

5 Na_2HPO_4 , 12 mM dextrose). To each aliquot of resuspended cells, 0.09 mL (250 μg) of the linear HZ24 plasmid (linearized by overnight digestion with Cla I (New England Biolabs) was added, and the cell/DNA solutions were transferred into 0.4 cm gap BTX (Gentronics) electroporation cuvettes at room temperature. A negative control electroporation was performed with no plasmid DNA mixed with the cells.

- 10 The cell/plasmid mixes were electroporated with a capacitor discharge of 330 V and 960 μF or at 350 V and 960 μF .

The cells were removed from the cuvettes after electroporation and transferred into 5 mL of Modified CD-CHO media for DHFR(-) cells, supplemented with 4 mM Glutamine and 18 ml/L Plurionic F68/L (Gibco), and allowed to grow in a well of a 6-

15 well tissue culture plate without selection for 2 days at 37° C in 5% CO_2 in a humidified incubator.

Two days post-electroporation, 0.5 mL of tissue culture media was removed from each well and tested for the presence of hyaluronidase activity, using the microturbidity assay described in Example 2.

20

Table 3: Initial Hyaluronidase Activity of HZ24 Transfected DG44 CHO cells at 40 hours post-transfection		
	Dilution	Activity Units/ml
Transfection 1 330V	1 to 10	0.25
Transfection 2 350V	1 to 10	0.52
Negative Control	1 to 10	0.015

Cells from Transfection 2 (350V) were collected from the tissue culture well, counted and diluted to 1×10^4 to 2×10^4 viable cells per mL. A 0.1 mL aliquot of the cell suspension was transferred to each well of five, 96 well round bottom tissue

culture plates. One hundred microliters of CD-CHO media (GIBCO) containing 4 mM GlutaMAX™-1 supplement (GIBCO™, Invitrogen Corporation) and without hypoxanthine and thymidine supplements were added to the wells containing cells (final volume 0.2 mL).

5 Ten clones were identified from the 5 plates grown without methotrexate.

Table 4. Hyaluronidase activity of identified clones

Plate/Well ID	Relative Hyaluronidase
1C3	261
2C2	261
3D3	261
3E5	243
3C6	174
2G8	103
1B9	304
2D9	273
4D10	302

Six HZ24 clones were expanded in culture and transferred into shaker flasks as single cell suspensions. Clones 3D3, 3E5, 2G8, 2D9, 1E11, and 4D10 were plated
10 into 96-well round bottom tissue culture plates using a two-dimensional infinite dilution strategy in which cells were diluted 1:2 down the plate, and 1:3 across the plate, starting at 5000 cells in the top left hand well. Diluted clones were grown in a background of 500 non-transfected DG44 CHO cells per well, to provide necessary growth factors for the initial days in culture. Ten plates were made per subclone, with
15 5 plates containing 50 nM methotrexate and 5 plates without methotrexate.

Clone 3D3 produced 24 visual subclones (13 from the no methotrexate treatment, and 11 from the 50 nM methotrexate treatment. Significant hyaluronidase activity was measured in the supernatants from 8 of the 24 subclones (>50 Units/mL), and these 8 subclones were expanded into T-25 tissue culture flasks. Clones isolated
20 from the methotrexate treatment protocol were expanded in the presence of 50 nM methotrexate. Clone 3D35M was further expanded in 500 nM methotrexate giving

rise to clones producing in excess of 1,000 Units/ml in shaker flasks (clone 3D35M; or Gen1 3D35M). A master cell bank (MCB) of the 3D35M cells was then prepared.

Example 2.

Determination of hyaluronidase activity of soluble rHuPH20

5 Hyaluronidase activity of soluble rHuPH20 in samples such as cell cultures, purification fractions and purified solutions was determined using a turbidimetric assay, which based on the formation of an insoluble precipitate when hyaluronic acid binds with serum albumin. The activity is measured by incubating soluble rHuPH20 with sodium hyaluronate (hyaluronic acid) for a set period of time (10 minutes) and
10 then precipitating the undigested sodium hyaluronate with the addition of acidified serum albumin. The turbidity of the resulting sample is measured at 640 nm after a 30 minute development period. The decrease in turbidity resulting from enzyme activity on the sodium hyaluronate substrate is a measure of the soluble rHuPH20 hyaluronidase activity. The method is performed using a calibration curve generated
15 with dilutions of a soluble rHuPH20 assay working reference standard, and sample activity measurements are made relative to this calibration curve. Dilutions of the sample were prepared in Enzyme Diluent Solutions. The Enzyme Diluent Solution was prepared by dissolving 33.0 ± 0.05 mg of hydrolyzed
20 gelatin in 25.0 mL of the 50 mM PIPES Reaction Buffer (140 mM NaCl, 50 mM PIPES, pH 5.5) and 25.0 mL of SWFI, and diluting 0.2 mL of 25% Buminat solution into the mixture and vortexing for 30 seconds. This was performed within 2 hours of use and stored on ice until needed. The samples were diluted to an estimated 1-2 U/mL. Generally, the maximum dilution per step did not exceed 1:100 and the initial sample size for the first dilution was not be less than 20 μ L. The minimum sample
25 volumes needed to perform the assay were: In-process Samples, FPLC Fractions: 80 μ L; Tissue Culture Supernatants: 1 mL; Concentrated Material 80 μ L; Purified or Final Step Material: 80 μ L. The dilutions were made in triplicate in a Low Protein Binding 96-well plate, and 30 μ L of each dilution was transferred to Optilux black/clear bottom plates (BD BioSciences).

30 Dilutions of known soluble rHuPH20 with a concentration of 2.5 U/mL were prepared in Enzyme Diluent Solution to generate a standard curve and added to the

Optilux plate in triplicate. The dilutions included 0 U/mL, 0.25 U/mL, 0.5 U/mL, 1.0 U/mL, 1.5 U/mL, 2.0 U/mL, and 2.5 U/mL. "Reagent blank" wells that contained 60 μ L of Enzyme Diluent Solution were included in the plate as a negative control. The plate was then covered and warmed on a heat block for 5 minutes at 37 °C. The cover
5 was removed and the plate was shaken for 10 seconds. After shaking, the plate was returned to the plate to the heat block and the MULTIDROP 384 Liquid Handling Device was primed with the warm 0.25 mg/mL sodium hyaluronate solution (prepared by dissolving 100 mg of sodium hyaluronate (LifeCore Biomedical) in 20.0 mL of SWFI. This was mixed by gently rotating and/or rocking at 2-8°C for 2-4
10 hours, or until completely dissolved). The reaction plate was transferred to the MULTIDROP 384 and the reaction was initiated by pressing the start key to dispense 30 μ L sodium hyaluronate into each well. The plate was then removed from the MULTIDROP 384 and shaken for 10 seconds before being transferred to a heat block with the plate cover replaced. The plate was incubated at 37 °C for 10 minutes
15 The MULTIDROP 384 was prepared to stop the reaction by priming the machine with Serum Working Solution and changing the volume setting to 240 μ L. (25 mL of Serum Stock Solution [1 volume of Horse Serum (Sigma) was diluted with 9 volumes of 500 mM Acetate Buffer Solution and the pH was adjusted to 3.1 with hydrochloric acid] in 75 mL of 500 mM Acetate Buffer Solution). The plate was
20 ~~removed from the heat block and placed onto the MULTIDROP 384 and 240 μ L of~~
serum Working Solutions was dispensed into the wells. The plate was removed and shaken on a plate reader for 10 seconds. After a further 15 minutes, the turbidity of the samples was measured at 640 nm and the hyaluronidase activity (in U/mL) of each sample was determined by fitting to the standard curve.
25 Specific activity (Units/mg) was calculated by dividing the hyaluronidase activity (U/ml) by the protein concentration (mg/mL).

Example 3

Production and Purification of Gen1 Human sPH20

A. 5 L Bioreactor Process

30 A vial of 3D35M was thawed and expanded from shaker flasks through 1 L spinner flasks in CD-CHO media (Invitrogen, Carlsbad Calif.) supplemented with 100

nM Methotrexate and GlutaMAX™-1 (Invitrogen). Cells were transferred from spinner flasks to a 5 L bioreactor (Braun) at an inoculation density of 4×10^5 viable cells per ml. Parameters were temperature setpoint 37°C, pH 7.2 (starting setpoint), with Dissolved Oxygen setpoint 25% and an air overlay of 0-100 cc/min. At 168 hrs,
5 250 ml of Feed #1 Medium (CD CHO with 50 g/L Glucose) was added. At 216 hours, 250 ml of Feed #2 Medium (CD CHO with 50 g/L Glucose and 10 mM Sodium Butyrate) was added, and at 264 hours 250 ml of Feed #2 Medium was added. This process resulted in a final productivity of 1600 Units per ml with a maximal cell density of 6×10^6 cells/ml. The addition of sodium butyrate was to dramatically
10 enhance the production of soluble rHuPH20 in the final stages of production.

Conditioned media from the 3D35M clone was clarified by depth filtration and tangential flow diafiltration into 10 mM Hepes pH 7.0. Soluble rHuPH20 was then purified by sequential chromatography on Q Sepharose (Pharmacia) ion exchange, Phenyl Sepharose (Pharmacia) hydrophobic interaction chromatography,
15 phenyl boronate (Prometix) and Hydroxapatite Chromatography (Biorad, Richmond, CA).

Soluble rHuPH20 bound to Q Sepharose and eluted at 400 mM NaCl in the same buffer. The eluate was diluted with 2M ammonium sulfate to a final concentration of 500 mM ammonium sulfate and passed through a Phenyl Sepharose
20 (low sub) column, followed by binding under the same conditions to a phenyl boronate resin. The soluble rHuPH20 was eluted from the phenyl sepharose resin in Hepes pH 6.9 after washing at pH 9.0 in 50 mM bicine without ammonium sulfate. The eluate was loaded onto a ceramic hydroxyapatite resin at pH 6.9 in 5 mM potassium phosphate and 1 mM CaCl₂ and eluted with 80 mM potassium phosphate,
25 pH 7.4 with 0.1 mM CaCl₂.

The resultant purified soluble rHuPH20 possessed a specific activity in excess of 65,000 USP Units/mg protein by way of the microturbidity assay (Example 2) using the USP reference standard. In one example, the specific activity of the native rHuPH20 was around 120,000 Units/mg, while PEGylated rHuPH20 (as described in
30 Example 7, below) has a specific activity of about 30,000 Units/mg. Purified sPH20 eluted as a single peak from 24 to 26 minutes from a Pharmacia 5RPC styrene

divinylbenzene column with a gradient between 0.1% TFA/H₂O and 0.1% TFA/90% acetonitrile/10% H₂O and resolved as a single broad 61 kDa band by SDS electrophoresis that reduced to a sharp 51 kDa band upon treatment with PNGASE-F. N-terminal amino acid sequencing revealed that the leader peptide had been efficiently removed.

B. Upstream Cell Culture Expansion Process into 100 L Bioreactor Cell Culture

A scaled-up process was used to separately purify soluble rHuPH20 from four different vials of 3D35M cell to produce 4 separate batches of sHuPH20; HUA0406C, HUA0410C, HUA0415C and HUA0420C. Each vial was separately expanded and cultured through a 125 L bioreactor, then purified using column chromatography. Samples were taken throughout the process to assess such parameters as enzyme yield. The description of the process provided below sets forth representative specifications for such things as bioreactor starting and feed media volumes, transfer cell densities, and wash and elution volumes. The exact numbers vary slightly with each batch, and are detailed in Tables 10 to 11.

Four vials of 3D35M cells were thawed in a 37°C water bath, CD CHO containing 100 nM methotrexate and 40 mL/L GlutaMAX was added and the cells were centrifuged. The cells were re-suspended in a 125 mL shake flask with 20 mL of fresh media and placed in a 37°C, 7% CO₂ incubator. The cells were expanded up to 40 mL in the 125 mL shake flask. When the cell density reached $1.5 - 2.5 \times 10^6$ cells/mL, the culture was expanded into a 125 mL spinner flask in a 100 mL culture volume. The flask was incubated at 37°C, 7% CO₂. When the cell density reached $1.5 - 2.5 \times 10^6$ cells/mL, the culture was expanded into a 250 mL spinner flask in 200 mL culture volume, and the flask was incubated at 37°C, 7% CO₂. When the cell density reached $1.5 - 2.5 \times 10^6$ cells/mL, the culture was expanded into a 1 L spinner flask in 800 mL culture volume and incubated at 37°C, 7% CO₂. When the cell density reached $1.5 - 2.5 \times 10^6$ cells/mL, the culture was expanded into a 6 L spinner flask in 5 L culture volume and incubated at 37°C, 7% CO₂. When the cell density reached $1.5 - 2.5 \times 10^6$ cells/mL, the culture was expanded into a 36 L spinner flask in 20 L culture volume and incubated at 37°C, 7% CO₂.

A 125 L reactor was sterilized with steam at 121°C, 20 PSI and 65 L of CD CHO media was added. Before use, the reactor was checked for contamination. When the cell density in the 36 L spinner flasks reached $1.8 - 2.5 \times 10^6$ cells/mL, 20 L cell culture were transferred from the 36L spinner flasks to the 125 L bioreactor (Braun), resulting a final volume of 85 L and a seeding density of approximately 4×10^5 cells/mL. Parameters were temperature set point, 37°C; pH: 7.2; Dissolved oxygen: $25\% \pm 10\%$; Impeller Speed 50 rpm; Vessel Pressure 3 psi; Air Sparge 1 L/min.; Air Overlay: 1 L/min. The reactor was sampled daily for cell counts, pH verification, media analysis, protein production and retention. Nutrient feeds were added during the run. At Day 6, 3.4 L of Feed #1 Medium (CD CHO + 50 g/L Glucose + 40 mL/L GlutaMAX™-1) was added, and culture temperature was changed to 36.5°C. At day 9, 3.5 L of Feed #2 (CD CHO + 50 g/L Glucose + 40 mL/L GlutaMAX™-1 + 1.1 g/L Sodium Butyrate) was added, and culture temperature was changed to 36°C. At day 11, 3.7 L of Feed #3 (CD CHO + 50 g/L Glucose + 40 mL/L GlutaMAX™-1 + 1.1 g/L Sodium Butyrate) was added, and the culture temperature was changed to 35.5°C. The reactor was harvested at 14 days or when the viability of the cells dropped below 50%. The process resulted in production of soluble rHuPH20 with an enzymatic activity of 1600 Units/ml with a maximal cell density of 8 million cells/mL. At harvest, the culture was sampled for mycoplasma, bioburden, endotoxin, and virus *in vitro* and *in vivo*, transmission electron microscopy (TEM) for viral particles, and enzyme activity.

The one hundred liter bioreactor cell culture harvest was filtered through a series of disposable capsule filters having a polyethersulfone medium (Sartorius): first through a 8.0 µm depth capsule, a 0.65 µm depth capsule, a 0.22 µm capsule, and finally through a 0.22 µm Sartopore 2000 cm² filter and into a 100 L sterile storage bag. The culture was concentrated 10× using two TFF with Spiral Polyethersulfone 30 kDa MWCO filters (Millipore), followed by a 6× buffer exchange with 10 mM HEPES, 25 mM Na₂SO₄, pH 7.0 into a 0.22 µm final filter into a 20 L sterile storage bag. Table 5 provides monitoring data related to the cell culture, harvest, concentration and buffer exchange steps.

Table 5. Monitoring data for cell culture, harvest, concentration and buffer exchange steps.

Parameter	HUA0406C	HUA04010C	HUA0415C	HUA0420C
Time from thaw to inoculate 100 L bioreactor (days)	21	19	17	18
100 L inoculation density ($\times 10^6$ cells/mL)	0.45	0.33	0.44	0.46
Doubling time in logarithmic growth (hr)	29.8	27.3	29.2	23.5
Max. cell density ($\times 10^6$ cells/mL)	5.65	8.70	6.07	9.70
Harvest viability (%)	41	48	41	41
Harvest titer (U/ml)	1964	1670	991	1319
Time in 100-L bioreactor (days)	13	13	12	13
Clarified harvest volume (mL)	81800	93300	91800	89100
Clarified harvest enzyme assay (U/mL)	2385	1768	1039	1425
Concentrate enzyme assay (U/mL)	22954	17091	8561	17785
Buffer exchanged concentrate enzyme assay (U/mL)	15829	11649	9915	8679
Filtered buffer exchanged concentrate enzyme assay (U/mL)	21550	10882	9471	8527
Buffer exchanged concentrate volume(mL)	10699	13578	12727	20500
Ratio enzyme units concentration/harvest	0.87	0.96	1.32	1.4

A Q Sepharose (Pharmacia) ion exchange column (3 L resin, Height = 20 cm,

- 5 Diameter = 14 cm) was prepared. Wash samples were collected for a determination of pH, conductivity and endotoxin (LAL) assay. The column was equilibrated with 5 column volumes of 10 mM Tris, 20 mM Na₂SO₄, pH 7.5. The concentrated, diafiltered harvest was loaded onto the Q column at a flow rate of 100 cm/hr. The column was washed with 5 column volumes of 10 mM Tris, 20 mM Na₂SO₄, pH 7.5 and 10 mM Hepes, 50 mM NaCl, pH 7.0. The protein was eluted with 10 mM Hepes, 400 mM NaCl, pH 7.0 and filtered through a 0.22 μ m final filter into a sterile bag.

- Phenyl-Sepharose (Pharmacia) hydrophobic interaction chromatography was next performed. A Phenyl-Sepharose (PS) column (9.1 L resin, Height = 29 cm, Diameter = 20cm) was prepared. The column was equilibrated with 5 column volumes of 5 mM potassium phosphate, 0.5 M ammonium sulfate, 0.1 mM CaCl₂, pH 7.0. The protein eluate from above was supplemented with 2M ammonium sulfate, 1

M potassium phosphate and 1 M CaCl_2 stock solutions to final concentrations of 5 mM, 0.5 M and 0.1 mM, respectively. The protein was loaded onto the PS column at a flow rate of 100 cm/hr. 5 mM potassium phosphate, 0.5 M ammonium sulfate and 0.1 mM CaCl_2 pH 7.0 was added at 100 cm/hr. The flow through was passed through
5 a 0.22 μm final filter into a sterile bag.

The PS-purified protein was the loaded onto an aminophenyl boronate column (ProMedics) (6.3 L resin, Height = 20 cm, Diameter = 20cm) that had been equilibrated with 5 column volumes of 5 mM potassium phosphate, 0.5 M ammonium sulfate. The protein was passed through the column at a flow rate of 100 cm/hr, and
10 the column was washed with 5 mM potassium phosphate, 0.5 M ammonium sulfate, pH 7.0. The column was then washed with 20 mM bicine, 100 mM NaCl, pH 9.0 and the protein eluted with 50 mM Hepes, 100 mM NaCl pH 6.9 through a sterile filter and into a 20 L sterile bag. The eluate was tested for bioburden, protein concentration and enzyme activity.

15 A hydroxyapatite (HAP) column (BioRad) (1.6 L resin, Height = 10 cm, Diameter = 14 cm) was equilibrated with 5 mM potassium phosphate, 100 mM NaCl, 0.1 mM CaCl_2 pH 7.0. Wash samples were collected and tested for pH, conductivity and endotoxin (LAL assay). The aminophenyl boronate purified protein was supplemented with potassium phosphate and CaCl_2 to yield final concentrations of 5
20 mM potassium phosphate and 0.1 mM CaCl_2 and loaded onto the HAP column at a flow rate of 100 cm/hr. The column was washed with 5 mM potassium phosphate pH 7.0, 100 mM NaCl, 0.1 mM CaCl_2 , then 10 mM potassium phosphate pH 7.0, 100 mM NaCl, 0.1 mM CaCl_2 pH. The protein was eluted with 70 mM potassium phosphate pH 7.0 and filtered through a 0.22 μm filter into a 5 L sterile storage bag.
25 The eluate was tested for bioburden, protein concentration and enzyme activity.

The HAP-purified protein was then pumped through a 20 nm viral removal filter via a pressure tank. The protein was added to the DV20 pressure tank and filter (Pall Corporation), passing through an Ultipor DV20 Filter with 20 nm pores (Pall Corporation) into a sterile 20 L storage bag. The filtrate was tested for protein
30 concentration, enzyme activity, oligosaccharide, monosaccharide and sialic acid profiling, and process-related impurities. The protein in the filtrate was then

concentrated to 1 mg/mL using a 10 kD molecular weight cut off (MWCO) Sartocore Slice tangential flow filtration (TFF) system (Sartorius). The filter was first prepared by washing with a Hepes/saline solution (10 mM Hepes, 130 mM NaCl, pH 7.0) and the permeate was sampled for pH and conductivity. Following concentration, the concentrated protein was sampled and tested for protein concentration and enzyme activity. A 6× buffer exchange was performed on the concentrated protein into the final buffer: 10 mM Hepes, 130 mM NaCl, pH 7.0. The concentrated protein was passed through a 0.22 µm filter into a 20 L sterile storage bag. The protein was sampled and tested for protein concentration, enzyme activity, free sulfhydryl groups, oligosaccharide profiling and osmolarity.

Tables 5 to 11 provide monitoring data related to each of the purification steps described above, for each 3D35M cell lot.

Table 6. Q sepharose column data

Parameter	HUA0406C	HUA0410C	HUA0415C	HUA0420C
Load volume (mL)	10647	13524	12852	20418
Load Volume/Resin Volume ratio	3.1	4.9	4.5	7.3
Column Volume (mL)	2770	3840	2850	2880
Eluate volume (mL)	6108	5923	5759	6284
Protein Conc. of Eluate (mg/mL)	2.8	3.05	2.80	2.86
Eluate Enzyme Assay (U/mL)	24493	26683	18321	21052
Enzyme Yield (%)	65	107	87	76

Table 7. Phenyl Sepharose column data

Parameter	HUA0406C	HUA0410C	HUA0415C	HUA0420C
Volume Before Stock Solution Addition (mL)	5670	5015	5694	6251
Load Volume (mL)	7599	6693	7631	8360
Column Volume (mL)	9106	9420	9340	9420
Load Volume/Resin Volume ratio	0.8	0.71	0.82	0.89
Eluate volume (mL)	16144	18010	16960	17328
Protein Conc. of Eluate (mg/mL)	0.4	0.33	0.33	0.38
Eluate Enzyme Assay (U/mL)	8806	6585	4472	7509
Protein Yield (%)	41	40	36	37
Enzyme Yield (%)	102	88	82	96

Table 8. Amino Phenyl Boronate column data

Parameter	HUA0406C	HUA0410C	HUA0415C	HUA0420C
Load Volume (mL)	16136	17958	16931	17884
Load Volume/Resin Volume ratio	2.99	3.15	3.08	2.98
Column Volume (mL)	5400	5700	5500	5300
Eluate volume (mL)	17595	22084	20686	19145
Protein Conc. of Eluate (mg/mL)	0.0	0.03	0.03	0.04
Protein Conc. of Filtered Eluate (mg/mL)	not tested	0.03	0.00	0.04
Eluate Enzyme Assay (U/mL)	4050	2410	1523	4721
Protein Yield (%)	0	11	11	12
Enzyme Yield (%)	not determined	41	40	69

Table 9. Hydroxyapatite column data

Parameter	HUA0406C	HUA0410C	HUA0415C	HUA0420C
Volume Before Stock Solution Addition (mL)	16345	20799	20640	19103
Load Volume/Resin Volume ratio	10.95	13.58	14.19	12.81
Column Volume (mL)	1500	1540	1462	1500
Load volume (mL)	16429	20917	20746	19213
Eluate volume (mL)	4100	2415	1936	2419
Protein Conc. of Eluate (mg/mL)	not tested	0.24	0.17	0.23
Protein Conc. of Filtered Eluate (mg/mL)	NA	NA	0.17	NA
Eluate Enzyme Assay (U/mL)	14051	29089	20424	29826
Protein Yield (%)	Not tested	93	53	73
Enzyme Yield (%)	87	118	140	104

5 **Table 10. DV20 filtration data**

Parameter	HUA0406C	HUA0410C	HUA0415C	HUA0420C
Start volume (mL)	4077	2233	1917	2419
Filtrate Volume (mL)	4602	3334	2963	3504
Protein Conc. of Filtrate (mg/mL)	0.1	NA	0.09	NA
Protein Conc. of Filtered Eluate (mg/mL)	NA	0.15	0.09	0.16
Protein Yield (%)	not tested	93	82	101

Table 11. Final concentration data

Parameter	HUA0406C	HUA0410C	HUA0415C	HUA0420C
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Start volume (mL)	4575	3298	2963	3492
Concentrate Volume (mL)	562	407	237	316
Protein Conc. of Concentrate (mg/mL)	0.9	1.24	1.16	1.73
Protein Yield (%)	111	102	103	98

Table 12. Buffer Exchange into Final Formulation data

Parameter	HUA0406C	HUA0410C	HUA0415C	HUA0420C
Start Volume (mL)	562	407	237	316
Final Volume Buffer Exchanged Concentrate (mL)	594	516	310	554
Protein Conc. of Concentrate (mg/mL)	1.00	0.97	0.98	1.00
Protein Conc. of Filtered Concentrate (mg/mL)	0.95	0.92	0.95	1.02
Protein Yield (%)	118	99	110	101

The purified and concentrated soluble rHuPH20 protein was aseptically filled into sterile vials with 5 mL and 1 mL fill volumes. The protein was passed through a 0.22 μ m filter to an operator controlled pump that was used to fill the vials using a gravimetric readout. The vials were closed with stoppers and secured with crimped caps. The closed vials were visually inspected for foreign particles and then labeled. Following labeling, the vials were flash-frozen by submersion in liquid nitrogen for no longer than 1 minute and stored at $\leq -15^{\circ}\text{C}$ ($-20 \pm 5^{\circ}\text{C}$).

Example 4

Production Gen2 Cells Containing Soluble human PH20 (rHuPH20)

The Gen1 3D35M cell line described in Example 1 was adapted to higher methotrexate levels to produce generation 2 (Gen2) clones. 3D35M cells were seeded from established methotrexate-containing cultures into CD CHO medium containing 4 mM GlutaMAX-1™ and 1.0 μ M methotrexate. The cells were adapted to a higher methotrexate level by growing and passaging them 9 times over a period of 46 days in a 37°C, 7% CO₂ humidified incubator. The amplified population of cells was cloned out by limiting dilution in 96-well tissue culture plates containing medium with 2.0 μ M methotrexate. After approximately 4 weeks, clones were identified and clone 3E10B was selected for expansion. 3E10B cells were grown in CD CHO

medium containing 4 mM GlutaMAX-1™ and 2.0 µM methotrexate for 20 passages. A master cell bank (MCB) of the 3E10B cell line was created and frozen and used for subsequent studies.

Amplification of the cell line continued by culturing 3E10B cells in CD CHO medium containing 4 mM GlutaMAX-1™ and 4.0 µM methotrexate. After the 12th passage, cells were frozen in vials as a research cell bank (RCB). One vial of the RCB was thawed and cultured in medium containing 8.0 µM methotrexate. After 5 days, the methotrexate concentration in the medium was increased to 16.0 µM, then 20.0 µM 18 days later. Cells from the 8th passage in medium containing 20.0 µM methotrexate were cloned out by limiting dilution in 96-well tissue culture plates containing CD CHO medium containing 4 mM GlutaMAX-1™ and 20.0 µM methotrexate. Clones were identified 5-6 weeks later and clone 2B2 was selected for expansion in medium containing 20.0 µM methotrexate. After the 11th passage, 2B2 cells were frozen in vials as a research cell bank (RCB).

The resultant 2B2 cells are dihydrofolate reductase deficient (dhfr-) DG44 CHO cells that express soluble recombinant human PH20 (rHuPH20). The soluble PH20 is present in 2B2 cells at a copy number of approximately 206 copies/cell. Southern blot analysis of Spe I-, Xba I- and BamH I/Hind III-digested genomic 2B2 cell DNA using a rHuPH20-specific probe revealed the following restriction digest profile: ~~one major hybridizing band of ~7.7 kb and four minor hybridizing bands~~ (~13.9, ~6.6, ~5.7 and ~4.6 kb) with DNA digested with Spe I; one major hybridizing band of ~5.0 kb and two minor hybridizing bands (~13.9 and ~6.5 kb) with DNA digested with Xba I; and one single hybridizing band of ~1.4 kb observed using 2B2 DNA digested with BamH I/Hind III. Sequence analysis of the mRNA transcript indicated that the derived cDNA (SEQ ID NO:56) was identical to the reference sequence (SEQ ID NO:47) except for one base pair difference at position 1131, which was observed to be a thymidine (T) instead of the expected cytosine (C). This is a silent mutation, with no effect on the amino acid sequence.

Example 5

A. Production of Gen2 soluble rHuPH20 in 300 L Bioreactor Cell Culture

A vial of HZ24-2B2 was thawed and expanded from shaker flasks through 36L spinner flasks in CD-CHO media (Invitrogen, Carlsbad, CA) supplemented with 20 μ M methotrexate and GlutaMAX-1™ (Invitrogen). Briefly, the a vial of cells was thawed in a 37°C water bath, media was added and the cells were centrifuged. The cells were re-suspended in a 125 mL shake flask with 20 mL of fresh media and placed in a 37°C, 7% CO₂ incubator. The cells were expanded up to 40 mL in the 125 mL shake flask. When the cell density reached greater than 1.5×10^6 cells/mL, the culture was expanded into a 125 mL spinner flask in a 100 mL culture volume. The flask was incubated at 37°C, 7% CO₂. When the cell density reached greater than 1.5×10^6 cells/mL, the culture was expanded into a 250 mL spinner flask in 200 mL culture volume, and the flask was incubated at 37°C, 7% CO₂. When the cell density reached greater than 1.5×10^6 cells/mL, the culture was expanded into a 1 L spinner flask in 800 mL culture volume and incubated at 37°C, 7% CO₂. When the cell density reached greater than 1.5×10^6 cells/mL the culture was expanded into a 6 L spinner flask in 5000 mL culture volume and incubated at 37°C, 7% CO₂. When the cell density reached greater than 1.5×10^6 cells/mL the culture was expanded into a 36 L spinner flask in 32 L culture volume and incubated at 37°C, 7% CO₂.

A 400 L reactor was sterilized and 230 mL of CD-CHO media was added. Before use, the reactor was checked for contamination. Approximately 30 L cells were transferred from the 36L spinner flasks to the 400 L bioreactor (Braun) at an inoculation density of 4.0×10^5 viable cells per ml and a total volume of 260L. Parameters were temperature set point, 37°C; Impeller Speed 40-55 RPM; Vessel Pressure: 3 psi; Air Sparge 0.5- 1.5 L/Min.; Air Overlay: 3 L/ min.. The reactor was sampled daily for cell counts, pH verification, media analysis, protein production and retention. Also, during the run nutrient feeds were added. At 120 hrs (day 5), 10.4L of Feed #1 Medium (4× CD-CHO + 33 g/L Glucose + 160 mL/L Glutamax-1™ + 83 mL/L Yeastolate + 33 mg/L rHuInsulin) was added. At 168 hours (day 7), 10.8 L of Feed #2 (2× CD-CHO + 33 g/L Glucose + 80 mL/L Glutamax-1™ + 167 mL/L Yeastolate + 0.92 g/L Sodium Butyrate) was added, and culture temperature was changed to 36.5°C. At 216 hours (day 9), 10.8 L of Feed #3 (1× CD-CHO + 50 g/L Glucose + 50 mL/L Glutamax-1™ + 250 mL/L Yeastolate + 1.80 g/L Sodium

Butyrate) was added, and culture temperature was changed to 36° C. At 264 hours (day 11), 10.8 L of Feed #4 (1× CD-CHO + 33 g/L Glucose + 33 mL/L Glutamax-1™ + 250 mL/L Yeastolate + 0.92 g/L Sodium Butyrate) was added, and culture temperature was changed to 35.5° C. The addition of the feed media was observed to
5 dramatically enhance the production of soluble rHuPH20 in the final stages of production. The reactor was harvested at 14 or 15 days or when the viability of the cells dropped below 40%. The process resulted in a final productivity of 17,000 Units per ml with a maximal cell density of 12 million cells/mL. At harvest, the culture was sampled for mycoplasma, bioburden, endotoxin and viral *in vitro* and *in*
10 *vivo*, Transmission Electron Microscopy (TEM) and enzyme activity.

The culture was pumped by a peristaltic pump through four Millistak filtration system modules (Millipore) in parallel, each containing a layer of diatomaceous earth graded to 4-8 µm and a layer of diatomaceous earth graded to 1.4-1.1 µm, followed by a cellulose membrane, then through a second single Millistak filtration system
15 (Millipore) containing a layer of diatomaceous earth graded to 0.4-0.11 µm and a layer of diatomaceous earth graded to <0.1 µm, followed by a cellulose membrane, and then through a 0.22 µm final filter into a sterile single use flexible bag with a 350 L capacity. The harvested cell culture fluid was supplemented with 10 mM EDTA and 10 mM Tris to a pH of 7.5. The culture was concentrated 10× with a tangential
20 flow filtration (TFF) apparatus using four Sartoslice TFF 30 kDa molecular weight cut-off (MWCO) polyether sulfone (PES) filter (Sartorius), followed by a 10× buffer exchange with 10 mM Tris, 20 mM Na₂SO₄, pH 7.5 into a 0.22 µm final filter into a 50 L sterile storage bag.

The concentrated, diafiltered harvest was inactivated for virus. Prior to viral
25 inactivation, a solution of 10% Triton X-100, 3% tri (n-butyl) phosphate (TNBP) was prepared. The concentrated, diafiltered harvest was exposed to 1% Triton X-100, 0.3% TNBP for 1 hour in a 36 L glass reaction vessel immediately prior to purification on the Q column.

B. Purification of Gen2 soluble rHuPH20

30 A Q Sepharose (Pharmacia) ion exchange column (9 L resin, H= 29 cm, D= 20 cm) was prepared. Wash samples were collected for a determination of pH,

conductivity and endotoxin (LAL) assay. The column was equilibrated with 5 column volumes of 10 mM Tris, 20 mM Na₂SO₄, pH 7.5. Following viral inactivation, the concentrated, diafiltered harvest was loaded onto the Q column at a flow rate of 100 cm/hr. The column was washed with 5 column volumes of 10 mM Tris, 20 mM Na₂SO₄, pH 7.5 and 10 mM Hepes, 50 mM NaCl, pH 7.0. The protein was eluted with 10 mM Hepes, 400 mM NaCl, pH 7.0 into a 0.22 µm final filter into sterile bag. The eluate sample was tested for bioburden, protein concentration and hyaluronidase activity. A₂₈₀ absorbance reading were taken at the beginning and end of the exchange..

10 Phenyl-Sepharose (Pharmacia) hydrophobic interaction chromatography was next performed. A Phenyl-Sepharose (PS) column (19-21 L resin, H=29 cm, D= 30 cm) was prepared. The wash was collected and sampled for pH, conductivity and endotoxin (LAL assay). The column was equilibrated with 5 column volumes of 5 mM potassium phosphate, 0.5 M ammonium sulfate, 0.1 mM CaCl₂, pH 7.0. The
15 protein eluate from the Q sepharose column was supplemented with 2M ammonium sulfate, 1 M potassium phosphate and 1 M CaCl₂ stock solutions to yield final concentrations of 5 mM, 0.5 M and 0.1 mM, respectively. The protein was loaded onto the PS column at a flow rate of 100 cm/hr and the column flow thru collected. The column was washed with 5 mM potassium phosphate, 0.5 M ammonium sulfate
20 ~~and 0.1 mM CaCl₂ pH 7.0 at 100 cm/hr and the wash was added to the collected flow~~
thru. Combined with the column wash, the flow through was passed through a 0.22 µm final filter into a sterile bag. The flow through was sampled for bioburden, protein concentration and enzyme activity.

 An aminophenyl boronate column (ProMedics) was prepared. The wash was
25 collected and sampled for pH, conductivity and endotoxin (LAL assay). The column was equilibrated with 5 column volumes of 5 mM potassium phosphate, 0.5 M ammonium sulfate. The PS flow through containing purified protein was loaded onto the aminophenyl boronate column at a flow rate of 100 cm/hr. The column was washed with 5 mM potassium phosphate, 0.5 M ammonium sulfate, pH 7.0. The
30 column was washed with 20 mM bicine, 0.5 M ammonium sulfate, pH 9.0. The column was washed with 20 mM bicine, 100 mM sodium chloride, pH 9.0. The

protein was eluted with 50 mM Hepes, 100 mM NaCl, pH 6.9 and passed through a sterile filter into a sterile bag. The eluted sample was tested for bioburden, protein concentration and enzyme activity.

5 The hydroxyapatite (HAP) column (Biorad) was prepared. The wash was collected and test for pH, conductivity and endotoxin (LAL assay). The column was equilibrated with 5 mM potassium phosphate, 100 mM NaCl, 0.1 mM CaCl_2 , pH 7.0. The aminophenyl boronate purified protein was supplemented to final concentrations of 5 mM potassium phosphate and 0.1 mM CaCl_2 and loaded onto the HAP column at a flow rate of 100 cm/hr. The column was washed with 5 mM potassium phosphate, 10 pH 7, 100 mM NaCl, 0.1 mM CaCl_2 . The column was next washed with 10 mM potassium phosphate, pH 7, 100 mM NaCl, 0.1 mM CaCl_2 . The protein was eluted with 70 mM potassium phosphate, pH 7.0 and passed through a 0.22 μm sterile filter into a sterile bag. The eluted sample was tested for bioburden, protein concentration and enzyme activity.

15 The HAP purified protein was then passed through a viral removal filter. The sterilized Viosart filter (Sartorius) was first prepared by washing with 2 L of 70 mM potassium phosphate, pH 7.0. Before use, the filtered buffer was sampled for pH and conductivity. The HAP purified protein was pumped via a peristaltic pump through the 20 nM viral removal filter. The filtered protein in 70 mM potassium phosphate, 20 pH 7.0 was passed through a 0.22 μm final filter into a sterile bag. The viral filtered sample was tested for protein concentration, enzyme activity, oligosaccharide, monosaccharide and sialic acid profiling. The sample also was tested for process related impurities.

The protein in the filtrate was then concentrated to 10 mg/mL using a 10 kD 25 molecular weight cut off (MWCO) Sartocore Slice tangential flow filtration (TFF) system (Sartorius). The filter was first prepared by washing with 10 mM histidine, 130 mM NaCl, pH 6.0 and the permeate was sampled for pH and conductivity. Following concentration, the concentrated protein was sampled and tested for protein concentration and enzyme activity. A 6 $\times$ buffer exchange was performed on the 30 concentrated protein into the final buffer: 10 mM histidine, 130 mM NaCl, pH 6.0. Following buffer exchange, the concentrated protein was passed through a 0.22 μm

filter into a 20 L sterile storage bag. The protein was sampled and tested for protein concentration, enzyme activity, free sulfhydryl groups, oligosaccharide profiling and osmolarity.

The sterile filtered bulk protein was then aseptically dispensed at 20 mL into 30 mL sterile Teflon vials (Nalgene). The vials were then flash frozen and stored at -20 ± 5°C.

C. Comparison of production and purification of Gen1 soluble rHuPH20 and Gen2 soluble rHuPH20

The production and purification of Gen2 soluble rHuPH20 in a 300L bioreactor cell culture contained some changes in the protocols compared to the production and purification Gen1 soluble rHuPH20 in a 100L bioreactor cell culture (described in Example 3.B). Table 13 sets forth exemplary differences, in addition to simple scale up changes, between the methods.

Table 13		
Process Difference	Gen1 soluble rHuPH20	Gen2 soluble rHuPH20
Cell line	3D35M	2B2
Media used to expand cell inoculum	Contains 0.10 µM methotrexate (0.045 mg/L)	Contains 20 µM methotrexate (9 mg/L)
Media in 6L cultures onwards	Contains 0.10 µM methotrexate	Contains no methotrexate
36 L spinner flask	No instrumentation	Equipped with instrumentation that monitors and controls pH, dissolved oxygen, sparge and overlay gas flow rate.
	20 L operating volume.	32 L operating volume
Final operating volume in bioreactor	Approx. 100 L in a 125 L bioreactor (initial culture volume + 65 L)	Approx. 300L in a 400L bioreactor (initial culture volume + 260L)
Culture media in final bioreactor	No rHuInsulin	5.0 mg/L rHuInsulin
Media feed volume	Scaled at 4% of the bioreactor cell culture volume i.e. 3.4, 3.5 and 3.7 L, resulting in a target bioreactor volume of ~92 L.	Scaled at 4% of the bioreactor cell culture volume i.e. 10.4, 10.8, 11.2 and 11.7 L, resulting in a target bioreactor volume of ~303L.
Media feed	Feed #1 Medium: CD	Feed #1 Medium: 4× CD

	<p>CHO + 50 g/L Glucose + 8mM GlutaMAX™-1</p> <p>Feed #2 (CD CHO + 50 g/L Glucose + 8 mM GlutaMAX + 1.1 g/L Sodium Butyrate</p> <p>Feed #3: CD CHO + 50 g/L Glucose + 8 mM GlutaMAX + 1.1 g/L Sodium Butyrate</p>	<p>CHO + 33 g/L Glucose + 32 mM Glutamax + 16.6 g/L Yeastolate + 33 mg/L rHuInsulin</p> <p>Feed #2: 2× CD CHO + 33 g/L Glucose + 16 mM Glutamax + 33.4 g/L Yeastolate + 0.92 g/L Sodium Butyrate</p> <p>Feed #3: 1× CD CHO + 50 g/L Glucose + 10 mM Glutamax + 50 g/L Yeastolate + 1.80 g/L Sodium Butyrate</p> <p>Feed #4: 1× CD CHO + 33 g/L Glucose + 6.6 mM Glutamax + 50 g/L Yeastolate + 0.92 g/L Sodium Butyrate</p>
Filtration of bioreactor cell culture	Four polyethersulfone filters (8.0 µm, 0.65 µm, 0.22 µm and 0.22 µm) in series	<p>1st stage - Four modules in parallel, each with a layer of diatomaceous earth graded to 4-8 µm and a layer of diatomaceous earth graded to 1.4-1.1 µm, followed by a cellulose membrane.</p> <p>2nd stage -single module containing a layer of diatomaceous earth graded to 0.4-0.11 µm and a layer of diatomaceous earth graded to <0.1 µm, followed by a cellulose membrane.</p> <p>3rd stage - 0.22 µm polyethersulfone filter</p>
	100 L storage bag	<p>300L storage bag</p> <p>Harvested cell culture is supplemented with 10 mM EDTA, 10 mM Tris to a pH of 7.5.</p>

Concentration and buffer exchange prior to chromatography	Concentrate with 2 TFF with Millipore Spiral Polyethersulfone 30K MWCO Filter Buffer Exchange the Concentrate 6× with 10 mM Hepes, 25 mM NaCl, pH 7.0 20L sterile storage bag	Concentrate using four Sartorius Sartoslice TFF 30K MWCO Filter Buffer Exchange the Concentrate 10× with 10 mM Tris, 20 mM Na ₂ SO ₄ , pH 7.5 50L sterile storage bag
Viral inactivation prior to chromatography	None	Viral inactivation performed with the addition of a 1% Triton X-100, 0.3% Tributyl Phosphate, pH 7.5,
1 st purification step (Q sepharose)	No absorbance reading	A280 measurements at the beginning and end
Viral filtration after chromatography	Pall DV-20 filter (20 nm)	Sartorius Virosart filter (20 nm)
Concentration and buffer exchange after chromatography	Hepes/saline pH 7.0 buffer Protein concentrated to 1 mg/ml	Histidine/saline, pH 6.0 buffer Protein concentrated to 10 mg/ml

Example 6

Targeting Hyaluronan with Recombinant Hyaluronidase (rHuPH20) in

5 Hyaluronan-Rich Tumors

A. *In vitro* Formation of Hyaluronan -Specific Pericellular Matrix Halos by Hyaluronan-Rich Tumor Cells

Hyaluronan-rich tumor cell cultures and hyaluronan-deficient tumor cell cultures were assessed for the ability to form pericellular matrices (halos) *in vitro*,
 10 using a particle exclusion assay and immunohistochemistry. For this study, low-density cultures of hyaluronan-rich human prostate tumor carcinomas (PC3) and low-density cultures of hyaluronan-deficient human lung carcinomas (NCI H460) were treated in cell media for 60 minutes at 37 °C, followed by incubation in the presence or absence of 0.5 mg/mL bovine aggrecan (commercially available) which is a large
 15 aggregating chondroitin sulfate proteoglycan at 37 °C for 60 minutes.

To assess halo formation by particle exclusion, small particles (5×10^6 formalin-fixed red blood cells(RBCs)) were added to the cultures. After the particles had settled, the cultures were viewed at 400X or 100X magnification.

To assess hyaluronan expression by immunohistochemistry, a subset of the
5 cultures were stained with biotin labeled hyaluronan binding protein (HABP-bio)
(Seikagaku, Japan). After washing to remove the primary reagent, a FITC-labeled
streptavidin (Vector Labs, Canada) was used as a secondary reagent. Nuclei were
counter-stained using a DAPI (4',6-diamidino-2-phenylindole) reagent. Micrographs
were captured via a Nikon Eclipse TE2000U microscope coupled to a Insight
10 FireWire digital camera (Diagnostic Instruments, Michigan).

Pericellular matrix halos were identified as the areas staining positive, by
immunohistochemistry, for HA, and the areas from which particles (RBCs) were
excluded in the particle exclusion assay. The results revealed halos (in a
representative experiment, roughly the size of a cell) in the hyaluronan-rich tumor
15 (PC3) cultures that had been pre-treated in the presence of aggrecan. By contrast,
halos did not form in the absence of aggrecan, nor in the cultures of hyaluronan-
deficient (NCI H460) tumor cells, suggesting that the formation of pericellular
matrices was dependent on hyaluronan expression.

To determine whether targeting hyaluronan could block the formation of these
20 halos, the particle exclusion study and the immunohistochemistry were carried out in
cell cultures that had been treated (prior to addition of aggrecan) for 60 minutes at 37
°C, in the presence or absence of a soluble recombinant human hyaluronidase
composition designated rHuPH20. rHuPH20 was included in the tumor cell cultures
at 1000 enzymatic units per mL, as determined using the method described in
25 Example 2, above. Immunofluorescent staining with an anti-hyaluronan antibody
(commercially available) revealed an absence of hyaluronan staining after incubation
with rHuPH20. Following incubation with rHuPH20, the particle assay was
performed as described above. Micrographs revealed that with rHuPH20, halos did
not form in the (PC3) tumor cell cultures. rHuPH20 had no effect on the hyaluronan-
30 deficient cell (NCI H460) cultures, which did not form pericellular matrices with or

without rHuPH20. These results support the indication that the *in vitro* halo formation in tumor cell cultures is hyaluronan-dependent.

The same particle exclusion assay was carried out with seven different types of human tumor cell lines, having varying degrees of HA expression, in the presence or absence of rHuPH20, as described in this Example, above. The cell lines used were BxPC3 (Pancreas tumor line) PC3 (prostate tumor line), MDA-MB-231 (breast tumor line), HCT 116 (colon tumor line), DU145 (prostate tumor line), MiaPaCa2 (pancreas tumor line) and the H460 (the NSC lung cancer line). A “halo per cell area” value was determined by measuring the average halo area in the culture dish (in pixels or mm²), divided by average cell area in pixels or mm². In order to compare the halo per cell area in the different tumor type cell cultures, the value for BxPC3 cells was set at 100, and the value for the HA-deficient cell line H460, was set at 0, and a “relative halo fraction” was determined for each cell type by comparison. The results are set forth in Table 14, below. The numbers listed represent an average of n = 25 measurements, plus or minus standard error of the mean (SEM).

Table 14: Relative Halo Fraction and HA Reduction by rHuPH20 in Tumor Cell Cultures

Human tumor type	Cell line	Relative HALO Fraction	
		-rHuPH20	+rHuPH20
Pancreas	BxPC3	100.0 ± 9.6	4.5 ± 0.8
Prostate	PC3	84.2 ± 4.6	14.2 ± 1.2
Breast	MDA-MB-231	82.1 ± 7.3	10.4 ± 1.3
Colon	HCT 116	50.8 ± 5.7	9.9 ± 0.9
Prostate	DU145	31.2 ± 4.3	5.5 ± 0.9
Pancreas	MiaPaCa 2	16.7 ± 1.4	4.2 ± 0.7
NSC Lung	H460	0.0 ± 0.8	- 2.3 ± 0.3

A timecourse study also was carried out using this assay to determine the duration of HA removal in the PC3 cultures following transient exposure of the cells to rHuPH20, followed by a “chase” period where the cells were incubated with cell media in the absence of the enzyme. As above, a “halo per cell area” value was

determined by measuring the average halo area, in pixels or mm², divided by average cell area in pixels or mm². Halo per cell area was calculated prior to treatment (time zero (0)), and the value set as 100 %. For each of six treated time-points, the cells were incubated in the presence of 1000 U/mL rHuPH20 for an hour, followed by a wash to remove the enzyme and a chase period, where the cells were incubated without the enzyme, for a total of 2, 4, 8, 16, 24 and 48 hours, respectively. For each time-point, halo per cell area was calculated and a relative halo fraction was determined by comparison to the 100 % time zero value. The results are set forth in Table 15, below. The numbers listed represent an average of n = 45 measurements, plus or minus standard error of the mean (SEM)

Table 15: Duration of HA Removal in PC3 Cell Cultures by rHuPH20

Total time (hours) post addition of 1000 U/mL rHuPH20 (including 1 hour incubation and chase)	Relative halo fraction (%)
0	100.00 ± 7.5
2	0.0 ± 2.7
4	3.2 ± 3.3
8	11.6 ± 4.4
16	64.4 ± 7.8
24	94.5 ± 5.6
48	92.7 ± 7.1

B. Intravenous rHuPH20 Administration in a Hyaluronan-Rich Tumor Model (Peritibial PC3 Prostate Carcinoma Xenograft Model) Reduces Distal Tumor Interstitial Fluid Pressure (IFP)

To analyze the effects of targeting hyaluronan in a hyaluronan-rich tumor, rHuPH20 was systemically administered in a peritibial prostate carcinoma (PC3) xenograft model. For this model, athymic male nude mice were inoculated with human prostate cancer cells, PC3 (1x10⁶ cells in a total volume of 0.05 mL) adjacent to the right tibia periosteum. All animal studies were performed in compliance with approved IACUC protocols.

To assess tumor interstitial fluid pressure (IFP) in these animals, Milar® Mikro-tip Catheter pressure transducers (SPR-320s) were connected to an ADInstruments® PowerLab 4/30 data acquisition unit and portable laptop computer for continuous IFP measurements (units = millimeters of mercury (mmHg)). The system was calibrated and considered stable when ambient pressure measurements did not deviate by more than ± 1.0 mmHg over 15-20 minutes. For probe placement, the pressure catheter was inserted into the inner bore of a 21 gauge needle, and the needle introduced into the center of the tumor. The needle was withdrawn around the transducer, while the pressure catheter was simultaneously held in position. The system then was used to measure IFP in the tumors (mmHg).

In one exemplary experiment, baseline distal tumor IFP measurements in 26 xenograft model animals using this method, revealed a correlation between tumor volume and IFP (correlation coefficient, $r = 0.5652$), suggesting that increased tumor volume may lead to increased IFP.

To determine whether systemic rHuPH20 treatment could reduce tumor IFP in this xenograft model, 10,000 units (as determined in Example 2) of rHuPH20, was administered in a 100 μ L dose by intravenous injection in the tail vein of three animals. Prior to dosing (injection) with rHuPH20, IFP was measured for approximately 20-30 minutes, to establish a baseline IFP measurement. IFP then was measured during and following injection. IFP was measured for 1-2 hours post-rHuPH20 administration. The intravenous rHuPH20 administration caused a reduction in distal tumor IFP. Within 10 minutes, tumor IFP was reduced by an average of 34 % compared to baseline. Further, tumor IFP levels dropped from 34.06 ± 15.9 mmHg to 14.86 ± 12.55 mmHg in one hour (an average of 38 % of the baseline level; a 61.3 % average reduction), the maximum reduction. Heat-inactivated rHuPH20, administered to animals as a control, did not decrease interstitial fluid pressure.

Consistent with rapid hyaluronan turnover, tumor IFP returned to baseline within 24 hours in all the mice. A repeat intravenous administration of 10,000 units rHuPH20, given 48 hours following the initial dose, revealed a similar drop in IFP (28.6 % of baseline, average). Measurements taken before and after rHuPH20

administration in non-tumor bearing limbs revealed no change in interstitial fluid pressure in these tissues. These data indicated that systemic, intravenous administration of hyaluronidase can be used to reduce interstitial fluid pressure in distal tumors.

5 Interstitial fluid pressure also was measured in a xenograft model generated similarly, by injection of the hyaluronan-deficient lung carcinoma cells (H460). While tumors in this model exhibited high interstitial fluid pressure, the effects of intravenous administration of rHuPH20 was much less pronounced in this model compared to the hyaluronan-rich tumor model, supporting the indication that the
10 effect of rHuPH20 on tumor interstitial pressure is hyaluronan-dependent.

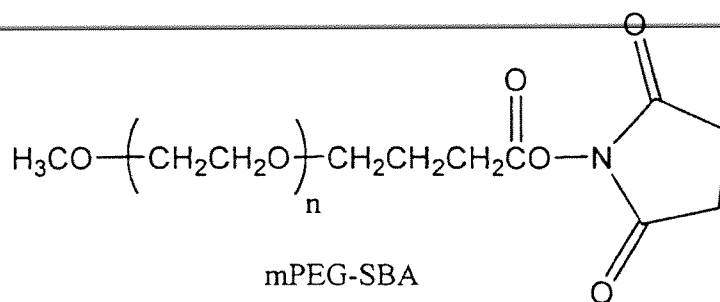
Example 7

PEGylation of rHuPH20

A. Conjugation of mPEG-SBA-30K to rHuPH20

In order to generate a PEGylated soluble human hyaluronidase, rHuPH20
15 (which is approximately 60 KDa in size) was covalently conjugated to a linear N-hydroxysuccinimidyl ester of methoxy poly(ethylene glycol) butanoic acid (mPEG-SBA-30K), having an approximate molecular weight of 30 kDa. The structure of mPEG-SBA is shown in scheme 2, below:

Scheme 2



20

Methods used to prepare the mPEG-SBA-30K that was used to PEGylate rHuPH20 are described, for example, in U.S. 5,672,662). Briefly, the mPEG-SBA-30K is made according to the following procedure:

A solution of ethyl malonate (2 equivalents) dissolved in dioxane is added
25 drop by drop to sodium hydride (2 equivalents) and toluene under a nitrogen atmosphere. mPEG methane sulfonate (1 equivalent, MW 30 kDa, Shearwater) is dissolved in toluene and added to the above mixture. The resulting mixture is

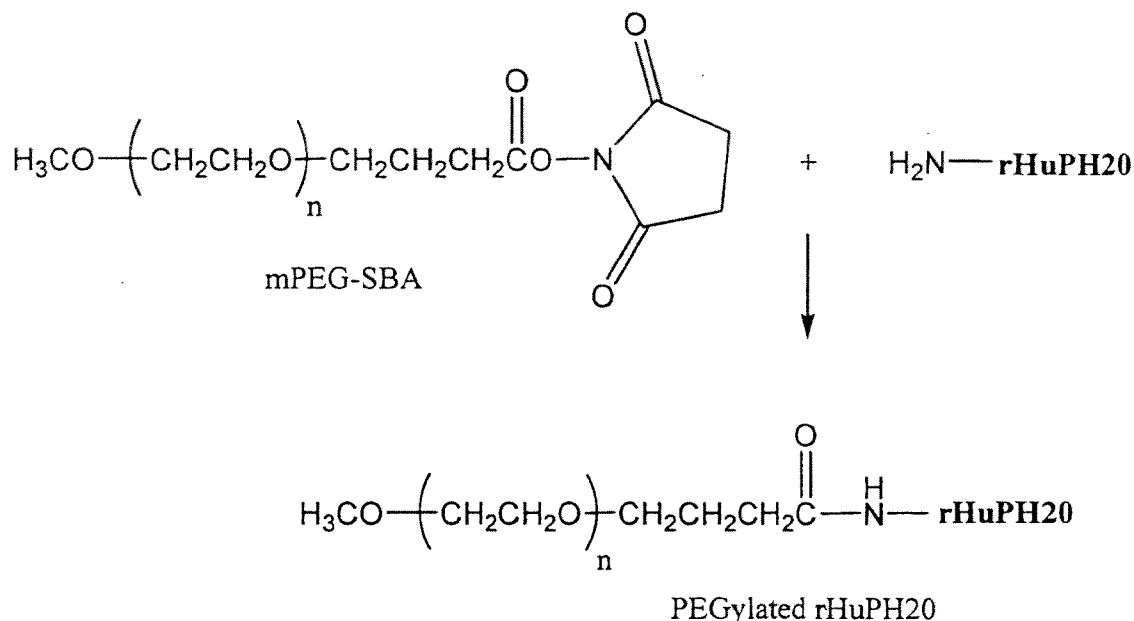
refluxed for approximately 18 hours. The reaction mixture is concentrated to half its original volume, extracted with 10% aqueous NaCl solution, extracted with 1% aqueous hydrochloric acid, and the aqueous extracts are combined. The collected aqueous layers are extracted with dichloromethane (3x) and the organic layer is dried with magnesium sulfate, filtered and evaporated to dryness. The resulting residue is dissolved in 1N sodium hydroxide containing sodium chloride and the mixture is stirred for 1 hour. The pH of the mixture is adjusted to approximately 3 by addition of 6N hydrochloric acid. The mixture is extracted with dichloromethane (2x).

The organic layer is dried over magnesium sulfate, filtered, concentrated, and poured into cold diethyl ether. The precipitate is collected by filtration and dried under vacuum. The resulting compound is dissolved in dioxane and refluxed for 8 hours and then concentrated to dryness. The resulting residue is dissolved in water and extracted with dichloromethane (2x), dried over magnesium sulfate, and the solution is concentrated by rotary evaporation and then poured into cold diethyl ether.

The precipitate is collected by filtration and dried under vacuum. The resulting compound (1 equivalent) is dissolved in dichloromethane and N-hydroxysuccinimide (2.1 equivalents) is added. The solution is cooled to 0 °C and a solution of dicyclohexylcarbodiimide (2.1 equivalents) in dichloromethane is added dropwise. The solution is stirred at room temperature for approximately 18 hours. The reaction mixture is filtered, concentrated and precipitated in diethyl ether. The precipitate is collected by filtration and dried under vacuum to afford mPEG-SBA-30K.

To make the PEGylated rHuPH20, mPEG-SBA-30K was coupled to the amino group(s) of rHuPH20 by covalent conjugation, providing stable amide bonds between rHuPH20 and mPEG, as shown in Scheme 3.

Scheme 3:



For the conjugation, the mPEG-SBA-30K was added in powder form to rHuPH20 (at a concentration of 10 mg/mL in 130 mM NaCl /10 mM HEPES; pH 7).

- 5 The PEG:rHuPH20 ratio was 10:1 (molar ratio). After the PEG had dissolved in the buffer, the solution was sterile-filtered (Corning 50 mL Tube top filter, polystyrene, cellulose acetate 0.22 μm membrane). The conjugation was carried out overnight, with stirring, at 4°C in a cold room.

Following conjugation, the solution was concentrated, using a 100,000

-
- 10 MWCO TFF membrane, and buffer exchanged against 130 mM NaCl /10 mM HEPES at pH 6.8. The resulting material, which was tested for enzyme activity, as described in Example 2, above, was diluted using 130 mM NaCl /10 mM HEPES at pH 6.8 to obtain a final enzyme activity of 100,000 U/mL (corresponding to approximately 2.5 mg peptide/mL). This PEGylated rHuPH20 material was filled, in
 - 15 1 mL volumes, into a 13-mm Type-1 glass vial with brombutyl seal, and stored frozen (frozen overnight in a -80°C freezer, then put in a -20°C freezer for longer storage).

B. Analysis of PEGylated rHuPH20

- 20 The PEGylated rHuPH20 material was assayed by gel electrophoresis. Three batches of PEGylated rHuPH20, made as in Example 7A above, revealed an identical pattern of multiple bands, representing unreacted PEG and multiple species of mPEG-rHuPH20 conjugates, which migrated at different distances. Based on comparison

with migration of a molecular weight marker, the bands representing the species ranged from approximately 90 KDa to 300 KDa, with three dark bands migrating above the 240 KDa marker. These data indicated that the PEGylated rHuPH20, generated by covalent conjugation of mPEG-SBA-30K, contained a heterogeneous mixture of PEGylated rHuPH20 species, likely including mono-, di- and tri- PEGylated proteins. The lack of a visible band at 60 KDa suggested that all the protein had reacted with the PEG, and that no detectable native rHuPH20 was present in the mixture.

C. Dose-dependent reduction of Hyaluronan-Specific Pericellular Matrix Halos in Hyaluronan-Rich Tumor Cells by PEGylated rHuPH20

With an assay similar to that described in Example 6A, the effects of various doses of PEGylated rHuPH20 on formation of pericellular matrices (halo's) in hyaluronan-rich human prostate tumor carcinoma (PC3) cells were assessed. For this assay, various low-density cultures of PC3 cells were treated with cell media alone (control; 0), or PEGylated rHuPH20 (at 3, 30, 300, 1000 or 3000 enzymatic units per mL (U/mL), as determined using the method described in Example 2, above), for 60 minutes at 37 °C. The cultures then were incubated with 0.5 mg/mL bovine aggrecan (commercially available; large aggregating chondroitin sulfate proteoglycan) at 37 °C for 60 minutes.

To visualize the amount of HA in the cultures using HA (hyaluronan) protein immunohistochemistry (IHC), cells then were stained with biotin labeled biotin labeled hyaluronan binding protein (HABP-bio) (Seikagaku, Japan). After washing to remove the primary reagent, a FITC-labeled streptavidin (Vector Labs, Canada) was used as a secondary reagent. Nuclei were counter-stained using a DAPI (4',6-diamidino-2-phenylindole) reagent. Micrographs were captured via a Nikon Eclipse TE2000U microscope coupled to a Insight FireWire digital camera (Diagnostic Instruments, Michigan).

For red blood cell (RBC) particle exclusion assay, 5×10^6 formalin-fixed red blood cells) were added to each culture. After the particles had settled, the cultures were viewed at 100x magnification. Pericellular matrix halos were identified as the

areas staining positive by IHC for HA, and the areas from which particles (RBCs) were excluded in the particle exclusion assay.

The “halo area” was determined by measuring the pixels staining positively for HA (immunohistochemistry), or the mm² of the area from which RBCs were excluded (particle exclusion assay). The average cell area was determined in a similar manner in pixels or mm² for each assay, respectively. The “halo area per cell area” then was derived by dividing the halo area by the average cell area for fields for each sample. “Percent halo” then was determined by setting the halo area per cell for the control sample (media alone) as 100 % and the halo area per cell for the 1000 U-PH20-treated hyaluronan-degraded H460 cells (from Example 6A, above) as zero. The results are presented in Table 16, below. This Table lists the average “percent halo” for each study condition (doses of PEGylated rHuPH20); each condition was performed in triplicate wells and 25 cells counted per well (total n=75 cells counted); standard error of the mean (SEM) is shown. The data revealed that the half maximal effective concentration (EC₅₀) of PEGylated rHuPH20 in reducing Halos in this study was 3.1 U/mL (0.09 µg/mL). The 75 % maximal effective concentration (EC₇₅) was 14.1 U/mL (0.4 µg/mL).

Table 16: Dose-Dependent Reduction in Halo Formation in Hyaluronan-Rich Tumor Cell Cultures Treated with PEGylated rHuPH20

Dose PEG-rHuPH20 (U/mL)	% Halo	SEM
0	100	4.2
3	51.5	3.1
30	12.3	1.4
300	9.8	1.0
1000	4.8	0.6
3000	0	0.5

D. PEGylated rHuPH20 Inhibits Colony Growth of PC3 Cells *in vitro*

To determine whether PEGylated rHuPH20 can inhibit anchorage-independent growth and proliferation of hyaluronan-rich prostate tumor cells (PC3) *in vitro*, a three-dimensional clonogenic assay was performed on cells. PC3 cells, at

approximately 80% confluency, were trypsinized, harvested, and washed once in completed medium. Cell density was adjusted to 8×10^4 /mL cells and suspended in Matrigel® (BD Biosciences, San Jose, CA) on ice. 0.025 mL of this cell / Matrigel® mixture were seeded onto a 48 well cell culture plate that had been pre-coated with
5 Matrigel® at 0.1 mL per well, and solidified at 37°C for 1 hour. For continuous exposure, over 17 days, to control API buffer and various concentrations of PEGylated rHuPH20, 0.6 mL/well of completed medium containing API buffer, 1, 3, 10 and 100 U/mL of PEGylated rHuPH20 were added to the top of the appropriate well. The wells were incubated at 37°C, in a humidified atmosphere with 5% CO₂ in
10 air for 17 days, fresh treatment medium, including the appropriate concentration of enzyme, where appropriate, was replace every 3-4 days during the 17 days period.

On day 17, growth of colonies was assessed by capturing images with a Nikon Eclipse TE2000U inverted microscope coupled to an Insight FireWire digital camera (Diagnostic Instruments, Michigan). The colony number and diameter of each colony
15 in μm were measured using ImageJ software (open source software, a publicly available program for display and analysis of images, for calculating area and pixel value) and coupled calibration function (colony volumes were calculated using colony diameter and using the formula: $\frac{4}{3} \pi r^3$).

Average colony volume of wells for each condition were determined and the
20 ~~effects of PEGylated rHuPH20 on colony volume assessed by comparing the average~~ colony volume in the control sample (API (active pharmaceutical ingredient) buffer (10 mM Hepes and 130 mM NaCl, pH 7.0) without enzyme) to the samples that were incubated in the presence of PEGylated rHuPH20. Inhibitory ratios were calculated using the formula:

25 **(mean volume of control - mean volume of treated) / (mean volume of control)*100.**

PEGylated rHuPH20 induced a dose-dependent inhibition of growth, evidenced by lower colony volume compared with control. Based on inhibitory ratios calculated using the above formula, the cultures incubated in the presence of PEGylated rHuPH20 at 1, 3, 10, and 100 U/mL exhibited an average reduction in
30 colony volume of 39%, 67%, 73%, and 75% respectively ($p < 0.01$ for the 3 U and 10 U samples; $p < 0.001$ for the 100 U samples; $n=6$), compared to cultures incubated with control buffer. Statistical differences were analyzed using the Mann-Whitney Test.

The IC₅₀ of PEGylated rHuPH20 in reducing colony volume, determined using the Graphpad Prism®4 program (GraphPad Software, Inc., La Jolla, CA), was approximately 1.67 U/mL. The average number of colonies was 10.17±1.56 per well in vehicle-treated (control) cultures and 11.50 ± 0.89 per well in the cultures treated with PEGylated rHuPH20 100U/mL. The difference in colony number was not significant between the control and the 100U/mL cultures (n=6, p>0.05). These results indicate that PEGylated rHuPH20 can inhibit proliferation and/or survival of hyaluronan rich cancer cells.

Example 8

10 **PEGylated rHuPH20: Increased Duration of *in vivo* action Compared with Native rHuPH20**

A. Evaluation of the pharmacodynamics of native rHuPH20 and PEGylated rHuPH20

1. Evaluation of the pharmacodynamics of native rHuPH20.

15 Ninety male CD-1 mice were obtained from Charles River Laboratories. The animals were of approximately 3 to 5 weeks old and weighed about 17 to 23 grams at the day of randomization. Animals were dosed via tail vein injection (intravenous) of either 0.398 mg/kg HUA (Lot No. HUA0703MA; generated using Gen1 production described in Example 3) or 0.433 mg/kg HUB (Lot No. HUB0702CA; generated
20 ~~using Gen2 production described in Example 4) rHuPH20. The intravenous~~ injection was by a slow push taking approximately 30 seconds. Six animals dosed with PH20 (HUA or HUB) were anesthetized/euthanized for blood collection at predose, at 1, 5, 10, 30 minutes, and at 1, 2, and 3 hours post dose. Plasma samples were harvested and stored frozen until rHuPH20 concentration measurement.

25 Plasma concentrations of rHuPH20 were determined using a 96-well plate-based enzymatic assay. The enzyme assay was a modification of the method described by Frost et al. (1997) (A Microtiter-Based Assay for Hyaluronidase Activity Not Requiring Specialized Reagents. Analytical Biochemistry (1997) 251:263-269) that provides a measure of rHuPH20 content in plasma specimens or tissue
30 homogenates. First, biotinylated HA (bHA) substrate was bound to plastic microtiter plates. rHuPH20 present in standards and specimens was incubated in these bHA-

coated plates for approximately 90 minutes at 37°C under optimized conditions. After stopping the enzyme reaction, remaining bound bHA was detected by initially adding a streptavidin-HRP conjugate for 60 minutes and then subsequently visualizing the response using TMB substrate. Since more rHuPH20 in a standard or sample would lead to less bHA available to bind SA-HRP, the optical density (450 nm) value was inversely proportional to the concentration of hyaluronidase activity in each specimen. The assay range was from approximately 0.013 to 1 U/mL. For a 50-fold sample dilution factor, the LLOQ was approximately 0.625 U/mL. Hence, the lower limit of quantitation (LLOQ) of the assay with a 1:100 dilution of the sample was 0.625 U/mL of enzyme. Pharmacokinetic (PK) analysis was conducted using WinNonlin Pro version 5.1 (Pharsight Corp., Mountain View, CA.). PK parameters were derived using non-compartmental methods. Summary statistics were computed using EXCEL (Microsoft Corp., Seattle, WA) or JMP v.5.0.1 (SAS Institute, Cary, NC.). ANOVAs were conducted using JMP v.5.0.1.

Peak plasma concentrations of rHuPH20 (HUA and HUB) was attained immediately upon initiation of IV infusion (at the 1-minute collection time). The average peak concentration (C_{max}) for HUA and HUB were 1148 and 1176 ng/mL, respectively. Decline from peak concentration was rapid and bi-phasic for both HUA and HUB. The initial distribution phase, between 1 and 5 minutes post dose, was followed by a slightly slower elimination phase. Plasma concentrations fell below the assay quantitation limit (0.625 U/mL) by 30 minutes post dose. The terminal half-life (T_{1/2 λz}) from the IV injection was 0.045 hour (2.6 minutes) for HUA and was 0.038 hours (2.3 minutes) for HUB. The short half-life represented rapid elimination of rHuPH20 in male CD-1 mice.

2. Evaluation of the pharmacodynamics of PEGylated rHuPH20

A single dose PK study was conducted in male IRC mice (Harlan) to characterize and compare the plasma concentration versus time profiles for two lots of PEGylated rHuPH20 (PEGPH20); lots 2005-9-14 (specific activity 38,000 U/mg) and lot 221-092 (specific activity 32,000 U/mg). Additionally, the PK concentration following repeated Monday-Thursday BIW (twice-a-week) IV injections were examined in one study group of animals.

PEGPH20 (made as described in Example 7A) was administered intravenously to male IRC mice, weighing more than 25 g.; the IV dose was 125,000 hyaluronidase activity units per kilogram body weight (U/kg). Blood samples were collected prior to dosing and at pre-determined times post dose. Due to the limited blood volume of a mouse, a sparse sampling scheme was used for sequential blood collections. Randomization of study animals and sparse blood collection times are shown in Table 16A.

TABLE 16A: Randomization and Sparse Blood Collection Scheme				
Group	N/group	Test Article	Dose	Time Points for Blood Withdrawals
1	3	PEGPH20 Lot #221-092	125,000 U/kg	5 min, 30 min, 1h, 24h
2	3	PEGPH20 Lot #2005-9-14	125,000 U/kg	5 min, 30 min, 1h, 24h
3	3	PEGPH20 Lot #221-092	125,000 U/kg	1h, 2h, 4h, 24h
4	3	PEGPH20 Lot #2005-9-14	125,000 U/kg	1h, 2h, 4h, 24h
5	3	PEGPH20 Lot #221-092	125,000 U/kg	Pre, 4h, 8h, 24h
6	3	PEGPH20 Lot #2005-9-14	125,000 U/kg	4h, 8h, 24h
7	3	PEGPH20 Lot #2005-9-14	125,000 U/kg per dose	1h, 4h, 24h, 48h, 72h – first dose 1h, 4h, 24h, 48h, 96h – second dose 1h, 4h, 24h, 48h – third dose

Plasma was prepared from collected blood samples and stored frozen at -70°C until analysis. Hyaluronidase activity in each plasma sample was determined by a micro turbidity microtiter plate based-assay described in Example 2. The lower limit of quantitation for the assay was 2.90 U/mL. Plasma concentration versus time data was analyzed by non-compartmental and compartmental methods using WinNonlin Pro version 5.1 (Pharsight Corp., Mountain View, CA). Derived PK parameters included AUC, Cmax, and Tmax. These parameters were compared between the two

lots of PEGPH20 to assess PK comparability. A general specific activity of 35,000 U/mg was used for conversion of U/mL to $\mu\text{g/mL}$. The 35,000 U/mg was an average of the specific activities of the 2 lots of PEGPH20 (lots 221-092 and 2005-9-14).

Systemic exposure defined by AUC and C_{max} were similar between the two
5 lots of PEGPH20 when administered intravenously to mice. The general PK profiles from the two lots were also similar with prolonged elimination half-life of about 10 to 11 hours and a slow systemic clearance of less than 10 mL/h-kg. Statistical comparison of mean and median plasma concentrations by blood collection times showed no significant differences between the 2 lots of PEGPH20. Repeated BIW
10 (Monday-Thursday) IV administration of PEGPH20 for a total of 3 doses gave expected plasma concentrations that could be predicted by the pharmacokinetics of the first dose. These results suggested that PEGylated rHuPH20 has a sustained *in vivo* enzymatic activity following administration by intravenous injection, having greater than a about 250-fold increase in half-life.

15 **B. In Vivo Activity in the Hyaluronan-Rich Tumor Model (Peritibial PC3 Prostate Carcinoma Xenograft Model)**

To compare the effects of PEGylated and native rHuPH20 following intravenous administration, the peritibial PC3 prostate carcinoma xenograft model animals, described in Example 6, above, were treated with native and PEGylated
20 rHuPH20.

1. Increased Duration of Hyaluronidase Enzymatic Activity in Plasma following administration of PEGylated rHuPH20

Two groups of peritibial PC3 prostate carcinoma xenograft model animals (athymic males bearing PC3 human prostate cancer xenografts; see Example 6,
25 above) were injected intravenously with 10,000 units of PEGylated rHuPH20 (made as described in Example 7A) and native rHuPH20, respectively.

Blood was taken from the animals prior to administration of 10,000 units of native rHuPH20 / PEGylated rHuPH20, and at various time-points following administration. Plasma was prepared and the enzymatic activity assay described in
30 Example 2, above, was used to determine the units of rHuPH20 activity per mL plasma at these time-points. The results revealed that the half-life of enzymatic

activity in the plasma after administration of native rHuPH20 was less than one minute (plasma half-life was 0.59 minutes in this experiment). By 10 minutes following administration of native rHuPH20, less than 5 enzymatic units per mL of plasma was observed.

- 5 By contrast, PEGylated rHuPH20 exhibited a plasma enzymatic half life of over 24 hours. At 60 minutes following administration of 10,000 enzymatic units of PEGylated rHuPH20, greater than 1000 enzymatic activity units (greater than 10 % of that injected) were observed per mL of plasma. These results suggested that PEGylated rHuPH20 has a sustained *in vivo* enzymatic activity following
- 10 administration by intravenous injection, having greater than a 2000-fold increase in half-life.

2. Increased Duration of Hyaluronan Reduction in Distal Tumors with PEGylated rHuPH20

- Peritibial tumors from the mice described in Example 8B.1, above, were
- 15 harvested and fixed in normal buffered formalin (NBF), prior to native/PEGylated rHuPH20 administration, and 45 minutes, 2 hours, 24 hours, 48 hours and 72 hours post- administration. Levels of hyaluronan (HA) in the tumors were assessed by immunohistochemistry (IHC). For IHC, 5 μ m tumor sections were stained using a biotin labeled hyaluronan binding protein (HABP-bio) (Seikagaku, Japan). After
- 20 ~~washing to remove the primary reagent, a FITC-labeled streptavidin (Vector Labs,~~
Canada) was used as a secondary reagent. Nuclei were counter-stained using a DAPI (4',6-diamidino-2-phenylindole) reagent. Micrographs were captured via a Nikon Eclipse TE2000U microscope coupled to a Insight FireWire digital camera (Diagnostic Instruments, Michigan).

- 25 Before rHuPH20 administration, an intense green staining was indicative of the presence of HA in the tumors. Forty-five (45) minutes after intravenous administration of 10,000 Units native rHuPH20, tumors displayed no visible HA staining. However, within 24 hours, HA levels in the tumors appeared to have returned to that observed prior to dosing, consistent with rapid hyaluronan turnover
- 30 and short half-life of native rHuPH20. Following administration of PEGylated rHuPH20, however, no HA staining was observed at 2 hours, 48 hours, nor 72 hours,

following administration. These results suggested that intravenous administration of PEGylated rHuPH20 results in sustained enzymatic activity at the tumor site, for at least 72 hours following administration.

3. Dose Response of HA-Degradation in Distal Tumors by PEGylated 5 rHuPH20

In another study, animals in an additional group of peritibial PC3 prostate carcinoma xenograft model animals were treated with vehicle and various amounts concentrations (10, 100, 500, 1000 and 10,000 enzymatic units (U) per mouse) of PEGylated rHuPH20. Three days (72 hours) following this treatment, peritibial
10 tumors from the mice were harvested and fixed in normal buffered formalin. Levels of hyaluronan (HA) in the tumors were assessed by immunohistochemistry (IHC), as described in Example 8B.2, above. Hematoxylin/Eosin (H&E) staining also was done on the tumor sections. The results revealed a dose-dependent reduction in HA staining in the tumor sections from animals treated with 100, 500, 1000 and 10,000 U
15 PEGylated rHuPH20. In the sections from animals receiving 1000 and 10,000 U PEGylated rHuPH20, almost no visible staining was observed. In the sections of animals that had received 500 U PEGylated rHuPH20, sporadic staining was observed, indicating that in this exemplary study, HA staining was completely removed only with doses greater than 500 (in this case, 1000) U. These results
20 indicated that a single systemic administration of PEGylated rHuPH20 can reduce hyaluronan in distal tumors for at least three days. The ED₅₀ (dose eliciting a 50 % reduction in HA staining) of the PEGylated rHuPH20 in this study was 13,000 enzymatic units per kilogram (U/Kg), which corresponds to 300 µg/Kg.

4. Duration of Action of PEGylated PH20 Removal of HA from Distal 25 PC3 Tumors

In a further study, PC3 prostate tumor model animals were treated with vehicle for ten days, or 10,000 U per mouse (approximately 15 mg/Kg) of PEGylated rHuPH20 for 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 days. After the treatment, peritibial tumors from the mice were harvested and fixed in normal buffered formalin and assessed for
30 hyaluronan (HA) expression via immunohistochemistry as described in Example

8B.2, above. HA staining was reduced at all time points, with no visible detection of HA staining out to day 5.

Example 9

Sustained IFP Suppression with PEGylated rHuPH20

5 A. PEG-rHuPH20 Administration Results in Rapid, Sustained, Dose-Dependent Reduction in Tumor IFP.

In order to compare the effects of systemic administration of PEGylated rHuPH20 (PEG rHuPH20) and native rHuPH20 on distal tumor IFP, the method described in Example 6B, above, was used to measure tumor IFP after intravenous
10 injection of various amounts of each enzyme in PC3 xenograft model animals.

First, reduction in tumor IFP was compared, using three mice per group, each mouse receiving a 10,000 unit dose of either PEG rHuPH20 or native rHuPH20. As noted in Example 6B, above, with administration of native rHuPH20, tumor IFP was reduced by an average of 34 % in 10 minutes and 61.3 % in one hour, following
15 administration. Administration of 10,000 units of PEG rHuPH20 resulted in a similar (22.4 %) reduction in the first ten minutes following injection. By one hour post-administration, however, the PEG rHuPH20 had reduced the distal tumor IFP by an average of 88.6 %, compared to baseline, indicating that PEGylated soluble hyaluronidase has an increased and/or sustained effect on IFP compared with native
20 enzyme.

Next, in order to determine whether the effect of rHuPH20 on interstitial fluid pressure were dose-dependent, the method described in Example 6B, above, was carried out using 10 groups of PC3 xenograft mice (7 mice per group), each group being treated as described above, with 100, 1000, 3000, 7000 (PEGylated rHuPH20
25 only) or 10,000 units of PEGylated or native rHuPH20, or control carrier buffer, was administered intravenously by tail vein injection. IFP was measured prior to, during and continuously following the administration. IFP was measured until 120 minutes post-administration.

Results revealed that intravenous administration of both native and PEGylated
30 rHuPH20 reduced distal tumor IFP in a dose-dependent manner. Several doses of PEGylated rHuPH20 resulted in greater decreases in IFP by one and two hours

following administration. For example, in this exemplary experiment, after 120 minutes, administration of 1000 units of native rHuPH20 resulted in an approximately 30 % reduction in tumor IFP (roughly 70 % compared to control), while administration of the same dose (1000 units) of PEG rHuPH20 reduced tumor IFP by greater than 50 % (roughly 48 % of control). IFP reduction at the highest doses (3,000 - 10,000 U/mouse) of PEGylated rHuPH20 was greater than 80% at 120 minutes. IC₅₀ (for % IFP inhibition at 120 minutes following PEGylated rHuPH20 treatment) was 807.6 U/mouse. The three highest dose (3000, 7000 and 10,000 U PEGylated rHuPH20/mouse) effects appeared to plateau at this time-point.

10 **B. Intravenous Administration of PEGylated rHuPH20 Results in Prolonged Tumor IFP Suppression**

To determine whether the increased duration of *in vivo* activity with PEGylated rHuPH20, as observed in Example 8, translated into prolonged suppression of tumor IFP, groups of PC3 xenograft model animals (4 animals per group) were injected intravenously with carrier buffer or 10,000 units of PEGylated rHuPH20 as described above, and IFP measured, as described in Example 6B, at 8 hours, 24 hours, 48 hours and 72 hours post administration.

Similar experiments had shown that distal tumor IFP returned to control levels by 24 hours following administration of native rHuPH20. By contrast, in this experiment, IFP was reduced by at least about 50 % at 8 hours, 24 hours and 48 hours following intravenous administration of a single dose of 10,000 units PEGylated rHuPH20. In this experiment, the average tumor IFP approached control levels by 72 hours. These results indicate that PEGylated rHuPH20 can be administered intravenously to effect prolonged interstitial fluid pressure decreases at distal tumor sites.

25 **C. Reduction in Tumor IFP by PEGylated rHuPH20 Administration is HA-Dependent**

To demonstrate that reduction in tumor IFP by PEGylated rHuPH20 was dependent on presence of hyaluronic acid (HA) in the tumor, the effect on IFP was assessed in five different tumor models. For this study, groups of animals for each tumor model were generated by inoculating male athymic nude (nu/nu) mice with the

appropriate cell lines: PC3 (described above, e.g. Example 6); BxPC3 (a human pancreatic carcinoma cell line); Mat LyLu (a rat prostatic carcinoma derived from the Dunning series of rat prostate carcinomas); Du145 (a human hormone refractory prostate carcinoma cell line); and NCI H460 (a human large cell lung carcinoma cell line), in the hind limb, peritibially, as described for the PC3 model (See Example 6, above).

As described in Example 17, below, tumors isolated from these mice were assessed by immunohistochemistry (IHC) for differences in the degree of HA expression. The IHC was performed on sections from the tumors and a scoring system was used, where a “+++” score indicated intense staining in greater than 80 % of the visual field, “+/-” indicated sparse staining and “-” indicated no visible staining. As noted in Example 17 and in Table 17 below, this study revealed a range of HA levels in tumors, ranging from +++ to +/-.

Animals from the PC3 prostate tumor model, BxPC3 pancreatic carcinoma tumor model, Mat LyLu prostate tumor model, Du145 human prostate tumor model, and NCI H460 lung carcinoma model, having tumors that had reached between 400 and 800 mm³ then were injected intravenously with 15 mg/Kg (10,000 U per mouse) PEGylated rHuPH20 or control buffer (API (active pharmaceutical ingredient) buffer (10 mM Hepes and 130 mM NaCl, pH 7.0) without enzyme). The relative amounts of tumor HA in each of these models, as determined as described in Example 17A, below, is set forth in Table 17, below.

IFP was measured, as described in Example 6B, above, prior to, during and continuously following the administration. IFP was measured until 120 minutes post-administration. Table 17, below, indicates the approximate relative reduction in IFP (compared to treatment with control buffer) at 120 minutes, for each tumor model. For these numbers, IFP was compared in the animals that received PEGylated rHuPH20, to that in animals receiving control buffer. The results indicate that PEGylated rHuPH20 intravenous administration reduces IFP in distal tumors in an HA-dependent manner.

Table 17: Reduction in Tumor IFP by PEGylated rHuPH20 Depends on HA amounts in Tumors

Tumor Model	Relative Tumor HA	Approximate Relative IFP Reduction
PC3	+++	84 %
BxPC3	+ / ++	30 %
Mat LyLu	+	25 %
Du145	+/-	19 %
NCI H460	+/-	16%

Example 10

Intravenous Administration of rHuPH20 Results in Increased Vascular Volume in Distal Tumors (Sustained Effects with PEGylated rHuPH20)

5 Transverse 2-dimensional ultrasound imaging was used to measure vascular volume before and after rHuPH20 administration in peritibial tumors in the xenograft model described in Example 6, above.

A VisualSonics Vevo 770® high resolution ultrasound (U/S) (VisualSonics, Inc., Toronto, Ontario, Canada) was used, in Contrast Mode, to obtain relative measurements of vascular volume in the tumors. During Contrast Mode image acquisition, non-targeted nitrogen/perfluorobutane filled MicroMarker® micro-bubbles (MBs) (VisualSonics, Inc.), which are not, but become trapped in the vascular space due to their size, were administered intravenously by tail vein injection. The MBs were hyper-echoic, thus providing strong ultrasound wave reflections and had a median diameter of 2.3 - 2.9 μ m. Thus, the MBs were confined to the vascular compartment following injection. Relative vascular volume was calculated, using the equation below, as ultrasound signal intensity after MB injection (B) minus ultrasound signal intensity prior to MB injection (A), per tumor region of interest (ROI):

$$20 \quad \text{Vasc Vol } (\tau_1) = [(B - A) / \text{ROI}]$$

The change in relative vascular volume was the difference in the vascular volume (as calculated with the equation above) at two time points (e.g. before and following administration of rHuPH20), as per the following equation:

$$\text{Change in Vasc Vol} = \text{Vasc Vol } (\tau_2) - \text{Vasc Vol } (\tau_1)$$

Using these methods, vascular volume in peritibial tumors was measured 8 hours, 24 hours, 48 hours and 72 hours following intravenous administration of soluble human hyaluronidase (native or PEGylated) to xenograft model animals, as described in Examples 6 and 9 above. At eight and twenty-four hours post-administration of native rHuPH20, distal tumor vascular volume was between 2 and 2.5-fold higher than after injection of carrier buffer alone. At eight and twenty-four hours post-administration of PEGylated rHuPH20, however, the vascular volume was between 3 and 4-fold higher than after injection of buffer alone. By 48 hours following administration of the PEGylated rHuPH20, the tumor vascular volume was still increased 3-fold compared to the vascular volume observed after injection with buffer alone. These results indicate that systemic administration of PEGylated rHuPH20 can effect sustained “enzymatic decompression” of tumor vessels, resulting in increased vascular volume.

Enzymatic decompression of tumor blood vessels was also assessed by immunohistochemistry (IHC) for visualization of blood vessels. Peritibial tumors from groups (of 3 PC3 xenograft model animals each), which had been treated with carrier buffer or 10,000 Units PEGylated rHuPH20 were harvested, fixed in formalin and embedded in paraffin.. For detection of blood vessels, sections from the tumors were stained using a rat anti-mouse CD31 antibody (Pharmingen, San Diego). After washing the primary antibody, the sections were stained with HRP-conjugated rabbit anti-rat IgG (Vector Labs, Canada). After washing the secondary antibody, a DAB substrate was used to visualize CD31 staining. Nuclei were counter stained with hematoxylin. Micro-vessel area was quantified using ImageJ open source software, a publicly available program for display and analysis of images, for calculating area and pixel value. In this case, the program was used to measure the area inside the CD31 positive microvessels. The results revealed an average CD31+ microvessel area of approximately 200 square micrometers (μm^2) in tumor sections from the control-treated animals, and of approximately 700 μm^2 in tumor sections from mice that had received intravenous doses of PEGylated rHuPH20 48 hours prior. This greater than three-fold increase supported the finding that PEGylated rHuPH20 administration effects a sustained enzymatic decompression of tumor blood vessels.

Example 11

Intravenous Administration of PEGylated rHuPH20 Results in Sustained Decrease in Tumor Water Content

5 Tumor water content was measured in groups of PC3 xenograft animals (9 mice per group) at various time points following intravenous administration, as described in the above Examples, of 10,000 Units PEG-rHuPH20 or carrier buffer alone. Water content was measured 2, 8, 24, 48, 72, 96 and 120 hours following administration.

10 To measure water content, PC3 tumors pieces were harvested, blotted, weighed, and snap frozen. Samples were subsequently placed in a lyophilizer, dried for over 48 hrs and weighed. Water weight was reported as the tissue wet weight to dry weight ratio. Results revealed that wet/dry weight ratio was significantly reduced ($p < 0.05$) in the mice having received PEG rHuPH20, relative to animals having received carrier buffer injection, at all time points measured.

15 Relative H₂O Diffusion Rate (ADC) - Animals were administered native rHuPH20, PEG-rHuPH20, or carrier buffer and Diffusion Weighted MRI measured over time to determine Apparent Diffusion Coefficients (ADCs) (see, for example, Park MJ et. al, *Korean J Radiol.* 2007 (5):390-6, *The role of diffusion-weighted imaging and the apparent diffusion coefficient (ADC) values for breast tumors*). The
20 ~~ADC value reflected tumor water content. At both 48 and 72 hrs after dosing, the~~
mean H₂O apparent diffusion coefficient (ADC) after PEG-rHuPH20 treatment was statistically different than treatment with carrier buffer alone. The reduction in water ADC reflected a decrease in water diffusion/translational motion in the tumors in animals treated intravenously by PEGylated rHuPH20. Thus, water diffusion was
25 significantly decreased in tumors in these animals at both 48 and 72 hours, relative to control. This result indicates that the interstitial volume fraction was decreased and/or that the tortuosity of interstitial fluid was decreased, following intravenous administration of PEGylated rHuPH20, for at least 72 hours.

Example 12

30 **Determination of sialic acid and monosaccharide content**

The sialic acid and monosaccharide content of soluble rHuPH20 can be assessed by reverse phase liquid chromatography (RPLC) following hydrolysis with trifluoroacetic acid. In one example, the sialic acid and monosaccharide content of purified hyaluronidase lot # HUB0701E (1.2 mg/mL; produced and purified essentially as described in Example 5) was determined. Briefly, 100 µg sample was hydrolyzed with 40 % (v/v) trifluoroacetic acid at 100°C for 4 hours in duplicate. Following hydrolysis, the samples were dried down and resuspended in 300 µL water. A 45 µL aliquot from each re-suspended sample was transferred to a new tube and dried down, and 10 µL of a 10 mg/mL sodium acetate solution was added to each.

The released monosaccharides were fluorescently labeled by the addition of 50 µL of a solution containing 30 mg/mL 2-aminobenzoic acid, 20 mg/mL sodium cyanoborohydride, approximately 40 mg/mL sodium acetate and 20 mg/mL boric acid in methanol. The mixture was incubated for 30 minutes at 80 °C in the dark. The derivatization reaction was quenched by the addition of 440 µL of mobile phase A (0.2% (v/v) n-butylamine, 0.5% (v/v) phosphoric acid, 1% (v/v) tetrahydrofuran). A matrix blank of water also was hydrolyzed and derivatized as described for the hyaluronidase sample as a negative control. The released monosaccharides were separated by RPLC using an Octadecyl (C₁₈) reverse phase column (4.6 x 250 mm, 5 µm particle size; J.T. Baker) and monitored by fluorescence detection (360 nm excitation, 425 nm emission). Quantitation of the monosaccharide content was made by comparison of the chromatograms from the hyaluronidase sample with chromatograms of monosaccharide standards including *N*-D-glucosamine (GlcN), *N*-D-galactosamine (GalN), galactose, fucose and mannose. Table 18 presents the molar ratio of each monosaccharide per hyaluronidase molecule.

Table 18. Monosaccharide content of soluble rHuPH20

Lot	Replicate	GlcN	GalN	Galactose	Mannose	Fucose
HUB0701E	1	14.28	0.07*	6.19	25.28	2.69
	2	13.66	0.08*	6.00	24.34	2.61
	Average	13.97	0.08*	6.10	24.81	2.65

* GalN results were below the limit of detection

Example 13

C-terminal heterogeneity of soluble rHuPH20 from 3D35M and 2B2 cells

C-terminal sequencing was performed on two lots of sHuPH20 produced and purified from 3D35M cells in a 100 L bioreactor volume (Lot HUA0505MA) and 2B2 cells in a 300L bioreactor volume (Lot HUB0701EB). The lots were separately digested with endoproteinase Asp-N, which specifically cleaves peptide bonds N-terminally at aspartic and cysteic acid. This releases the C-terminal portion of the soluble rHuPH20 at the aspartic acid at position 431 of SEQ ID NO:4. The C-terminal fragments were separated and characterized to determine the sequence and abundance of each population in Lot HUA0505MA and Lot HUB0701EB.

It was observed that the soluble rHuPH20 preparations from 3D35M cells and 2B2 cells displayed heterogeneity, and contained polypeptides that differed from one another in their C-terminal sequence (Tables 19 and 20). This heterogeneity is likely the result of C-terminal cleavage of the expressed 447 amino acid polypeptide (SEQ ID NO:4) by peptidases present in the cell culture medium or other solutions during the production and purification process. The polypeptides in the soluble rHuPH20 preparations have amino acid sequences corresponding to amino acids 1-447, 1-446, 1-445, 1-444 and 1-443 of the soluble rHuPH20 sequence set forth SEQ ID NO:4. The full amino acid sequence of each of these polypeptides is forth in SEQ ID NOS: 4 to 8, respectively. As noted in tables 19 and 20, the abundance of each polypeptide in the soluble rHuPH20 preparations from 3D35M cells and 2B2 cells differs.

Table 19. Analysis of C-terminal fragments from Lot HUA0505MA

Fragment	Amino acid position (relative to SEQ ID NO: 4)	Sequence	Theor. mass	Exp. Mass	Error	Elution time	Abundance
D28a	431-447	DAFKLPPMETEEPQIFY (SEQ ID NO:57)	2053.97	2054.42	0.45	99.87	0.2%
D28b	431-446	DAFKLPPMETEEPQIF (SEQ ID NO:58)	1890.91	1891.28	0.37	97.02	18.4%
D28c	431-445	DAFKLPPMETEEPQI (SEQ ID NO 59)	1743.84	1744.17	0.33	86.4	11.8%
D28d	431-444	DAFKLPPMETEEPQ	1630.70	1631.07	0.32	74.15	56.1%

		(SEQ ID NO:60)					
D28e	431-443	DAFKLPPMETEEP (SEQ ID NO:61)	1502.70	1502.98	0.28	77.36	13.6%
D28f	431-442	DAFKLPPMETEE (SEQ ID NO:62)	1405.64	ND	N/A	N/A	0.0%

Table 20 Analysis of C-terminal fragments from Lot HUB0701EB

Fragment	Amino acid position (relative to SEQ ID NO: 4)	Sequence	Theor. mass	Exp. Mass	Error	Elution time	Abundance
D28a	431-477	DAFKLPPMETEEPQIFY (SEQ ID NO:57)	2053.97	2054.42	0.45	99.89	1.9%
D28b	431-446	DAFKLPPMETEEPQIF (SEQ ID NO:58)	1890.91	1891.36	0.45	96.92	46.7%
D28c	431-445	DAFKLPPMETEEPQI (SEQ ID NO:59)	1743.84	1744.24	0.40	85.98	16.7%
D28d	431-444	DAFKLPPMETEEPQ (SEQ ID NO:60)	1630.70	1631.14	0.39	73.9	27.8%
D28e	431-443	DAFKLPPMETEEP (SEQ ID NO:61)	1502.70	1503.03	0.33	77.02	6.9%
D28f	431-442	DAFKLPPMETEE (SEQ ID NO:62)	1405.64	ND	N/A	N/A	0.0%

5

Example 14.

Anti-tumor effects of PEGylated rHuPH20 and cytotoxic agents following intravenous administration in prostate cancer models.

The anti-tumor effect of co-administration of PEGylated rHuPH20 and the cytotoxic agents docetaxel (Taxotere®; Sanofi Aventis) and liposomal doxorubicin (Doxil®; Ortho Biotech), was assessed using a hormone refractory prostate cancer xenograft model and bone metastasis model.

10

A. Prostate cancer xenograft model

To establish PC3 peritibial hormone refractory prostate cancer xenograft model, athymic male nude mice were inoculated intramuscularly with human PC3 prostate cancer cells (1×10^6 cells per mouse in a total volume of 0.05 mL) adjacent to the right tibial periosteum to generate tumors with a mean interstitial fluid pressure of
5 34 ± 16 mmHg. The tumors were allowed to grow to a mean tumor volume of 400-500 mm³ before initiation of treatment with either cytotoxic agent/PEGylated rHuPH20, cytotoxic agent alone or PEGylated rHuPH20 alone. Tumor growth was monitored using VisualSonics Imaging Micro-ultrasound system.

Mice in one group were intravenously administered 10 mg/kg Taxotere® (docetaxel) and 10,000 U/mouse PEGylated rHuPH20 on days 0 and 7. This was performed by
10 first drawing 0.1 mL Taxotere® (docetaxel) into a syringe, followed by 0.1 mL PEGylated rHuPH20, then immediately injecting the 0.2 mL solution into the tail vein of a mouse. These mice also received additional doses of 10,000 U/mouse PEGylated rHuPH20 in 0.2 mL intravenously on days 3 and 10. A control group of mice (G2)
15 received 10 mg/kg docetaxel Q7Dx2 intravenously on days 0 and 7. Another control group of mice received 30 mg/kg docetaxel Q7Dx2 intravenously on days 0 and 7. An additional control group of mice were administered 10,000 U/mouse PEGylated rHuPH20 intravenously on days 0, 3, 7 and 10. A final control group that received only API buffer also was included in the study (G1). The tumor volume was
20 ~~measured at various time points (Figure 1 and Table 21) and the percentage survival~~ to 1500 mm³ tumor volume also was observed (Figure 2). Table 22 presents analysis of the effect of co-administration of Taxotere® (docetaxel) and PEGylated rHuPH20 on survival. The Median Survival Time (MST) (in days) was calculated by median time to 1500 mm³ tumor volume based on individual animals in each group. The %
25 T/C is calculated by $\%T/C = (T/C) \times 100$, where T is the median survival time to 1500 mm³ tumor volume in the treated group and C is the median survival time to 1500 mm³ tumor volume in the API buffer control group. By National Cancer Institute (NCI) criteria, a product with a %T/C over 125 % is considered active. The Increase in Life Span (ILS) is a measure of anti-tumor activity calculated as $[(T-C)/C] \times 100$,
30 where (T-C) is the difference in the median day to 1500 mm³ tumor volume between

the treated (T) and control (C) groups. By NCI criteria, a product with a %ILS over 25% is considered effective.

Co-administration of Taxotere® (docetaxel) and PEGylated rHuPH20 resulted in a synergistic tumor growth inhibition over a period of 73 days that was significantly superior to that of docetaxel alone at 10 mg/kg given by Q7Dx2 or PEGylated rHuPH20 alone, and an increase in survival time (defined as time to reach tumor volume of 1500 mm³).

10 **Table 21. Effect of co-administration of Taxotere® (docetaxel) and PEGylated rHuPH20 on tumor volume.**

Treatment		Tumor volume (mm ³) at various days post-treatment									
		-1	4	13	21	27	35	42	50	61	73
API buffer (std dev)	Av	472.06	794.35	1077.2	1920.6	2549.4					
	SE	126.29	176.47	240.71	711.18	1095.7					
Taxotere® (docetaxel) (30 mg/kg) alone	Av	471.34	497.04	401.12	409.02	291.65	213.73	147.05	130.2	343.03	865.5
	SE										
Taxotere® (docetaxel) (10 mg/kg) alone	Av	470.39	687.27	869.25	1011.1	1260.7	1441.1	1552.1			
	SE										
PEGylated rHuPH20 alone	Av	469.47	506.38	602.98	1213.6	2024.7					
	SE										
Taxotere® (docetaxel) and PEGylated rHuPH20	Av	497	419.94	392.34	431.73	367.97	348.28	576.69	720.43	1106.2	1424.1
	SE										
		65.52	74.99	119.47	120.87	82.74	70.4	179.41	184.55	289.82	407.88

Table 22. Effect of co-administration of Taxotere® (docetaxel) and PEGylated rHuPH20 on survival.

Treatment	No. of Mice	Average Survival Time	MST	%T/C	%ILS	P-value*
Control (API Buffer)	6	23.01 ± 4.52	20.7	-	-	-
Taxotere® (docetaxel) (10 mg/kg) alone	6	38.96 ± 7.07	32.9	157	59	NS vs. API control
PEGylated rHuPH20 alone	5	27.22 ± 3.87	24.7	119	19	NS vs. API control
Taxotere®	7	64.71 ± 3.20	67.2	319	225	p=0.0002

(docetaxel) and PEGylated rHuPH20						API control; p=0.003 vs. Taxotere® (docetaxel) alone
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To assess the anti-tumor effects of co-administration of PEGylated rHuPH20 and liposomal doxorubicin, mice harboring PC3 tumors were intravenously administered 6 mg/kg Doxil® liposomal doxorubicin and 10,000 U/mouse PEGylated rHuPH20 on Days 0, 7 and 14. This was performed by first drawing 0.1 mL Doxil® liposomal doxorubicin into a syringe, followed by 0.1 mL PEGylated rHuPH20, then immediately injecting the 0.2 mL solution into the tail vein of a mouse. These mice also received additional doses of 10,000 U/mouse PEGylated rHuPH20 in 0.2 mL on days 3, 5, 10, 12. A control group of mice were intravenously administered 6 mg/kg Doxil® liposomal doxorubicin on days 0, 7 and 14. Another control groups of mice were administered doses of 10,000 U/mouse PEGylated rHuPH20 in 0.2 mL on days 0, 3, 5, 7, 10, 12 and 14. A final control group that received only API buffer also was included in the study. Tumor volume was observed at various time points (Figure 3 and Table 23). It was observed that the anti-tumor effects of co-administered PEGylated rHuPH20 and liposomal doxorubicin was statistically significantly superior to that of liposomal doxorubicin alone.

Table 23. Effect of co-administration of Doxil® liposomal doxorubicin and PEGylated rHuPH20 on tumor volume.

Treatment		Tumor volume (mm ³) at various days post-treatment						
		-1	6	13	18	24	27	36
Control (API Buffer)	Av.	532.95	926.12	1908.97	2322			
	SE	55.69	170.25	453.4	469.96			
PEGylated rHuPH20 alone	Av.	467.52	483.42	850.46	1096.05	1495.48	1996.86	
	SE	60.26	104.18	290.35	358.28	457.49	626.1	
Doxil® liposomal doxorubicin (10 mg/kg) alone	Av.	454.89	880.01	1237.57	1236.616	1256.25	1327.96	
	SE	65.21	229.44	317.69	260.81	400.68	415.97	
Doxil® liposomal doxorubicin and	Av.	452.95	352.28	451.97	497.52	561.28	770.27	1227.18
	SE	56.58	55.78	53.37	80.12	73.6	55.91	114.98

PEGylated rHuPH20								
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B. PC-3M-luc-C6 bone metastasis model

The PC-3M-luc-C6 bone metastasis model was established in nude mice as described by Jenkins *et al.* (Clin Exp Metast (2003) 20:745-756). Due to the bioluminescence of the PC-3M-luc-C6 cells, in addition to monitoring the survival of the mice, the mice were imaged to visualize the PC-3M-luc-C6 metastases. Briefly, 7-10 week old nu/nu male mice (Charles River Laboratories) were anesthetized and injected with 3×10^6 PC-3M-luc-C6 cells (a bioluminescent human prostate carcinoma cell line) suspended in 50 μ L sterile DPBS by intracardial injection on day 0. Mice were injected into the left ventricle of the heart by non-surgical means to increase the potential for metastasis from PC-3M-luc-C6 cells. The intracardiac injection of tumor cells bypasses the lungs and serves as a method to disperse cells into general circulation in order to allow seeding of metastasis in multiple tissues within an animal. A satisfactory injection into the left ventricle was detectable within minutes by bioluminescent imaging (BLI) and was identified by an immediate but transient systemic bioluminescence over the entire animal, as indicated by complete blue signal BLI of mice. An unsuccessful implantation produced a more localized signal generally isolated and sustained over time solely within the thoracic region of the animal. The animals that showed high bioluminescent signal in the chest on days 0 and 11 were excluded from the study. Mice with successful intra-cardiac injection of PC-3M-luc-C6 cells on day 0 ($n = 10-13$ mice/Grp) were imaged twice a week for up to four weeks and subsequently followed up for survival till day 49 and beyond that time point. In all the mice, early indications of metastasis to various tissues were observed within fourteen days after injection of cells. The observed patterns of metastasis indicated lesions developing in the thorax, jaw and/or leg of mice.

On day 14, the mice were subjected to one of several treatment regimens. A set of control mice were administered API buffer (10 mM Hepes and 130 mM NaCl, pH 7.0) only on days 14, 16, 18, 21, 23, 25, 28, 30, and 32. Two other sets of mice received 30 mg/kg Taxotere® (docetaxel) Q7Dx2 on days 14 and 21, or 10 mg/kg Taxotere® (docetaxel) Q7Dx3 on days 14, 21 and 28, respectively. Another group of

mice were administered 10,000 U/mouse PEGylated rHuPH20 intravenously on days 14, 16, 18, 21, 23, 25, 28, 30, 32 and 35. Two other groups of mice received either admixed PEGylated rHuPH20/Taxotere® (docetaxel) or sequentially administered PEGylated rHuPH20 then Taxotere® (docetaxel). The first of these groups were
5 intravenously administered 10 mg/kg Taxotere® (docetaxel) and 10,000 U/mouse PEGylated rHuPH20 on days 14, 21 and 28 post injection. This was performed by first drawing 0.1 mL Taxotere® (docetaxel) into a syringe, followed by 0.1 mL PEGylated rHuPH20, then immediately injecting the 0.2 mL solution into the tail vein of a mouse. These mice also received additional doses of 10,000 U/mouse PEGylated
10 rHuPH20 in 0.2 mL intravenously on days 16, 18, 23, 25, 30 and 32. The second of these groups received the same dosing, but instead of an admixture of 10 mg/kg Taxotere® (docetaxel)/10,000 U PEGylated rHuPH20, the 10,000 Unit PEGylated rHuPH20 dose was administered two hours prior to administration of the 10 mg/kg Taxotere® (docetaxel) dose.

15 The mice were imaged as described previously (Jenkins *et al.* (2003) Clin Exp Metast 20:745-756) using an IVIS® Imaging System (Xenogen) and analyzed using Living Image® software (Xenogen) on days 0, 11, 15, 18, 22, 25 and 29 to visualize the bioluminescent PC-3M-luc-C6 metastases. The initial signals at days 0 and 11 were confirmed by subsequent images till day 29, and nearly all metastatic sites
20 showed a gradual increase in bioluminescence over time. The *in vivo* bioluminescent signal from the jaw region of mice corresponds to micrometastases in the dental pulp, mandible or cervical lymph nodes. Lower limb *in vivo* signals corresponded to metastatic lesions identified in the tibia or femur leg bones. The thoracic signals that are observed *in vivo* are associated with residual tumor cells that had seeded into the
25 heart, lung or the pleural surface of the chest cavity. Mice with images indicating a successful systemic injection on day 0 and showing *in vivo* evidence of metastasis by day 29 were included in the analysis of the treatment groups.

Taxotere® (docetaxel) administered intravenously at 30 mg/kg on Days 14 and 21 represents the highest dose level at MTD (maximum tolerated dose). Images
30 taken from day 11 to day 29 indicate that the drug has significant inhibitory effect at all metastatic sites in the treated mice. The images on Day 11 show mice with

representative metastatic patterns that gradually disappear with time as indicated by the decrease in bioluminescence intensity detectable at day 29 in the 10 out of 11 mice remaining alive at Day 29. A similar trend, but to a lesser extent, of luminescence decrease was observed in the images of 10 mg/kg Taxotere® (docetaxel) treated mice, with 9 out of 12 surviving animals at day 29 post-tumor cell injection. In contrast, control PEGylated rHuPH20 treated animals showed evidence of a subsequent relapse in whole animal bioluminescence, indicated by a rebound signal at day 29 as seen from the images of 6 surviving mice. Images at day 29 of mice that received admixed PEGylated rHuPH20/Taxotere® (docetaxel) and mice that received sequentially administered PEGylated rHuPH20 and Taxotere® (docetaxel) demonstrated unchanged to reduced levels of BLI signal amount and intensity, indicating stabilization of tumor dissemination to bone sites. In particular, four mice that received sequential doses of PEGylated rHuPH20 and Taxotere® (docetaxel) appeared to have become completely signal free on day 29, indicating possible cures or long term survivors effects.

The survival rates of each group of mice 49 days after intracardial injection of PC-3M-luc-C6 are presented in Table 24. It was observed that co-administration of 10,000 units PEGylated rHuPH20 and 10 mg/kg Taxotere® (docetaxel) resulted in greater anti-tumor activity than treatment with 10 mg/kg Taxotere® (docetaxel) alone.

Table 24. Effect of co-administration of PEGylated rHuPH20 and Taxotere® (docetaxel) of survival in a PC-3M-luc-C6 bone metastasis model

Treatment	Number of. Animals	No. of animals alive at day 49	% survival
API buffer	13	3	23.08
Taxotere® (docetaxel) (30 mg/kg)	11	10	90.91
Taxotere® (docetaxel) (10 mg/kg)	12	9	75.00
PEGylated rHuPH20	10	4	40.00
PEGylated rHuPH20 and Taxotere®	13	13	100

(docetaxel) (admixed)			
PEGylated rHuPH20 and Taxotere® (docetaxel) (sequentially 2 hrs apart)	11	10	90.91

Example 15.

Toxicity Studies - PEGylated rHuPH20 alone and in combination with docetaxel

For a toxicity study of PEGylated rHuPH20 alone and in combination with Taxotere® (docetaxel), nude mice were administered various doses of PEGylated rHuPH20 with 10 mg/kg Taxotere® (docetaxel). Control groups of mice receiving PEGylated rHuPH20 alone, Taxotere® (docetaxel) alone or API buffer alone also were included in the study. Table 25 sets forth the treatment regimen for each group of mice. For toxicity studies, body weights and in-life observation were monitored over the course of study for up to 26 days. Complete blood chemistry with differential and serum clinical chemistry was assessed on whole blood and serum samples by an external Contract Research Organization, BioQuant Inc (San Diego, CA).

Tables 26 and 27 and Figures 4 and 5 set forth the changes in body weight following administration of PEGylated rHuPH20 alone, PEGylated rHuPH20/Taxotere® (docetaxel) and Taxotere® (docetaxel) alone. Table 27 and Figure 6 set forth the number of granulocytes in the blood of mice administered PEGylated rHuPH20 alone, PEGylated rHuPH20/Taxotere® (docetaxel) and Taxotere® (docetaxel) alone, and Table 28 and Figure 7 set forth the serum albumin levels in mice administered PEGylated rHuPH20 alone, PEGylated rHuPH20/Taxotere® (docetaxel) and Taxotere® (docetaxel) alone. The normal range for number of granulocytes in the blood of healthy mice is $1.2-6.8 \times 10^3$ cells/ μ L, and normal serum albumin ranges are 2.5-4.8 g/dL. It was observed that co-administration of docetaxel and PEGylated rHuPH20 was well tolerated with no significant increase in neutropenia (as indicated by no significant reduction in granulocytes) and was better tolerated compared to docetaxel treatment alone at

maximum tolerated dose (MTD) of 30 mg/kg. Administration of PEGylated rHuPH20 alone at doses of up to 30,000 U/mouse also was well tolerated.

Table 25. Treatment regime for PEGylated rHuPH20/Taxotere toxicity study.

Group No.	Dose Route	Dose	Dose Volume (mL/mouse)	Dose Frequency
1	IV	API Buffer (control group)	0.3	Study days 0,3,7,10,14,17,21,24
2	IV	PEGylated rHuPH20 (3,000 U/mouse)	0.3	Study days 0,3,7,10,14,17,21,24
3	IV	PEGylated rHuPH20 (7,000 U/mouse)	0.3	Study days 0,3,7,10,14,17,21,24
4	IV	PEGylated rHuPH20 (10,000 U/mouse)	0.3	Study days 0,3,7,10,14,17,21,24
5	IV	PEGylated rHuPH20 (30,000 U/mouse)	0.3	Study days 0,3,7,10,14,17,21,24
6	IV	PEGylated rHuPH20 (3,000 U/mouse) + Taxotere® (docetaxel) (10 mg/kg)	0.3	Study days 0,7,14,21 plus PEG alone on days 3,10,17,24
7	IV	PEGylated rHuPH20 (7,000 U/mouse) + Taxotere® (docetaxel) (10 mg/kg)	0.3	Study days 0,7,14,21 plus PEG alone on days 3,10,17,24
8	IV	PEGylated rHuPH20 (10,000 U/mouse) + Taxotere® (docetaxel) (10 mg/kg)	0.3	Study days 0,7,14,21 plus PEG alone on days 3,10,17,24
9	IV	Taxotere® (docetaxel) (10 mg/kg)	0.3	Study days 0,7,14 plus API buffer alone on days 3,10,17
10	IV	Taxotere® (docetaxel) (30 mg/kg)	0.3	Study days 0,7,14 plus API buffer alone on days 3,10,17

5 **Table 26. Change in body weight following administration of PEGylated rHuPH20 alone.**

Day	Percentage change in body weight				
	API Buffer (control group)	PEGylated rHuPH20 (3,000 U/mouse)	PEGylated rHuPH20 (7,000 U/mouse)	PEGylated rHuPH20 (10,000 U/mouse)	PEGylated rHuPH20 (30,000 U/mouse)

	Av.	SE	Av.	SE	Av.	SE	Av.	SE	Av.	SE
3	-4.3	1.4	-7.8	2.1	-12	2	-12.2	0.7	-13.7	1.3
5	-0.2	1.7	-8	1	-12.8	2.9	-9.2	0.8	-12.6	1.4
7	-2.4	1.5	-8.4	1.5	-11.3	3.4	-7.9	1	-11.1	1.8
10	-2.8	0.8	-9.5	1.2	-9.9	3.2	-7.2	1.1	-10.1	2.4
12	-1	1	-8.5	1	-8.4	2.7	-5.6	2.3	-8.9	2.1
14	2.4	1.5	-3	1.2	-3.9	2.3	-2.8	4.2	-3.9	2.3
17	-1.9	1.1	-5	0.7	-4.7	2.4	-1.2	8	-4.7	2.2
19	0	1.7	-0.6	1	-1.3	3	0.5	0.8	-2.8	2.9
21	1.5	2.2	0.5	1.5	0.7	2.5	2.1	2.5	-0.9	3
24	0.9	1.9	1.2	2	-0.4	1.5	5.1	2.9	2.6	2.4
26	-0.2	1.9	-0.4	1.5	2.1	1.2	6.7	5.3	4.5	3.2

Table 27. Change in body weight following administration of PEGylated rHuPH20/Taxotere® (docetaxel) or Taxotere® (docetaxel) alone.

Day	Percentage change in body weight									
	API Buffer (control group)		PEGylated rHuPH20 (3,000 U/mouse) + Taxotere® (docetaxel) (10 mg/kg)		PEGylated rHuPH20 (7,000 U/mouse) + Taxotere® (docetaxel) (10 mg/kg)		PEGylated rHuPH20 (10,000 U/mouse) + Taxotere® (docetaxel) (10 mg/kg)		Taxotere® (docetaxel) (10 mg/kg)	
	Av.	SE	Av.	SE	Av.	SE	Av.	SE	Av.	SE
3	-4.3	1.4	-14.9	3	-13.9	2.6	-11.8	1.2	-4	0.6
5	-0.2	1.7	-15.9	3	-15.3	3.3	-12.9	1.3	-3.1	1.5
7	-2.4	1.5	-16.8	2.8	-13.4	1.3	-12.7	0.6	-5.5	1.8
10	-2.8	0.8	-17.1	2.2	-12.1	2.5	-12.8	1.1	-4.9	2
12	-1	1	-17	2.2	-14.7	3.6	-13	1.3	-5.8	2.2
14	2.4	1.5	-15.2	3.5	-10.3	4	-8.4	0.7		
17	-1.9	1.1	-16.7	3.1	-10.2	3.7	-10.5	1.2		

19	0	1.7	-15.3	2.3	-9	3.6	-8.6	2.2		
21	1.5	2.2								
24	0.9	1.9								
26	-0.2	1.9								

Table 28. Number of granulocyte in the blood of mice following administration of PEGylated rHuPH20/Taxotere® (docetaxel) or Taxotere® (docetaxel) alone.

Treatment	Number of granulocytes in the blood ($1 \times 10^3/\mu\text{L}$)			
	Mouse 1	Mouse 2	Mouse 3	Mean
API Buffer	2.9	4.1	3.8	3.6
PEG rHuPH20 (3,000)	3.1	3.1	5.5	3.9
PEG rHuPH20 (7,000 U)	5.5	2.7	8.5	5.6
PEG rHuPH20 (10,000 U)	5.7	8.4	10.8	8.3
PEG rHuPH20 (30,000 U)	5.4	3	5.5	4.6
PEG rHuPH20 (3,000 U) + Taxotere® (docetaxel)	3.3	2.4	1.1	2.3
PEG rHuPH20 (7,000 U) + Taxotere® (docetaxel)	1.6	1.5	2.8	1.9
PEG rHuPH20 (10,000 U) + Taxotere® (docetaxel)	4.1	1.2	2.2	2.5
Taxotere® (docetaxel) (10 mg/kg)	3.2	0.9	0.7	1.6
Taxotere® (docetaxel) (30 mg/kg)	1.1	2.4	1.7	1.7

5 **Table 29. Albumin levels in mice following administration of PEGylated rHuPH20/Taxotere® (docetaxel) or Taxotere® (docetaxel) alone.**

Treatment	Albumin levels (g/dL)			
	Mouse 1	Mouse 2	Mouse 3	Mean

API Buffer	2.4	2.3	2.3	2.3
PEG rHuPH20 (3,000)	1.9	2	1.9	1.9
PEG rHuPH20 (7,000 U)	2	1.7	2	1.9
PEG rHuPH20 (10,000 U)	1.7	1.8	1.7	1.7
PEG rHuPH20 (30,000 U)	1.9	1.9	1.7	1.8
PEG rHuPH20 (3,000 U) + Taxotere® (docetaxel)	0.5	1.8	1.9	1.4
PEG rHuPH20 (7,000 U) + Taxotere® (docetaxel)	1.6	1.8	1.8	1.7
PEG rHuPH20 (10,000 U) + Taxotere® (docetaxel)	1.7	2.2	1.7	1.9
Taxotere® (docetaxel) (10 mg/kg)	2.1	2		2.0
Taxotere® (docetaxel) (30 mg/kg)	1.3	1.1	0.5	1

Example 16

Antitumor Activity of PEGylated rHuPH20 Alone in PC3 Xenograft Model

To demonstrate antitumor activity of PEGylated rHuPH20 alone, the PC3
5 xenograft model described in the Examples above was used to measure tumor growth following repeated and sustained administration.

A. Repeated Intravenous administration of PEGylated rHuPH20 produces anti-tumor effects in the PC3 human prostate cancer model.

The PC3 human prostate cancer model was generated as described above.
10 Athymic male nude mice were inoculated intramuscularly (IM) with human PC3 prostate cancer cells (1×10^6 cells per mouse in a total volume of 0.05 mL) adjacent to the right tibial periosteum to generate tumors with high interstitial fluid pressure. Tumors were allowed to grow to a mean tumor volume of 400-500 mm³ before

initiation of intravenous treatment with control vehicle and varying enzymatic unit (U) amounts of PEGPH20, as follows. Five groups of mice were used. Control vehicle was administered to group 1 (eleven animals); 1000 U PEGylated rHuPH20 was administered to each animal in group 2 (eight animals); 3,000 U PEGylated rHuPH20 was administered to each animal in group 3 (eight animals); 10,000 U PEGylated rHuPH20 was administered to each animal in group 4 (eight animals); and 30,000 U PEGylated rHuPH20 was administered to each animal in group 5 (eleven animals). PEGylated rHuPH20 or control vehicle was administered to each animal, three times per week (Q3W), every other day (EOD), Monday, Wednesday and Friday, for a total of eight doses: one dose on day 0, and then on day 3, 5, 7, 10, 12, 14 and 17.

Tumor volume (mm^3) was measured over the course of the study in each group of animals, at days 2, 4, 7, 11, 14 and 18 by capturing images using the VisualSonic ultrasound system and using an ultrasound imaging software program. The data are presented in Figure 8 and in Table 30, below. Error bars in Figure 8 represent standard error, which also is listed in Table 30. The results demonstrated that each dose of repeated intravenous PEGylated rHuPH20 caused a reduction in tumor growth, as evidenced by smaller tumor volumes at each post-treatment time-point, compared with the control group.

Table 30: Repeated Systemic Administration of PEGylated rHuPH20 Reduces Tumor Growth in PC3 Tumor Model

Amount PEGylated rHuPH20 per mouse (each treatment)		Tumor volume (mm^3) at various days compared to initiation of treatment					
		-2	4	7	11	14	18
0 (control)	Average	406.97	795.93	1017.15	1291.00	1733.69	1942.10
	Standard Error	15.86	52.61	70.82	75.72	88.88	181.76
1,000 U	Average	418.48	456.01	586.78	929.09	1291.94	1465.20
	Standard Error	18.14	41.75	69.07	113.70	128.89	205.63
3,000 U	Average	422.24	496.53	567.40	905.50	1281.85	1409.97
	Standard Error	17.13	27.68	32.58	53.39	132.05	192.96
10,000 U	Average	423.19	429.38	607.00	865.88	1180.50	1405.52
	Standard Error	16.79	14.10	34.13	57.35	84.83	89.91
30,000 U	Average	416.25	470.78	589.66	815.17	1039.30	1303.49
	Standard Error	13.42	27.90	39.04	73.87	119.10	127.75

To demonstrate removal of HA in the tumors of treated animals, tumors from animals in each group also were isolated, fixed and sectioned, as described in

Example 8B.2, above. 5 μ m sections were stained, as described in that Example. For immunohistochemistry, 5 μ m tumor sections were stained using a biotin labeled hyaluronan binding protein (HABP-bio) (Seikagaku, Japan). After washing to remove the primary reagent, a FITC-labeled streptavidin (Vector Labs, Canada) was used as a secondary reagent. Nuclei were counter-stained using a DAPI (4',6-diamidino-2-phenylindole) reagent. Micrographs were captured via a Nikon Eclipse TE2000U microscope coupled to a Insight FireWire digital camera (Diagnostic Instruments, Michigan). The results revealed no visible staining in tumors of each of the treated animals, demonstrating that systemic administration of 1,000, 3,000, 10,000 and 30,000 U PEGylated rHuPH20 removed all detectable hyaluronan in distal tumors in this human prostate cancer animal model.

B. Sustained, Systemic Exposure to PEGylated rHuPH20 Reduces Tumor Volume.

Ten PC-3 peritibial tumor-bearing mice were generated as described above, by peritibial implantation by inoculating athymic nude mice, intramuscularly (IM), with human PC3 prostate cancer cells (1×10^6 cells per mouse, total volume of 0.05 mL) adjacent to the right tibial periosteum. PC-3 peritibial tumor bearing mice with approximately 300 mm³ tumor volumes were implanted sub-cutaneously (SC) with Alza pumps (Alzet® mini-osmotic pumps, Model No. 2002, Durect Corporation, Cupertino, CA), containing control buffer (API buffer) (5 animals – control group) or PEGylated rHuPH20 (5 animals), at approximately 20,000 enzymatic units (U) per 0.2 ml volume (0.7 mg/mouse (35 mg/Kg) administered to each mouse over 12 days). For each group, the pump delivered 0.5 microliters per hour, over 14 days, which, for the group treated with PEGylated rHuPH20 was equal to 50 U per hour; 1,200 U per day.

Tumor volume (mm³) was measured prior to treatment at day -1, and at termination of the study at day 12, by capturing images using the VisualSonic ultrasound system and using an ultrasound imaging software program. The results are set forth in Table 31, below. As noted in the Table, one mouse in the control group was found dead before the conclusion of the study.

Table 31: Continuous Administration of PEGylated rHuPH20 Reduces PC3 Tumor Volume

Treatment Groups		Tumor Volume (mm ³)	
		Day -1	Day 12
Control (API Buffer)	Mouse 1	160.82	Found dead
	Mouse 2	245.18	777.22
	Mouse 2	254.59	983.69
	Mouse 4	355.1	743.72
	Mouse 5	442.09	964.37
	Average ($\pm$ SE)	291.56 $\pm$ 48.55	867.25 $\pm$ 62.15
20,000 U (total) per mouse PEGylated rHuPH20	Mouse 1	178.91	324.27
	Mouse 2	212.19	439.67
	Mouse 2	262.65	264
	Mouse 4	286.13	426.5
	Mouse 5	560.42	245.08
	Average $\pm$ SE	300.06 $\pm$ 67.62	339.904 $\pm$ 45.03

As set forth in Table 31, sustained intravenous exposure to PEGylated rHuPH20 (20,000 U total, over 12 days) reduced tumor growth, compared to the control animals. A significant difference ($p < 0.0015$) was observed at day 12, between tumor volumes in the control group and the group treated with PEGylated rHuPH20. Thus, continuous administration of PEGylated rHuPH20 has antitumor effects in a human prostate tumor xenograft model.

Body weights of the mice also were measured on days -1, 0, 4, 6, 8 and day 11. These results are set forth in Table 32, below.

Table 32: Body Weights after Continuous Administration of Control Buffer or PEGylated rHuPH20

Treatment Groups		Day 0		Day 4		Day 6		Day 8		Day 11	
		BW (g)	% Ch	BW (g)	% Ch	BW (g)	% Ch	BW (g)	% Ch	BW (g)	% Ch
Control (API Buffer)	Mouse 1	23.75	0.00	20.54	-13.52	20.46	-13.85	20.31	-14.48	fd	N/A
	Mouse 2	22.8	0.00	23.33	2.32	23.67	3.82	23.24	1.93	23.64	3.68
	Mouse 2	23.27	0.00	22.15	-4.81	22.02	-5.37	22.45	-3.52	23	-1.16
	Mouse 4	21.82	0.00	21.82	0.00	21.57	-1.15	21.96	0.64	22.41	2.70
	Mouse 5	25.32	0.00	23.26	-8.14	23.12	-8.69	23.55	-6.99	24.29	-4.07

	Avg	23.39	0	22.22	-4.83	22.17	-5.05	22.30	-4.49	23.33	0.29
20,000 U (total) per mouse PEGyla ted rHuPH 20	Mouse 1	25.76	0.00	23.92	-7.14	24.06	-6.60	24.79	-3.77	26.08	1.24
	Mouse 2	24.24	0.00	23.01	-5.07	23.41	-3.42	23.38	-3.55	23.32	-3.80
	Mouse 2	24.70	0.00	23.1	-6.48	23.36	-5.43	23.42	-5.18	23.93	-3.12
	Mouse 4	22.97	0.00	22.6	-1.61	23.36	1.70	22.64	-1.44	23.01	0.17
	Mouse 5	21.26	0.00	19.71	-7.29	20.02	-5.83	20.93	-1.55	21.31	0.24
	Avg	23.79	0.00	22.47	-5.52	22.84	-3.92	23.03	-3.10	23.53	-1.05

* Avg = average; BW (g) = body weight in grams; % Ch = percent change from day zero; fd = found dead.

5

Example 17

In vivo HA Reducing Activity and Antitumor Activity of PEGylated rHuPH20 in Multiple HA-Expressing Tumors

A. Systemic Administration of PEGylated rHuPH20 Removes HA in a Plurality of HA-Expressing Tumors

10 Expression of hyaluronan (HA) was assessed, as described in Example 8B.2, above, in various tumors in animal models of tumors (listed in Table 33, below) two hours after intravenous administration of vehicle or PEGylated rHuPH20 (10,000 U/mouse = 15 mg/Kg). The models were generated, as described below, from various cancerous cell lines.

15 (i) Cell lines

The cancerous cell lines used to make the animal tumor models are listed in Table 33, below. Human hormone refractory prostate cancer cell lines (PC3, DU145); human pancreatic cancer cell lines (MIAPACA II, BXPC3); human breast cancer cell line (MDA MB 231); human colon cancer cell lines (HCT116, HT29) and
20 human non small cell lung cancer cell line (NCI H460) were purchased from ATCC (Manassas, VA). The Dunning rat prostate cancer cell line (Mat LyLu) was a kind gift from the Department of Medicine, Physiology and Oncology, McGill University Health Centre, Montreal, Quebec, H3A 1A1 Canada. The mouse breast cell line (4T1-GFP) was a gift from Sidney Kimmel Cancer Center (SKCC, San Diego, CA). The
25 human pancreatic cancer cell line, Capan-1 H2B, was a gift from Sidney Kimmel Cancer Center (SKCC, San Diego, CA).

The PC3 cells were maintained in Ham's F12K medium (Mediatech Inc.) with 2 mM L-glutamine, adjusted to contain 1.5 g/L sodium bicarbonate, with 10% Fetal Bovine Serum (FBS). MIA PACA II cells were maintained in Dulbecco's Modified Eagle's Medium with 10% FBS (Mediatech Inc.) Mat LyLu cells were maintained in RPMI 1640 with 10% FBS, 2 mM L-glutamine and 250 nM dexamethasone. Other cells were maintained in RPMI 1640 medium with 10% FBS. The PC3 human prostate cancer animal model was generated as described in Example 8, above. Animals were selected with tumor volumes that had reached approximately 400-800 mm³, and then treated by intravenous injection of vehicle or PEGylated rHuPH20 (10,000 U/mouse = 15 mg/Kg).

(ii) Animal tumor models

PC3 and Du145 human prostate xenograft models

The PC3 and Du145 xenograft tumor models were generated as follows, using PC3 and Du145 cells, respectively. Tumor cells at approximately 80% confluency were trypsinized, harvested, washed once in HBSS (Hank's balance salt solution, Mediatech Inc.), and re-suspended in 50% Matrigel® in HBSS at 2×10^7 cells/mL on ice before inoculation into animals. Athymic male nude mice were inoculated intramuscularly (IM) with 0.05 mL of this cell suspension, peritibially, in the left hind leg (adjacent to the tibia periosteum). When the volume of tumors in the animals appeared to reach 400 to 500 mm³, actual tumor volumes were determined using VisualSonics ultrasound, using two perpendicular axial dimensions.

Animals were randomly grouped into two groups of 3-4 animals each, and treated by intravenous injection of vehicle (API buffer) and PEGylated rHuPH20 (10,000 U/mouse = approximately 15 mg/Kg), respectively. Tumor tissues were harvested 2 hours after the treatment and fixed in 10% Neutral buffered Formalin solution (NBF) for further histological evaluation.

MIAPACA II, BXPC3 (human pancreatic cancer models), HCT116, HT29 (human colon cancer models) and NCI H460 (human non-small lung cancer model)

The MIAPACA II, BXPC3, HCT116, HT29 and NCI H460 xenograft tumor models were generated as follows, using the MIAPACA II, BXPC3, HCT116, HT29 and NCI H460 cells, respectively. Tumor cells at approximately 80% confluency were

trypsinized, harvested, washed once in HBSS (Hank's balance salt solution, Mediatech Inc.), and re-suspended in HBSS at 2×10^7 cells/mL on ice before inoculation into animals. Athymic female nude mice were inoculated intramuscularly (IM) with 0.05 mL of cell suspension, peritibially, in the left hind leg (adjacent to the tibia periosteum). When the volume of tumors in the animals appeared to reach 400 to 500 mm³, actual tumor volumes were determined using VisualSonics ultrasound. Animals were randomly grouped into two groups of 3-4 animals each, and treated by intravenous injection of vehicle (API buffer) and PEGylated rHuPH20 (10,000 U/mouse = approximately 15 mg/Kg), respectively. Tumor tissues were harvested 2 hours after the treatment and fixed in 10% Neutral buffered Formalin solution (NBF) for further histological evaluation.

MDA MB 231 human breast cancer xenograft model

The MDA MB 231 human breast tumor xenograft model was generated as follows, using the MDA MB 231 cell line. Tumor cells at approximately 80% confluency were trypsinized, harvested, washed once in HBSS (Hank's balance salt solution, Mediatech Inc.), and resuspended in 50% Matrigel® in HBSS solution at 4×10^7 cells /mL, on ice, prior to inoculation. Athymic female nude mice were inoculated orthotopically in the mammary fat pad with 0.05 mL of the cell-Matrigel® suspension. Due to poor tumor take, tumor tissue transplantation was performed to generate more tumor bearing animals for the study. Animals having tumor volumes of more than 500 mm³ were selected and tumors excised from these animals, rinsed in sterile medium and minced with a blade into 1 mm cubes. These tumor tissues then were implanted into the mammary fat pads of female nude mice using a Trocar. The length (L) and width (W) of the solid tumor mass were measured with a caliper twice weekly and the tumor volume (TV) was calculated as: $(L \times W^2)/2$. When the volume of their tumors reached about 1000 mm³, animals were divided randomly into two groups of 5 mice each, and animals in the two groups treated by intravenous injection of vehicle or PEGylated rHuPH20 (3000 U/mouse = approximately 4.5 mg/Kg), respectively. Tumor tissue were harvested 3 days after the treatment and fixed in 10% Neutral buffered Formalin solution (NBF) for further histological evaluation.

Capan-1 human pancreatic tumor xenograft model

The Capan-1 human pancreatic xenograft tumor model was generated, as follows, using the Capan-1 HB2 cell line. Tumor cells at approximately 80% confluency were trypsinized, harvested, washed once in HBSS (Hank's balance salt solution, Mediatech Inc.), and resuspended in HBSS solution at 5×10^7 cells/mL, on ice, prior to inoculation. Athymic female nude mice were inoculated subcutaneously with 0.1 mL of the cell suspension. The length (L) and width (W) of the solid tumor mass were measured by caliper twice weekly and the tumor volume (TV) was calculated as: $(L \times W^2)/2$. When the volume of their tumors reached approximately about 500 - 700 mm³, animals were divided randomly into two groups of 3-4 mice each. Animals in these groups were treated by intravenous injection of vehicle (API buffer) and PEGylated rHuPH20 (5000 U/mouse = approximately 7.5 mg/Kg), respectively. Tumor tissues were harvested 3 days after the treatment and fixed in 10% Neutral buffered Formalin solution (NBF) for further histological evaluation.

4T1-GFP breast cancer tumor Model

The 4T1-GFP breast cancer model was generated as follows, using the 4T1-GFP cell line. 4T1-GFP tumor cells at ~ 80% confluency were trypsinized, harvested, washed once in HBSS (Hank's balance salt solution, Mediatech Inc.), and resuspended in HBSS at 2×10^6 cells/mL on ice, prior to inoculation. Female Balb/c mice were inoculated orthotopically in the mammary fat pad with 0.05 mL of the cell suspension. The length (L) and width (W) of the solid tumor masses were measured by caliper twice weekly and the tumor volume (TV) was calculated as: $(L \times W^2)/2$. When the tumor volume reached approximately 500 - 700 mm³, animals were divided randomly into two groups of 3-4 mice each, and the groups treated by intravenous injection of vehicle and PEGylated rHuPH20 (10,000 U/mouse = approximately 15 mg/Kg), respectively. Tumor tissue were harvested 2 hours after the treatment and fixed in 10% Neutral buffered Formalin solution (NBF) for further histological evaluation.

Mat LyLu rat prostate tumor Model

The Mat LyLu tumor model was generated using the Mat LyLu cell line, as follows. Tumor cells at approximately 80% confluency were trypsinized, harvested, washed once in HBSS (Hank's balance salt solution, Mediatech Inc.), and

resuspended in HBSS at 1×10^6 cells/mL on ice, prior to inoculation. Athymic male nude mice were inoculated intramuscularly with rat Mat LyLu prostate cancer cells (2×10^5 cells per mouse in a total volume of 0.04 mL) adjacent to the right tibial periosteum. When tumor volume appeared to reach 400 to 500 mm³, actual volumes were determined using VisualSonics ultrasound. Animals were randomly put into two groups of 3-4 each, which were treated by intravenous injection of vehicle and PEGylated rHuPH20, respectively. Tumor tissues were harvested 2 hours after the treatment and fixed in 10% Neutral buffered Formalin solution (NBF) for further histological evaluation.

10 **(iii) Immunohistochemistry**

The fixed tumors from each animal model (Example 17A(ii), above) were cut into 5 µm sections. To assess amount of hyaluronan (HA) in the harvested tumor, the sections were stained using a biotin labeled hyaluronan binding protein (HABP-bio) (Seikagaku, Japan). After washing to remove the primary reagent, a FITC-labeled streptavidin (Vector Labs, Canada), or a Texas Red-labeled streptavidin (for the 4T1-GFP tumor sections; Vector Labs, Canada) was used as a secondary reagent. Nuclei were counter-stained using a DAPI (4',6-diamidino-2-phenylindole) reagent. Micrographs were captured via a Nikon Eclipse TE2000U microscope coupled to a Insight FireWire digital camera (Diagnostic Instruments, Michigan).

20 HA expression in the sections was graded by the level of fluorescent intensity in the sections (in the tumor area and the associated stromal area), using a scoring system ranging from +++ (intense staining in greater than 80 % of the visual field), to +/- (sparse staining), to - (no visible staining). The results are presented in Table 33, below. The table indicates the name of each cell line used to generate the mouse xenograft models, the type of tumor and species the cell line was isolated from. Degree of HA expression is indicated for each tumor type model, after treatment with vehicle and PEGylated rHuPH20. The results indicate that HA expression was reduced by treatment with PEGylated rHuPH20 in all HA-expressing tumors. As noted in the Table, the HT29 tumor sections and the Capan-1 tumor sections (from animals injected with vehicle) exhibited negative staining in pericellular areas and positive staining in stroma, prior to PEGylated.

Table 33: Hyaluronan (HA) Expression Reduction in HA-Expressing Tumors Following Systemic Administration with PEGylated rHuPH20

Tumor cell line name	Tumor Type	Tumor Source Species	HA expression in Tumor tissue	
			Vehicle	PEGylated rHuPH20
PC-3	Prostate	Human	+++	-
4T1	Breast	Mouse	++	-
MIA PACA II	Pancreas	Human	++	-
BxPC3	Pancreas	Human	+ / ++	-
MatLyLu	Prostate	Rat	+	-
MDA MB 231	Breast	Human	+	-
HCT 116	Colon	Human	+ / -	-
HT29*	Colon	Human	+ / -	-
NCI H460	NSCLC	Human	+ / -	-
Du145	Prostate	Human	+ / -	-
Capan-1*	Pancreas	Human	-	-

* Negative staining in pericellular areas; positive staining in stroma

B. Dose-Dependent Removal of Tumor HA by PEGylated rHuPH20 in Exemplary HA-Rich Tumors

Animals in one exemplary HA-rich (++) tumor model, 4T1-GFP, generated as described above, were treated with vehicle and various amounts (10, 100, 1,000, 3,000 and 10,000 enzymatic units (U) per mouse) of PEGylated rHuPH20. Three days (72 hours) after administration of this treatment, peritibial tumors from the mice were harvested and fixed in normal buffered formalin. Levels of hyaluronan (HA) in the tumors were assessed by immunohistochemistry (IHC), as described in Example 17A, above, using avidin-Texas Red as a secondary. Hematoxylin/Eosin (H&E) staining also was done on the tumor sections. The results revealed a dose-dependent reduction in HA staining in the tumor sections from animals treated with 100, 1,000, 3,000 and 10,000 U PEGylated rHuPH20. In the sections from animals receiving 1,000, 3,000 and 10,000 U PEGylated rHuPH20, no visible staining was observed. These results indicated that a single systemic administration of PEGylated rHuPH20 can reduce hyaluronan in a breast distal tumor model for at least three days.

C. PEGylated rHuPH20 Administration Inhibits Tumor Growth and Increases Survival in HA-Rich Tumor Models

To demonstrate that repeated administration of PEGylated rHuPH20 alone produces anti-tumor effects in a plurality of tumor model systems, tumor growth and survival was assessed in three different mouse tumor models generated using cell lines listed in the previous example. In these studies, two different prostate cancer models (PC3 (HA +++), as determined in Example 17A, above) and Mat LyLu (HA +, as determined in Example 17A, above)) and one breast cancer model (4T1-GFP (HA ++, as determined in Example 17A, above) were treated with PEGylated rHuPH20.

For these studies, a total of six groups of mice (one control and experimental group for each tumor model, respectively (n = 6-8 animals per group) were dosed as described below with PEGylated rHuPH20 and assessed for tumor volume and survival. For each group, tumor growth was measured before treatment and then monitored over the course of treatment using the VisualSonics Imaging Micro-ultrasound system. The effect of PEGylated rHuPH20 treatment on animal survival was assessed by equating a 1500 mm³ endpoint tumor volume to a moribund state, as described below.

The PC3 human prostate cancer model was generated as described above. Athymic male nude mice were inoculated intramuscularly with human PC3 prostate cancer cells (1x10⁶ cells per mouse in a total volume of 0.05 mL) adjacent to the right tibial periosteum to generate tumors with high interstitial fluid pressure. Tumors were allowed to grow to a mean tumor volume of 400-500 mm³ before initiation of intravenous treatment with 3,000 enzymatic units (U) per mouse of PEGylated rHuPH20, or with control vehicle (API-buffer). n = 7 mice per group. The animals were treated every third day on a Q3dx9 schedule (once every three days, total of nine doses), on days 0, 3, 6, 9, 12, 15, 18, 21 and 24.

As described above, the 4T1-GFP breast tumor model was generated by inoculating BALB/c female mice orthotopically in the mammary fat pad with 4T1-GFP breast cancer cells (5x10⁵ cells per mouse in a total volume of 0.05 mL).

Tumors were allowed to grow to a mean tumor volume of 100 mm³ before initiation of intravenous treatment. For treatment, mice were injected, every other day (EOD) for a total of six doses (Q2dx6 regimen), on days 0, 2, 4, 7, 9 and 11, with API-buffer

(Control group) or with 3,000 enzymatic units (U) per mouse, PEGylated rHuPH20. n = 6-8 mice per group.

For the Mat LyLu prostate tumor model, athymic male nude mice were inoculated intramuscularly with rat Mat LyLu prostate cancer cells (2×10^5 cells per mouse in a total volume of 0.04 mL) adjacent to the right tibial periosteum to generate tumors with high interstitial fluid pressure. Tumors were allowed to grow to a mean tumor volume of 100 mm^3 before initiation of treatment. For treatment, animals were dosed intravenously with API-buffer (Control group) or with 3000 U PEGylated rHuPH20. n = 6-8 animals per group. Dosing was administered every other day, three times weekly, on Monday, Wednesday and Friday (Q2dx6), on days 0, 2, 4, 7, 9 and 11. Mat LyLu tumors in the control group, however, had reached critical volume or greater than $2,000 \text{ mm}^3$ by day 11, and thus were sacrificed for humane reasons.

For each group of PC3-tumor bearing animals, tumor growth was monitored using the VisualSonics (VS) Imaging Micro-ultrasound system. Tumor growth in the 4T-1 and Mat LyLu-tumor bearing animals was measured with calipers. Serial tumor volumes were obtained by bi-dimensional diameter measurements with calipers, using the formula $0.4 (a \times b^2)$ where “a” is the major tumor diameter and “b” the minor perpendicular diameter.

The results of this study, which assessed tumor volume and survival over time, are discussed in the following sections. The tumor volume data are described in section (i), below, and are presented in Tables 33-36, and in the top panel of Figure 9. The survival data are described in section (ii), below, and are presented in Table 38 and in the bottom panel of Figure 9.

(i) Tumor Volume Effects

The VS-ultrasound and caliper tumor volumes were expressed in mm^3 . For the two groups (control and 3000 U PEG-rHuPH20) of PC3 tumor-bearing animals, measurements were taken on days -1, 3, 6, 10, 13, 17, 20, 24, 27, 31, 34, 38, 41 and 45. The results are set forth in Table 34. Animals with tumors reaching over 1500 mm^3 were sacrificed for humane reasons as described below. Thus, for the PC3 model, no measurements were made after day 27, as 100 % of the animals had reached this tumor weight. For the two groups of 4T1-GFP-bearing tumors, the

caliper measurements were taken on days 0, 2, 4, 7, 9, 11, 14, 16, 18, and 21. The results are set forth in Table 35. For the two groups of Mat Ly Lu-bearing tumors, the caliper measurements were taken on days 0, 2, 4, 7 and 9. The results are set forth in Table 36.

5 **Table 34. Repeated Systemic PEGylated rHuPH20 Administration Effects Tumor Volume over Time in PC3 Prostate Tumor Model**

TUMOR VOLUME (mm ³) at day compared to initiation of treatment	TREATMENT (U/mouse rHuPH20)			
	0 (API Buffer)		3000	
	Average	SEM	Average	SEM
Day -1	440.31	27.05	439.00	28.01
Day 3	494.05	15.99	298.65	29.98
Day 6	774.09	40.86	486.72	30.37
Day 10	1043.11	98.11	575.10	50.58
Day 17	1606.10	168.80	783.10	86.90
Day 20	1930.10	242.00	1009.30	134.10
Day 24	2450.20	239.20	1065.50	153.80
Day 27	2926.20	281.70	1205.50	193.10
Day 31	N/A	N/A	1364.90	231.80
Day 34	N/A	N/A	1520.80	268.10
Day 38	N/A	N/A	1714.40	321.90
Day 41	N/A	N/A	1963.80	379.90

*NA = not applicable – all animals sacrificed due to tumor volumes over 1500 mm³.

10 **Table 35. Repeated Systemic PEGylated rHuPH20 Administration Effects Tumor Volume over Time in 4T1 Breast Tumor Model**

TUMOR VOLUME (mm ³) at day compared to	TREATMENT (U/mouse rHuPH20)			
	0 (API Buffer)		3000	
	Average	SEM	Average	SEM

initiation of treatment				
Day 0	63.27	11.13	67.36	10.75
Day 2	159.02	14.45	147.41	10.40
Day 4	226.97	22.63	158.66	22.71
Day 7	271.64	28.77	184.21	17.47
Day 9	438.28	62.57	251.37	28.77
Day 11	638.49	113.56	336.46	39.99
Day 14	1099.31	254.54	483.68	93.73
Day 16	1468.76	260.78	650.66	115.87
Day 18	1707.37	225.72	861.36	171.18
Day 21	2254.99	375.00	1227.40	352.71
Day 25	2693.19	279.51	1536.89	398.65

Table 36. Repeated Systemic PEGylated rHuPH20 Administration Effects Tumor Volume over Time in Mat LyLu Prostate Tumor Model

TUMOR VOLUME (mm ³) at day compared to initiation of treatment	TREATMENT (U/mouse rHuPH20)			
	0 (API Buffer)		3000	
	Average	SEM	Average	SEM
Day 0	76.10	10.80	75.70	17.80
Day 2	355.70	35.20	208.00	56.90
Day 4	853.00	29.80	470.90	61.20
Day 7	2248.80	96.50	1518.90	158.10
Day 9	3571.00	305.40	2384.40	382.10

5

As indicated by the tumor volume measurements, repeated intravenous administration of PEGylated rHuPH20 reduced tumor growth in each of the models, with a correlation to the amount of HA in the tumors, as determined in Example 17A,

above. For example, a significant difference in tumor volume between the PEGylated rHuPH20 treated and control groups in each tumor model ($p = 0.001$ at day 27 in the PC3 prostate cancer model; $p = 0.04$ at day 14 in the 4T1 breast cancer model; and $p = 0.02$ at day 9 in the Mat LyLu prostate cancer model).

5 Percent Tumor Growth Inhibition (TGI) for each respective tumor model was calculated using the following equation:

$$\% \text{ TGI} = [1 - (T_n - T_0) \div (C_n - C_0)] \times 100\%$$

where “ T_n ” is the average tumor volume for the treatment group (animals receiving PEGylated rHuPH20) at day “ n ” (here, day 27, day 14 and day 9 for PC3, 10 4T1 and Mat LyLu models, respectively) after the final dose of PEGylated rHuPH20; “ T_0 ” is the average tumor volume in that treatment group at day 0, before treatment; “ C_n ” is the average tumor volume for the corresponding control group at day “ n ” (here, day 27, day 14 and day 9 for PC3, 4T1 and Mat LyLu models, respectively; some time after the final dose of vehicle); and “ C_0 ” is the average tumor volume in 15 the control group at day 0, before treatment. Time to Tumor Volume (TTV) for the respective tumor model was calculated as the average time to reach tumor volume of 1,500 mm³.

Table 37. Tumor Growth Inhibition

	TUMOR MODEL		
	PC3 (n = day 27)	4T1 (n = day 14)	Mat LyLu (n = day 9)
$C_n - C_0$	2485.89 $\pm$ 270.94	1049.74 $\pm$ 262.07	3494.96 $\pm$ 304.94
$T_n - T_0$	766.26 $\pm$ 217.15	412.36 $\pm$ 81.90	2308.63 $\pm$ 397.76
TGI	70 %	60 %	34 %

20 As demonstrated in Table 37, the results of this study revealed a 70 %, 60 % and 34 % Tumor Growth Inhibition (TGI) by systemic PEGylated rHuPH20 administration, in the PC3 prostate, 4T1 breast and Mat LyLu prostate tumor models, respectively (at day 27, day 14 and day 9 post administration, respectively).

As demonstrated in Figure 9 and in Tables 34-37, the effect on tumor volume 25 correlated with the level of tumor HA expression, as determined in Example 17A, above. For example, the tumor volume was most reduced (TGI = 70 %) by PEGylated rHuPH20 in the model with the highest relative HA expression in tumors

(PC3 (HA +++), and least (TGI = 34 %) in the model with the lowest relative HA expression in the tumors (Mat LyLu (HA +). The reduction in tumor volume was intermediate (TGI = 60 %) in the 4T1-GFP model, which displayed a relative HA level of ++ in Example 17A, above.

5 **(ii) Survival effects**

Effect of systemic, repeated, PEGylated rHuPH20 administration on survival was assessed in each model by determining the percentage of “surviving” animals at each time-point in each group. For this study, a tumor volume of greater than or equal to 1500 mm³ was selected as an endpoint, which was considered analogous to a moribund (non-surviving) state. Thus, animals with tumor volumes below 1500 mm³ were considered surviving, while animals with tumor weights 1500 mm³ or greater were considered morbid.

The effects of survival by repeated administration of PEGylated rHuPH20 on the various models are set forth in Figure 9 (bottom panel). As demonstrated in Figure 9 (bottom panel), treatment with PEGylated rHuPH20 resulted in a 2-fold, 1.7-fold and 1.5-fold delay in reaching the designated moribund state, in the PC3, 4T1-GFP and Mat LyLu tumor models, respectively, compared to control treatment. Thus, the effect on survival also correlated with the relative expression of HA in the tumors, as measured in Example 17A, above.

20 The Median Survival Time (MST), in days, was determined for each animal group, as the median time before mice in each group reached the designated 1500 mm³ tumor volume endpoint. Percent ILS (Increase in Life Span), a measure of anti-tumor activity by the PEGylated rHuPH20 compared to control, was calculated for each model, using the following equation:

25
$$\% \text{ ILS} = [(T-C)/C] \times 100$$

where “T” is the MST of the group treated with PEGylated rHuPH20 and “C” is the MST for the control group. By National Cancer Institute (NCI) criteria, a treatment with a % ILS greater than 25% is considered effective. The results are summarized in Table 38, below.

30 **Table 38: Median Survival Time and Increase in Life Span**

TREATMENT	MODEL
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	PC3		4T1		Mat LyLu	
	MST	ILS	MST	ILS	MST	ILS
Control	16.13	N/A	16.57	N/A	5.67	N/A
3000 U PEG-PH20	31.83	97 %	28.66	73 %	8.83	56 %

N/A = not applicable

As set forth in Table 38, above, the systemic treatment with PEGylated rHuPH20 caused an increase in life span (ILS) of 97 %, 73 % and 56 %, in the PC3 prostate, 4T1 breast and Mat LyLu prostate tumor models, respectively, again correlating with the measured relative HA levels in the tumors as described in Example 17A, above (+++, ++ and + respectively).

These results demonstrate that repeated, systemic administration of PEGylated soluble hyaluronidase, alone, is effective as an anti-cancer treatment in a number of HA-rich tumor models, and that the degree of anti-tumor effects are correlated with the expression of HA in the tumors.

(iii) Reduction of HA in Tumors

Further, tumors were harvested after the final PEGylated rHuPH20 or control treatment, for immunohistochemical detection of tumor tissue hyaluronan. For this study, PC-3 tumors were harvested 72 hours following the final treatment (day 20), 4T1-GFP tumors were harvested 72 hours following the final treatment (day 14) and the Mat LyLu tumors were harvested 24 hours following the final treatment (day 12).

The harvested tumors were fixed in normal buffered formalin (NBF) and 5 μ m sections cut and stained using a biotin labeled hyaluronan binding protein (HABP-bio) (Seikagaku, Japan). After washing to remove the primary reagent, a FITC-labeled streptavidin (Vector Labs, Canada), for the PC-3 and Mat LyLu tumors, or a Texas Red-labeled streptavidin was used as a secondary reagent. Nuclei were counter-stained using a DAPI (4',6-diamidino-2-phenylindole) reagent. Micrographs were captured via a Nikon Eclipse TE2000U inverted fluorescent microscope coupled to a Insight FireWire digital camera (Diagnostic Instruments, Michigan) or ZEISS overhead scope (Carl Zeiss, Inc.) that has the same imaging system. The results revealed strong HA staining in the control tumors, but no detectable hyaluronan staining in the tumors from the animals that had been treated with PEGylated

rHuPH20, demonstrating the sustained reduction in tumor hyaluronan caused by the treatment.

Example 18

5 **Blood Brain Barrier Permeability of PEGylated rHuPH20 and Treatment of Brain Tumor Model**

The study was carried out to demonstrate that PEGylated rHuPH20 could reduce hyaluronic acid (HA) in brain tumors in an animal model and could increase survival in combination with radiotherapy.

A. Brain tumor model

10 To generate the brain tumor model, 4×10^4 PC3 cells in 2 μ l of Hanks medium were injected into the right cerebral hemisphere with a stereotaxic frame of each mouse in various groups, as follows.

B. Blood Brain Barrier Permeability and degradation of brain HA by intravenously administered PEGylated rHuPH20

15 This study was carried out to demonstrate that intravenously administered PEGylated rHuPH20 can degrade HA in brain tumors. 3,000 enzymatic units (U) PEGylated rHuPH20, and control buffer (API) were administered intravenously to different groups of brain tumor model animals described in Example 18A, above. The animals were sacrificed four hours later. Brains were subsequently collected and
20 sectioned for analysis.

For assessment of compromised Blood Brain Barrier (BBB) in the tumor model animals, a BBB permeability assay was performed on a subset of the animals. For this assay, 0.1 mL of 2 % Evan's Blue Dye was injected intravenously into the tail vein of these mice, one hour prior to sacrifice. Cerebral sections were made and
25 visualized as described below. Blue dye in cerebral sections indicated the presence of Albumin, a large serum protein (67 kDa) that had complexed with Evans Blue, indicating increased BBB permeability (the BBB's relative "leakiness").

For assessment of the amount of HA in the brain tumors following treatment, brain sections were incubated a biotin labeled hyaluronan binding protein (HABP-bio)
30 (Seikagaku, Japan). After washing to remove the primary reagent, a FITC-labeled streptavidin (Vector Labs, Canada) was used as a secondary reagent. Nuclei were counter-stained using a DAPI (4',6-diamidino-2-phenylindole) reagent.

Hematoxylin/Eosin staining also was done to identify the tumor in the brain. Micrographs were captured via a Nikon Eclipse TE2000U microscope coupled to a Insight FireWire digital camera (Diagnostic Instruments, Michigan) or a Leitz overhead scope that has the same image capturing system.

5 The results revealed a strong HA staining in control animals (API buffer), but no detectable HA staining in the animals that had been injected with PEGylated rHuPH20, demonstrating that this enzyme can be used to reduce HA in brain tumors when administered intravenously.

C. Survival of animals after PEGylated rHuPH20 treatment

10 To assess survival of brain tumor model animals after treatment with PEGylated rHuPH20 alone, twenty of the PC3 brain tumor model mice (described in Example 18A, above) were stayed on Day 12 post injection. The mice were injected in the tail vein, twice a week for four weeks, either with 5000 enzymatic units (U) of PEGylated rHuPH20 (ten mice), or API buffer (ten control mice).

15 Survival rate of the animals was monitored over time by monitoring death of the animals. The results, set forth in Figure 10, indicated no significant change in survival between the PEGylated rHuPH20 treated and control animals.

D. Effects on survival of PEGylated rHuPH20 in combination with radiotherapy

20 To assess survival of brain tumor model animals after treatment with PEGylated rHuPH20 in combination with radiation, three groups of the PC3 brain tumor model mice (described in Example 18A, above) were used.

For this study, twenty-seven tumor-bearing mice were staged into three groups (A, B and C) based on body weights at day 13, post-inoculation. For each group, dosing was initiated on Day 13 post inoculation of the PC3 cells. Each
25 treatment/control was administered using a q3d x 4 regime (every third day, total of four times). At each administration, Group A, the control group, was given intravenous injection of 100 μ L API buffer alone. Group B was treated with whole-brain radiation (5 Gy). Group C, the combination therapy group, was treated with 5000 enzymatic units of PEGylated rHuPH20 (in 100 μ L API buffer), followed four
30 hours later by whole-brain radiation (3 Gy). This dosing regime is set forth in Table 39, below.

Table 39. Dosing Regime

Group	Treatment	Dosage	Route	Regimen	Number of animals
A	API buffer	100 µl	iv	q3d x 4	9
B	Radiation	3 Gy	WBR **	q3d x 4	9
C	Radiation PEGPH20*	3 Gy 5000 U	WBR iv	q3d x 4	9

* PEGPH20 administrated 4 hours prior to radiation.

** WBR, Whole Brain Radiation

Survival of animals in each group was monitored over time by monitoring death of the animals. The results, which are set forth in Figure 11 and in Table 40, indicated improved survival with combination treatment (radiation and PEGylated rHuPH20; Group C) over control (no treatment) (up to 45 % longer survival), and an improvement in survival with combination treatment (radiation and PEGylated rHuPH20; Group C) over radiation alone (Group B) (up to 10 % longer survival).

Table 40.

Group	Treatment	Median Survival Day	% longer survival compared to no treatment (negative control)	% longer survival compared to single radiation treatment (positive control)
A	API	45.5	N/A	N/A
B	Radiation	60	31	N/A
C	Radiation+ PEGPH20	66	45	10

Example 19

Pharmacokinetic Properties of PEGylated rHuPH20 in Animals

The pharmacokinetic properties of pegylated rHuPH20 (made with mPEG-SBA-30K as described in Example 7, above) were evaluated in ICR mice from Harlan (Indianapolis, Indiana), rats, and Cynomolgus monkeys by measuring plasma hyaluronidase in blood samples, at a number of time-points following intravenous administration of the agent.

In mice, the study was carried out over a period of seventy hours. At “time zero” of this study, female ICR mice from Harlan, weighing 19-25 grams each, were

injected, via the lateral tail vein, with approximately 3000 U purified, pegylated rHuPH20, which had been diluted to 0.5 mg/ml. In most cases, blood from the retro-orbital plexus by glass capillary was taken five minutes post-treatment, for later evaluation. Animals then were returned to their cages. At various time-points following administration of PEGylated rHuPH20, blood samples were taken from the retro-orbital plexus by glass capillary from animals that had been briefly anesthetized with 5% isoflurane. The blood from each sample was mixed with EDTA in a purple-top Microtainer blood collection tube to prevent coagulation. The tubes were centrifuged at 5000 rpm on a table-top centrifuge for 15 minutes, and the plasma transferred to a new Eppendorf tube. Plasma was frozen at -20 °C, before being assayed to determine hyaluronidase activity.

For determination of plasma hyaluronidase activity at the various time-points, a standard micro turbidity plate assay, as described in Example 2, above, was performed on the plasma to measure the amount of hyaluronidase activity in the plasma. Because of the low total blood volumes in mice, only two or three blood draws were carried out per animal, over the course of the study. Accordingly, pharmacokinetic curves were fitted to multiple animals across the entire time course. PK curve fitting was done using either GraphPad Prism® (GraphPad Software, Inc., La Jolla, CA) software or WinNonLin software (Pharsight® Corporation, Mountain View, CA).

Similar studies were performed in rats and Cynomolgus monkeys. In rats, the study was carried out over a period of 96 hours. In a study similar to that described above for mice, rats were administered 125,000 U/kg (approximately 4 mg/kg) pegylated rHuPH20. Plasma hyaluronidase activity was monitored at various time-points using a standard micro turbidity plate assay, as described in Example 2, above. Three rats were evaluated per time point. In Cynomolgus monkeys, a MPI 28 day intravenous dose study was performed wherein pegylated rHuPH20 was administered to two female monkeys in escalating doses of 0.5, 2.0 and 6.0 mg/kg. The monkeys were allowed to clear all pegylated rHuPH20 prior to subsequent dosing. Plasma hyaluronidase activity was monitored at various time-points over 96 hours using a standard micro turbidity plate assay, as described in Example 2, above. Intravenous

dose comparisons of pegylated rHuPH20 for mice, rats and Cynomolgus monkeys are set forth in Table 41.

Table 41

Species	Route	Dose
mouse	IV	100000 U/mouse
Rat	IV	125000 U/kg 85000 U/kg 56077 U/kg
Cyno Mk	IV	15000 U/kg (0.5 mg/kg) 60000 U/kg (2.0 mg/kg) 180000 U/kg (6.0 mg/kg)

5 Pharmacokinetic regression curves were fitted to the derived data, and plasma half-life ($T_{1/2}$) was determined using the following equations in GraphPad Prism® (GraphPad Software, Inc., La Jolla, CA)

Plasma half life was determined using Prism software for non-linear regression. The standardized formula for half life curve fitting after intravenous
10 injection is:

$$C_p = C_0 \exp(-k_{el})t$$

$$\text{where } k_{el} = 0.693/T_{1/2}$$

t = time after test article administration

C_0 is theoretical starting value

15 C_p is the measured value at time t

The following equations were used:

One component exponential decay:

$$Y = \text{span1} * \exp(-k_{el}X) + \text{plateau};$$

where k_{el} is the decay rate constant equal to $0.693/T_{1/2}$ and plateau is the

20 asymptotic value as decay approaches infinity.

Two-component exponential decay:

$$Y = \text{span1} * \exp(-k_{1el}X) + \text{span2} * \exp(-k_{2el}X) + \text{plateau};$$

where k_{1el} and k_{2el} are decay rate constants for span 1 and span 2,

respectively, and plateau is the asymptotic value as decay approaches infinity.

The results of the studies above, derived by 2-compartment modeling of concentration-time data, are set forth in Table 42, below, where the PK parameter descriptions are set forth in Table 43, T1/2 alpha is the distribution phase half-life, T1/2 beta is the elimination phase half-life, and MRT is the mean residence time. In mice, the alpha phase (distribution phase) half life and the beta phase (elimination phase) half life of the enzyme in the plasma were 0.696 hours and 19.23 hours, respectively. The results in mice are presented in Figure 12, where initial rapid degradation of plasma hyaluronidase is observed (distribution phase) followed by a slow gradual decay over time (elimination phase). In rats, the alpha phase (distribution phase) half life and the beta phase (elimination phase) half life of the enzyme in the plasma were 1.13 hours and 7.48 hours, respectively. In Cynomolgus monkeys, the alpha phase (distribution phase) half life and the beta phase (elimination phase) half life of the enzyme in the plasma were 1.13 hours and 21.75 hours, respectively.

Table 42. Median PK Parameters of PEG-rHuPH20 in Mouse, Rat, and Cynomolgus Monkeys (Derived by 2-Compartment Modeling of Concentration-Time Data)

	Mouse	Rat	Cyno Mk
Body Wt. (kg)	0.026	0.292	1.920
BSA (m ²)	0.0079	0.0419	0.1539
V ₁ (mL/kg)	38.44	72.00	55.83
k ₁₀ (1/hr)	0.161	0.384	0.046
k ₁₂ (1/hr)	0.65	0.15	0.31
k ₂₁ (1/hr)	0.22	0.15	0.40
CL (mL/h-kg)	6.20	30.33	2.54
CL ₂ (mL/h-kg)	24.91	11.52	17.60
V ₂ (mL/kg)	112.03	85.66	17.63
T1/2 α (h)	0.696	1.13	1.13
T1/2 β (h)	19.23	7.48	21.75
MRT (h)	24.32	5.81	30.76

Table 43. PK Parameter descriptions

PK Parameter	Description
CL ₁	Clearance from the Central Compartment (mL/h-kg)
CL ₂ or Q _D	Distribution rate (mL/h-kg)
k ₁₀	Elimination rate constant (h ⁻¹)

k ₁₂	Distribution rate constant from the Central to Peripheral Compartment (h ⁻¹)
k ₂₁	Distribution rate constant from the Peripheral to Central Compartment (h ⁻¹)
V ₁	Volume of the Central Compartment (mL/kg)
V ₂	Volume of the Peripheral Compartment (mL/kg)

Example 20

Pharmacokinetic Analysis of Plasma Hyaluronidase Levels After Single Dose Intravenous Administration of PEGPH20

5 Pharmacokinetic (PK) studies were conducted in ICR mice from Harlan (Indianapolis, Indiana) and in Sprague-Dawley rats (Harlan) following single dose administration of PEGPH20. Following injection of PEGPH20 as described below, plasma levels of PEGPH20 were determined by measuring hyaluronidase activity using a modified turbidometric assay as described in Example 2. Briefly, plasma
10 samples containing hyaluronidase were incubated with hyaluronate (HA) for 60 minutes. Undigested HA was precipitated with the addition of acidified serum. The turbidity of the resulting sample was measured at 640 nm after a 30 minute development period, and the resulting turbidity was a measure of the hyaluronidase activity present. The assay was run under pH conditions that suppress endogenous
15 lysosomal hyaluronidase activity in plasma, which normally exhibits activity at acidic pH. PEGPH20 concentrations were expressed as units of hyaluronidase activity (U)/mL as interpolated from a calibration curve, or as µg/mL calculated using the specific activity (in U/mg) of the PEGPH20 lot. Generally, for rats, the lower level of
20 quantitation was 10 U/mL and the assay was applicable to measuring up to 100 u/mL hyaluronidase activity.

A. Mice

Male ICR mice (3/group) received a single IV dose (125,000 U/kg; 3.3 mg/kg) of PEGPH20 and plasma samples were collected at selected intervals from 5 minutes to 24 hours post-dose. Plasma samples were analyzed for hyaluronidase activity as
25 described above. The results show that following IV injection, the plasma concentration of PEGPH20 declined in a biphasic manner. The initial rapid decline represented distribution from the systemic circulation and was followed by a slower decrease representing the elimination phase. Pharmacokinetic parameters derived from non-compartmental analysis of the hyaluronidase activity over time are set forth

in Table 44. The Values are mean data derived from 3 mice using non-compartmental analysis. The data show that the elimination half-life ($t_{1/2} \lambda_z$) of PEGPH20 was approximately 10 hours. The mean volume of distribution (V_z) was approximately 122 mL/kg, which is approximately twice the expected plasma volume in mice (i.e. 50 mL/kg; Davies and Morris 1993), suggesting that PEGPH20 was not widely distributed to peripheral tissues.

TABLE 44: Mean PK Parameters After Single IV Dose (125,000 U/kg; 3.3 mg/kg) Administration of PEGPH20 to Male Mice	
PK Parameter	Value
C_0 (U/mL)	1,898
C_0 (μ g/mL)	50.0
C_{max} (U/mL)	1,727
C_{max} (μ g /mL)	45.5
$t_{1/2} \lambda_z$ (h)	10.3
$AUC_{0-\infty}$ (U·h/mL)	15,244
$AUC_{0-\infty}$ (μ g·h/mL)	401
V_z (mL/kg)	121.8
V_{ss} (mL/kg)	109.7
CL (mL/h·kg)	8.2
MRT (h)	13.4

PK = pharmacokinetic; U = units of enzymatic activity; IV = intravenous; C_0 = plasma concentration back-extrapolated to time 0; C_{max} = maximum observed plasma concentration; $t_{1/2} \lambda_z$ = terminal half-life; $AUC_{0-\infty}$ = area under the plasma concentration versus time curve from time 0 and extrapolated to infinity; V_z = volume of distribution; V_{ss} = volume of distribution at steady state; CL = absolute clearance; MRT = mean residence time

B. Rats

Male Sprague-Dawley rats (3/group) received a single IV dose (125,000 U/kg; 3 mg/kg) of PEGPH20 and plasma samples were collected at selected intervals from 5 minutes to 96 hours post-dose. Plasma samples were analyzed for hyaluronidase activity as described above. The results show that following IV injection, the plasma concentration of PEGPH20 declined in a biphasic manner. The initial rapid decline represented a distribution from the systemic circulation and was followed by a slower decrease representing the elimination phase. Pharmacokinetic parameters derived from non-compartmental analysis of the hyaluronidase activity over time are set forth in Table 45. The Values are mean data derived from 3 rats using non-compartmental

analysis. The data show that the elimination half-life ($t_{1/2}$) of PEGPH20 was approximately 8 hours, which was more rapid than seen in mice. This was also reflected in a shorter mean residence time (MRT) and faster absolute clearance (CL) than observed in mice. The mean volume of distribution (V_z) was approximately 296 mL/kg, which is greater than the expected plasma volume in rats (i.e. 31 mL/kg; Davies and Morris 1993), and approximates the expected extracellular fluid volume in rats (i.e. 297 mL/kg; Davies and Morris 1993).

TABLE 45: Mean PK Parameters After Single IV Dose (125000 U/kg; 3	
PK Parameter	Value
C_0 (U/mL)	1,228
C_0 (µg/mL)	30.0
C_{max} (U/mL)	1,256
C_{max} (µg /mL)	30.6
$t_{1/2}$ λz (h)	7.7
$AUC_{0-\infty}$ (U·h/mL)	4,713
$AUC_{0-\infty}$ (µg·h/mL)	115
V_z (mL/kg)	296.1
V_{ss} (mL/kg)	174.9
CL (mL/h·kg)	26.6
MRT (h)	6.6

PK = pharmacokinetic; U = units of enzymatic activity; IV = intravenous; C_0 = plasma concentration back-extrapolated to time 0; C_{max} = maximum observed plasma concentration; $t_{1/2}$ λz = terminal half-life; $AUC_{0-\infty}$ = area under the plasma concentration versus time curve from time 0 and extrapolated to infinity; V_z = volume of distribution; V_{ss} = volume of distribution at steady state; CL = absolute clearance; MRT = mean residence time

C. Summary

In single intravenous dose studies in mice and rats, plasma hyaluronidase activity declined in a biphasic manner, with a rapid initial distribution phase and a slower elimination phase. Mean elimination half-lives ($t_{1/2}$) were approximately 10 hours in mice and 8 hours in rats. The mean volume of distribution (V_z) was 122 mL/kg for mice, suggesting that PEGPH20 was not widely distributed into peripheral tissues in mice. The mean V_z was higher in rats at 296 mL/kg.

Example 21

Dose Range and Pharmacokinetic Study of PEGPH20 Following a Single Intravenous Dose Administration in Cynomolgus Monkeys

Pharmacokinetic (PK) studies were conducted in Cynomolgus Monkeys following single dose administration of PEGPH20 at various dose ranges. Following
5 injection of PEGPH20 as described below, plasma levels of PEGPH20 were determined by measuring hyaluronidase activity using a modified turbidometric assay as described in Example 20. As with rats, the lower level of quantitation was 10 U/mL and the assay was applicable to measuring up to 100 u/mL hyaluronidase activity.

10 Male cynomolgus monkeys (1 to 5/group) received a single IV dose of PEGPH20 at concentrations ranging from 1 mg/kg (38,000 U/kg), 3 mg/kg (114,000 U/kg), 6 mg/kg (228,000 U/kg), 12.5 mg/kg (412,500 U/kg) and 33 mg/kg (1,089,000 U/kg) and plasma samples were collected at selected intervals from 0.5 to 72 hours post-dose. Plasma samples were analyzed for hyaluronidase activity as described
15 above. The results show that the decline in plasma concentration was monophasic because the rapid distribution phase was not captured with the first PK blood collection time at 0.5 hours post-dose. Pharmacokinetic parameters derived from non-compartmental analysis of the hyaluronidase activity over time are set forth in Table 46. The Values are mean data derived from 1 to 5 monkeys using non-
20 compartmental analysis as set forth in the Table.

The data show that the elimination half-life ($t_{1/2\lambda z}$) of PEGPH20 was ranged from 39 to 53 hours (1.6 to 2.2 days). The long half-life resulted from a slow systemic clearance of approximately 1.0 mL/h kg. Maximum plasma concentration as determined by back extrapolation to time immediately after IV injection [C_0], terminal
25 elimination rate $k_{\lambda z}$, and absolute clearance (CL) provided estimates of the volume of distribution (V_z). The range of the mean V_z was from 70 to 93 mL/kg, which is approximately twice the expected plasma volume in monkeys (i.e. 45 mL/kg; Davies and Morris 1993), suggesting that PEGPH20 was not widely distributed to peripheral tissues.

30 Dose linearity was examined using area under the plasma concentration-time curve, the extrapolated maximal concentration [C_0], and the observed C_{max} . Correlation of log-transformed PK parameters with log-transformed IV dose revealed

linear relationships with slopes equal to 1.0, suggesting that the PK of PEGPH20 in monkeys was linear with respect to dose. Systemic exposure and observed maximal plasma concentration increased proportionally with increases in dose.

TABLE 46: Mean PK Parameters After Single IV Dose Administration of					
	Value				
Dose (U/kg):	38,000	114,000	228,000	412,500	1,089,000
Dose (mg/kg):	1	3	6	12.5	33
N:	3	3	5	1	1
PK Parameter					
C ₀ (U/mL)	813	1,653	4,558	6,320	18,601
C ₀ (µg/mL)	21.4	43.5	119.9	191.5	563.7
C _{max} (U/mL)	712	1,763	4,503	5,770	17,084
C _{max} (µg/mL)	18.8	46.4	118.5	174.9	517.7
t _{1/2 λz} (h)	39.7	49.1	41.0	53.2	52.3
AUC _{0-∞} (U·h/mL)	23,654	88,630	195,117	372,399	961,009
AUC _{0-∞}	622	2,332	5,135	11,285	29,121
V _z (mL/kg)	92.5	92.8	70.0	85.0	85.6
V _{ss} (mL/kg)	81.7	86.6	66.2	81.2	81.7
CL (mL/h·kg)	1.6	1.4	1.2	1.1	1.1
MRT (h)	50.9	66.6	56.0	73.3	72.1

5 PK = pharmacokinetic; U = units of enzymatic activity; IV = intravenous; C₀ = plasma concentration back-extrapolated to time 0; C_{max} = maximum observed plasma concentration; t_{1/2 λz} = terminal half-life; AUC_{0-∞} = area under the plasma concentration versus time curve from time 0 and extrapolated to infinity; V_z = volume of distribution; V_{ss} = volume of distribution at steady state; CL = absolute clearance; MRT = mean residence time

10 Summary

In single intravenous dose studies in monkeys, plasma hyaluronidase activity declined in a biphasic manner, with a rapid initial distribution phase and a slower elimination phase. Mean elimination half-lives (t_{1/2}) was approximately 40 to 53 hours in monkeys. The mean volume of distribution (V_z) was 70 to 93 mL/kg for
15 monkeys, suggesting that PEGPH20 was not widely distributed into peripheral tissues. In the monkey study evaluating several dose levels of PEGPH20, the maximum observed plasma concentration (C_{max}) and area under curve for plasma concentration versus time (AUC) increased linearly with increasing PEGPH20 dose.

A 4-week repeat-dose toxicity study was conducted in Sprague-Dawley rats following IV administration of PEGPH20. Four groups of 3 rats/gender/group received intravenous twice-weekly doses of the vehicle (10 mM Hepes pH 7.0 + 130 mM NaCl) or 0.5, 5 or 25 mg/kg of PEGPH20, respectively, for 4 consecutive weeks. Blood samples were collected from each animal on Days 1 and 25 at predetermined times post-dosing. Plasma samples were analyzed for hyaluronidase activity as described in Example 20. Toxicokinetic parameters were derived for each IV dose using non-compartmental modeling of the plasma concentration-versus-time data. The derived TK parameters are set forth in Table 47. Analysis of plasma concentration versus time after twice-weekly IV dose administration of PEGPH20 showed that exposures (C_{max} and AUC) increased with increasing PEGPH20 dose. Toxicokinetics were approximately linear over the range of doses evaluated.

20 Elimination half-life ($t_{1/2}$) increased with increasing PEGPH20 dose. Exposures were generally similar between genders for any given dose level and dosing day. Following repeated IV dosing of PEGPH20, there was a trend toward increased AUC on Day 25 compared with Day 1 in the 5 mg/kg and 25 mg/kg dose groups. However, Day 25 AUC exposures remained < 2-fold the exposures observed on Day 1 within any given dose group. Exposures as determined by C_{max} generally remained consistent from Day 1 to Day 25 within any given dose group.

Parameter	0.5 mg/kg		5 mg/kg		25 mg/kg	
	Day 1	Day 25	Day 1	Day 25	Day 1	Day 25
Males						

TABLE 47: Mean TK Parameters After Twice –Weekly IV Administration of PEGPH20 to Rats for 4 Consecutive Weeks

Parameter	0.5 mg/kg		5 mg/kg		25 mg/kg	
	Day 1	Day 25	Day 1	Day 25	Day 1	Day 25
C _{max} (U/mL)	174	203	1,760	2,490	11,500	10,900
t _½ (h)	2.90	3.28	8.71	23.9	12.6	24.0
AUC _{0-t} (U·h/mL)	674	695	12,000	20,700	100,000	195,000
C _{max} Exposure Ratio (C _{max} on Day 25/ C _{max} on Day 1)	1.17		1.41		0.95	
AUC Exposure Ratio (AUC _{0-t} on Day 25/ AUC _{0-t} on Day 1)	1.03		1.73		1.95	
Females						
C _{max} (U/mL)	196	171	1,830	1,960	10,300	10,100
t _½ (h)	2.42	3.75	9.43	13.3	11.6	21.1
AUC _{0-t} (U·h/mL)	491	608	10,300	13,400	105,000	154,000
C _{max} Exposure Ratio (C _{max} on Day 25/ C _{max} on Day 1)	0.87		1.07		0.98	
AUC Exposure Ratio (AUC _{0-t} on Day 25/ AUC _{0-t} on Day 1)	1.24		1.30		1.47	
C _{max} Gender Ratio (C _{max} (males)/ C _{max} (females))	0.89	1.19	0.96	1.27	1.12	1.08
AUC Gender Ratio (AUC _{0-t} (males)/ AUC _{0-t} (females))	1.37	1.14	1.17	1.54	0.95	1.27

TK = toxicokinetic; IV = intravenous; U = units of enzymatic activity; C_{max} = maximum observed plasma concentration; t_{1/2} = elimination half-life; AUC_{0-t} = area under the plasma concentration versus time curve from time 0 to the last measured time point

- 5 The twice-weekly IV administration was well tolerated in rats. There was a transient decrease in body weight, which returned towards pre-study values by the end of the dosing period. This effect was attributed to PEGPH20 pharmacology as a decrease in extracellular hyaluronan and its associated water. There was a modest

prolongation of activated partial thromboplastin time (APTT), although prothrombin time was unchanged. There was no hemorrhage observed performed grossly at necropsy or microscopically in any tissue examined. Thus, due to the assessment that APTT prolongation was an adverse event in rats, the no observed adverse effect level (NOAEL) was defined as 5 mg/kg/dose in rats. No changes in food consumption or behavior was observed. Since no other test article-related findings were observed, the 25 mg/kg/dose would be the designated NOAEL, except for the prolonged APTT observed.

B. Monkeys

1. Repeat Dose Toxicity Study

Female cynomolgus monkeys (N = 3) received a 6 mg/kg (198,000-222,000 U/kg) IV dose of PEGPH20 on study days 1, 3, 8, 11, 15, 22, 25, and 29. Plasma samples were collected at selected intervals from 0.083 to 96 hours post-dose on Days 1, 3, 8, 11 and 25. Plasma samples were analyzed for hyaluronidase activity using a micro-turbidity plate assay as described in Example 20. Toxicokinetic parameters were derived for each IV dose using non-compartmental modeling of the plasma concentration-versus-time data. Each dose was modeled independently of the previous sequential dose. The results are shown in Table 48. TK analysis of the plasma concentration-versus-time data after repeated IV dose administration of PEGPH20 to female monkeys showed that the decline in plasma concentration following IV injection of a 6 mg/kg (~210,000 U/kg) dose of PEGPH20 was bi-phasic. There was a rapid initial decline in concentration followed by a slower decrease in concentration with time. Estimates of the volume of distribution (V_z) suggested that PEGPH20 was not extensively distributed to peripheral tissues. The systemic clearance of PEGPH20 was slow with mean rate ranging from 0.9 to 1.6 mL/h-kg as determined on Study Days 1, 3, 8, and 11. The slow clearance contributed to a long elimination half-life ($t_{1/2}$). The observed mean elimination half-life ($t_{1/2}$) for PEGPH20 ranged from 28 to 64 hours (1.2 to 2.7 days). An accelerated clearance for PEGPH20 was observed in two of three animals on study day 25. Plasma samples collected on study day 25 were re-assayed to confirm PEGPH20 concentrations and the mean concentrations were reported.

TABLE 48: TK Parameters After Repeat IV Dose Administration of PEGPH20

TK Parameter	Animal No.	Day				
		1	3	8	11	25
C ₀ (U/mL)	1	5,044	4,915	4,395	5,170	4,209
	2	5,090	5,722	3,245	3,142	4,233
	3	4,338	ND	3,517	4,649	5,851
	Mean	4,824	5,319	3,719	4,320	4,765
C ₀ (µg/mL)	1	144.1	140.4	125.6	147.7	120.3
	2	145.4	163.49	92.7	89.8	121.0

TABLE 48: TK Parameters After Repeat IV Dose Administration of PEGPH20						
TK Parameter	Animal No.	Day				
		1	3	8	11	25
	3	123.9	ND	100.5	132.8	167.2
	Mean	137.8	152.0	106.3	123.4	136.1
C_{max} (U/mL)	1	4,762	4,620	4,002	4,836	4,006
	2	4,986	5,361	3,578	3,038	4,070
	3	4,143	ND	3,243	4,309	5,832
	Mean	4630	4,991	3,608	4,061	4,636
C_{max} (µg /mL)	1	136.1	132.0	114.3	138.2	114.5
	2	142.5	153.2	102.2	86.8	116.3
	3	118.4	ND	92.7	123.1	166.6
	Mean	132.3	142.6	103.1	116.0	132.5
t_½ λ_Z (h)	1	33.4	40.7	66.6	48.9	10.0
	2	30.2	49.0	62.3	63.4	14.0
	3	33.7	ND	63.8	51.3	60.2
	Mean	32.5	44.8	64.2	54.5	28.1
AUC_{0-∞} (U·h/mL)	1	140,017	207,696	209,290	169,919	62,524
	2	147,554	269,928	234,010	169,380	67,016
	3	135,337	ND	159,178	153,185	366,069
	Mean	140,969	238,812	200,826	164,162	165,203
AUC_{0-∞} (µg·h/mL)	1	4,000	5,934	5,980	4,855	1,786
	2	4,216	7,712	6,686	4,839	1,915
	3	3,867	ND	4,548	4,377	10,459
	Mean	4,028	6,823	5,738	4,690	4,720
V_Z (mL/kg)	1	76.5	62.7	101.9	92.1	45.8
	2	65.6	58.2	85.2	119.9	59.6
	3	79.8	ND	128.4	107.2	47.0
	Mean	74.0	60.4	105.2	106.4	50.8
V_{ss} (mL/kg)	1	75.8	61.5	99.4	90.1	49.8
	2	64.9	57.2	83.9	118.6	58.9
	3	79.8	ND	124.9	104.8	46.3
	Mean	73.5	59.3	102.7	104.5	51.6
CL (mL/h·kg)	1	1.59	1.07	1.06	1.31	3.17
	2	1.50	0.82	0.95	1.31	2.95
	3	1.64	ND	1.39	1.45	0.54

TABLE 48: TK Parameters After Repeat IV Dose Administration of PEGPH20

TK Parameter	Animal No.	Day				
		1	3	8	11	25
	Mean	1.58	0.95	1.13	1.36	2.22
MRT (h)	1	47.8	57.5	93.7	69.0	15.7
	2	43.1	69.5	88.4	90.5	19.9
	3	48.6	ND	89.6	72.3	85.6
	Mean	46.5	63.5	90.6	77.3	40.4

TK = toxicokinetic; IV = intravenous; U = units of enzymatic activity; C_0 = plasma concentration back-extrapolated to time 0; ND = not determined; C_{max} = maximum observed plasma concentration; $t_{1/2}$ = terminal half-life; $AUC_{0-\infty}$ = area under the plasma concentration versus time curve from time 0 and extrapolated to infinity; V_z = volume of distribution; V_{ss} = volume of distribution at steady state; CL = absolute clearance; MRT = mean residence time

2. Four-Week Repeat Intravenous Dose Toxicity Study

A 4-week repeat-dose toxicity study was conducted in cynomolgus following IV administration of PEGPH20. Four groups of monkeys (6 animals per gender, with the exception of the group that received 0.2 mg/kg/dose of PEGPH20, which was 4 animals per gender) received IV twice-weekly doses of vehicle, 0.2, 2.0 or 10.5 mg/kg/dose of PEGPH20, respectively, for 4 consecutive weeks. Blood samples were collected from each animal on Days 1 and 25 at pre-dose and at 0.5, 1, 2, 4, 8, 12, 24, 48 and 72 hours post-dose. Additional blood samples were collected on Days 4, 8, 11, 15, 18 and 22 at pre-dose and at two minutes post-dose. Plasma samples were analyzed for hyaluronidase activity using turbidometric assay described in Example 20. Toxicokinetic parameters were derived for each IV dose using non-compartmental modeling of the plasma concentration-versus-time data. The results are set forth in Table 49. TK analysis of the plasma concentration-versus-time data after twice-weekly IV dose administration of PEGPH20 to monkeys for 4 consecutive weeks demonstrated that exposures (C_{max} and AUC) increased with increasing PEGPH20 dose. Toxicokinetics were approximately linear over the range of doses evaluated. Elimination half-life ($t_{1/2}$) increased with increasing PEGPH20 dose. On repeated dosing, $t_{1/2}$ decreased in the low- and mid-dose males. Exposure data for the low-dose also showed animals with loss of exposure on repeated dosing. Exposures

were generally similar between genders for any given dose level and dosing day. Following repeated IV dosing of PEGPH20, there was a trend toward increased C_{max} and AUC on Day 25 compared with Day 1 in the 2 mg/kg and 10.5 mg/kg dose groups. However, Day 25 AUC exposures remained <2-fold the exposures observed on Day 1 within any given dose group. A loss of exposure for PEGPH20 was observed in the 0.2 mg/kg group in 3 of 8 animals on Study Day 25.

TABLE 49: Mean TK Parameters After Twice –Weekly IV Administration of						
Parameter	0.2 mg/kg		2 mg/kg		10.5 mg/kg	
	Day 1	Day 25	Day 1	Day 25	Day 1	Day 25
Males						
C _{max} (U/mL)	114	92.5	1,130	1,330	6,410	9,490
t _½ (h)	33.5	17.0	40.9	16.7	50.1	78.1
AUC _{0-t}	2,750	1,790	32,500	36,200	225,000	429,000
C _{max} Exposure Ratio (C _{max} on Day 25/ C _{max} on Day 1)	0.77		1.18		1.48	
AUC Exposure Ratio (AUC _{0-t} on Day 25/ AUC _{0-t} on Day 1)	0.65		1.11		1.91	
Females						
C _{max} (U/mL)	113	97.3	1,310	1,560	6,450	8,340
t _½ (h)	29.0	42.0	37.7	44.8	51.1	42.4
AUC _{0-t} (U·h/mL)	2,650	1,680	35,600	56,300	230,000	332,000
C _{max} Exposure Ratio ((C _{max} on Day 25/ C _{max} on Day 1)	0.74		1.19		1.29	
AUC Exposure Ratio ((AUC _{0-t} on Day 25/ AUC _{0-t} on Day 1)	0.61		1.58		1.44	
C _{max} Gender Ratio (C _{max} (males)/ C _{max} (females))	1.01	0.95	0.86	0.85	0.99	1.14

AUC Gender Ratio (AUC _{0-t} (males)/ AUC _{0-t} (females))	1.04	1.07	0.91	0.64	0.98	1.29
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TK = toxicokinetic; IV = intravenous; U = units of enzymatic activity; C_{max} = maximum observed plasma concentration; t_{1/2} = elimination half-life; AUC_{0-t} = area under the plasma concentration versus time curve from time 0 to the last measured time point

The twice-weekly IV administration was well tolerated in monkeys. Like rats, there was a transient decrease in body weight, which returned towards pre-study values by the end of the dosing period. Unlike rats, there was no prolonged APTT and the only change in coagulation parameters was limited to a transient and dose-independent increase in plasma fibrinogen, which is consistent with a transient acute-phase response to PEGPH20 administration. Changes in limb joints were observed in monkeys, with a dose-related decrease in range of motion at the knee and elbow, which showed partial to full recovery following cessation of dosing. Also, there was a moderate decrease in soft tissue mass (skeletal muscle) in a single high-dose animal observed by radiologic examination. This is consistent with the pharmacologic effect of PEGPH20 to remove hyaluronan and its associated extracellular water from tissues. There was no associated histopathologic changes of the knee joint or skeletal muscle, nor abnormal radiography findings of the knee joint itself. No changes in food consumption or behavior was observed. Since no test article-related findings were observed, the high-dose of 10.5 mg/kg/dose was the designated NOAEL monkeys.

C. Summary

In a repeat IV dose in monkeys in which a 6 mg/kg (approximately 210,000 U/kg) dose of PEGPH20 was administered 8 times over a 29-day period, exposures (as assessed by mean C_{max} and AUC values) were similar over the study period. The systemic clearance (CL) of PEGPH20 was slow with mean rates of 0.9 to 1.6 mL/h kg. The slow clearance contributed to a long elimination half-life, which ranged from 28 to 64 hours (1.2 to 2.7 days).

The toxicology studies in rats and monkeys, in which animals received PEGPH20 at varying doses twice-weekly for 4 consecutive weeks, showed that exposures (C_{max} and AUC values) increased linearly with increase dose and elimination half-life increased with increasing dose. The mean values of elimination half-life in rats ranged from approximately 3 hours (0.5 mg/kg dose) to 24 hours (25

mg/kg dose) and in monkeys ranged from 17 hours (0.2 mg/kg dose) to 78 hours (10.5 mg/kg dose). In both species, exposures were generally similar between genders for any given dose level and dosing day. A loss of exposure, however, was observed in the 0.2 mg/kg group in 3 of 8 animals on Day 25. In rats, there was a trend toward increased AUC on Day 25 compared with Day 1 in the 5 mg/kg and 25 mg/kg dose groups, nevertheless, day 25 AUC exposures remained < 2-fold the exposures observed on day 1 within any given dose group. Exposures as determined by C_{max} generally remained consistent from Day 1 to Day 25 within any given dose group in rats. In monkeys, there was a trend toward increased C_{max} and AUC on Day 25 compared with Day 1 in the 2 mg/kg and 10.5 mg/kg dose groups. Similar to rats, Day 25 AUC exposures in monkeys remained < 2-fold the exposures observed on Day 1 within any given dose group.

Example 23

Allometric Scaling Model

Single-dose intravenous pharmacokinetic studies in mice, rats and monkeys described in Example 20 formed the basis of an allometric scaling model applicable to large molecules that are retained largely within the vascular system, which was used to estimate the pharmacologically active dose (PAD) and the dosing frequency for PEGPH20 in humans. Due to the large molecular size of PEGPH20 (molecular weight is estimated to be between 100 and 270 kDa), the conventional Interspecies Scaling Factor technique used for small molecules is not applicable to molecules confined to the intravascular compartment.

Allometric modeling was performed from the PK parameters observed in mice, rats and monkeys by scaling the parameters allometrically to the animals' body surface area and to the animals' body weight. Derived interspecies allometric relationships were used to calculate/predict primary PK parameters for humans. The parameters calculated were systemic clearance (CL), peripheral clearance (CL₂), volume of the central compartment (V₁), volume of the peripheral compartment (V₂), elimination rate constant from the central compartment (k₁₀), and the distribution rate constants between the central and peripheral compartment (k₁₂ and k₂₁). With the assumption of proportional increase in systemic exposure with increases of IV dose,

the concentration-time profiles for PEGPH20 in humans were simulated with a 2-compartment model for IV dose levels of 1500, 3,000, 6,000, 12,000, 24,000 and 48,000 U/kg. Twice-a-week (BIW) dose administrations of Monday-Thursday or Monday-Friday schedule were examined and demonstrated that clinical doses $\geq 1,500$ U/kg (50 $\mu\text{g/kg}$) and a Monday-Thursday BIW dose schedule are projected to maintain a threshold plasma concentration (C_{trough}) above the pharmacologically effective concentration of ≥ 10 U/mL.

The human dose of 0.05 mg/kg is consistent with the conventional 1/10 safety factor for rodents where the 5 mg/kg dose level was defined as the NOAEL in rats.

10 The lowest dose in the monkey study was 0.2 mg/kg and the highest does was 10.5 mg/kg. Taking these two dose levels, respectively, and noting that they were not associated with adverse events, the human dose of 0.05 mg/kg represents a 4-fold and 210-fold safety factor vs. doses administered to primates.

15 Since modifications will be apparent to those of skill in this art, it is intended that this invention be limited only by the scope of the appended claims.

CLAIMS:

1. Use of a soluble hyaluronidase composition formulated as a medicament for maintaining a plasma level of the hyaluronidase enzyme in plasma at a level of at least 3 U/ml for at least a week for treating a disease or condition in which a hyaluronidase substrate accumulates, wherein the composition comprises a soluble hyaluronidase conjugated to a polymer in an amount sufficient to maintain the level.
2. A pharmaceutical composition for use in treating a disease or condition in which a hyaluronidase substrate accumulates, comprising a soluble hyaluronidase in an amount sufficient for maintaining a plasma level of the hyaluronidase enzyme in plasma at a level of at least 3 U/mL for at least a week, wherein the soluble hyaluronidase is conjugated to a polymer.
3. The use of claim 1 or composition of claim 2, wherein the composition is for administration at least twice a week for more than at least one week.
4. The use or composition of any of claims 1-3, wherein:
the composition contains at least or about 2.0 mg – 60 mg of the soluble hyaluronidase conjugated to a polymer; and
the soluble hyaluronidase conjugated to a polymer has a specific activity of at least or about 20,000 U/mg, 25,000 U/mg, 30,000 U/mg, 31,000 U/mg, 32,000 U/mg, 33,000 U/mg, 34,000 U/mg, 35,000 U/mg, 36,000 U/mg, 37,000 U/mg, 38, 000 U/mg, 39,000 U/mg, 40,000 U/mg, 45,000 U/mg, 50,000 U/mg, 55,000 U/mg, 60,000 U/mg or more.
5. The use or composition of any of claims 1-4, wherein the composition is at least 10 mL/per administration.
6. The use or composition of any of claims 1-5, wherein the composition is formulated for administration orally, intravenously (IV), subcutaneously, intramuscularly, intra-tumorally, radermally, topically, transdermally, rectally or sub-epidermally.
7. The use or composition of any of claims 1-6, wherein the composition is formulated for intravenous administration.

8. The use or composition of any of claims 1-7, wherein the composition further comprises histidine and/or NaCl.

9. The use or composition of claim 8, wherein the composition is formulated with at or about 10 mM histidine and/or at or about 130 mM NaCl.

5 10. The use or composition of any of claims 1-9, wherein the composition has a pH that is or is about 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1 or 7.2.

11. The use or composition of claim 10 that has a pH of or about 6.5.

12. The use or composition of any of claims 1-11, wherein the level of hyaluronidase in the plasma is at least or about 3 U/mL– 12 U/mL.

10 13. The use or composition of any of claims 1-11, wherein the level of hyaluronidase in the plasma is at least or at least about 5 U/mL, 6 U/mL, 7 U/mL, 8 U/mL, 9 U/mL, 10 U/mL, 15 U/mL, 20 U/mL, 30 U/mL, 40 U/mL, 45 U/mL or 50 U/mL.

14. The use or composition of any of claims 1-13, wherein the level of
15 hyaluronidase in the plasma is least or about 10 U/mL.

15. The use or composition of any of claims 1-14, wherein the polymer is a sialation or pegylation moiety.

16. The use or composition of any of claims 1-15, wherein the hyaluronidase substrate is hyaluronan.

20 ~~17. The use or composition of any of claims 1-16, wherein the soluble~~
hyaluronidase is a soluble PH20.

18. The use or composition of claim 17, wherein the PH20 is selected from among an ovine, mouse, monkey, bovine, bacterial and human PH20.

19. The use or composition of claim 17 or claim 18, wherein the soluble
25 form of PH20 is a soluble PH20 that has been truncated to remove a C-terminal GPI.

20. The use or composition of any of claims 1-19, wherein the soluble hyaluronidase has a sequence of amino acids included in SEQ ID NO:1 or a sequence that has at least about 91% amino acid sequence identity with a sequence of amino acids included in SEQ ID NO:1, whereby the soluble hyaluronidase is soluble, N-
30 glycosylated and neutral active.

21. The use or composition of any of claims 1-20, wherein the soluble hyaluronidase includes a sequence of amino acids set forth in SEQ ID NO:1 that is truncated at an amino acid residue that is or is between amino acid residues 467 to 483.

5 22. The use or composition of any of claims 1-21, wherein the soluble hyaluronidase includes a sequence of amino acids set forth in SEQ ID NO:1 that is truncated at an amino acid residue selected from among 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482 and 483.

23. The use or composition of claim 22, wherein the soluble hyaluronidase
10 is secreted in CHO cells.

24. The use or composition of any of claims 1-23, wherein the soluble hyaluronidase has the sequence of amino acids set forth as amino acids 36-467, 36-468, 36-469, 36-470, 36-471, 36-472, 36-473, 36-474, 36-475, 36-476, 36-477, 36-478, 36-479, 36-480, 36-481, 36-482, or 36-483 of SEQ ID NO:1, or has at least
15 about 91% amino acid sequence identity with a sequence of amino acids set forth as amino acids 36-467, 36-468, 36-469, 36-470, 36-471, 36-472, 36-473, 36-474, 36-475, 36-476, 36-477, 36-478, 36-479, 36-480, 36-481, 36-482, or 36-483 of SEQ ID NO:1.

25. The use or composition of any claims 1-24, wherein the soluble
20 ~~hyaluronidase is selected from among polypeptides containing a sequence of amino~~
acids set forth in any of SEQ ID NOS: 4-9 and 46-48, and allelic variants, species variants and other variants thereof.

26. The use or composition of any of claims 1-25, wherein the soluble hyaluronidase is a polypeptide encoded by a sequence of nucleic acids that encodes a
25 sequence of amino acids set forth in SEQ ID NO:4.

27. The use or composition of any of claims 1-26, wherein the soluble hyaluronidase is selected from among polypeptides encoded by a sequence of nucleic acids that encodes a sequence of amino acids set forth in any of SEQ ID NOS: 4-9.

28. The use or composition of any of claims 1-27, wherein the soluble
30 hyaluronidase is produced by expression in CHO cells.

29. The use or composition of claim 28, wherein the soluble hyaluronidase is designated rHuPH20.

30. The use or composition of any of claims 1-29, wherein the soluble hyaluronidase is glycosylated.

5 31. The use or composition of any of claims 1-30, wherein the polymer conjugated to the soluble hyaluronidase comprises a pegylation moiety (PEG).

32. The use or composition of claim 31, wherein the polymer is selected from among methoxy-poly(ethylene glycol)-succinimidyl butanoate (mPEG-SBA) (5 kDa); methoxy-poly(ethylene glycol)-succinimidyl butanoate (mPEG-SBA) (20 kDa);
10 methoxy-poly(ethylene glycol)-succinimidyl butanoate (mPEG-SBA) (30 kDa); methoxy-poly(ethylene glycol)-succinimidyl α -methylbutanoate (mPEG-SMB) (20 kDa); methoxy-poly(ethylene glycol)-succinimidyl α -methylbutanoate (mPEG-SMB) (30 kDa); methoxy-poly(ethylene glycol)-butyraldehyde (mPEG-butyraldehyde) (30 kDa), methoxy-poly(ethylene glycol)-succinimidyl propionate (mPEG-SPA) (20
15 kDa); methoxy-poly(ethylene glycol)-succinimidyl propionate (mPEG-SPA) (30 kDa); (methoxy-poly(ethylene glycol))₂-N-hydroxysuccinimide ester (mPEG₂-NHS) (10 kDa branched); (methoxy-poly(ethylene glycol))₂-N-hydroxysuccinimide ester (mPEG₂-NHS) (20 kDa branched); (methoxy-poly(ethylene glycol))₂-N-hydroxysuccinimide ester (mPEG₂-NHS) (40 kDa branched); (methoxy-poly(ethylene
20 glycol))₂-N-hydroxysuccinimide ester (mPEG₂-NHS) (60 kDa branched); biotin-poly(ethylene glycol)-N-hydroxysuccinimide ester (biotin-PEG-NHS) (5 kDa biotinylated); poly(ethylene glycol)-p-nitrophenyl carbonate (PEG-p-nitrophenyl-carbonate) (30 kDa); and poly(ethylene glycol)-propionaldehyde (PEG-propionaldehyde) (30 kDa).

25 33. The use or composition of claim 32, wherein the PEG is a branched or linear PEG.

34. The use or composition of claim 33, wherein the PEG is a linear PEG.

35. The use or composition of any of claims 31-34, wherein the PEG is a methoxy-PEG (mPEG).

30 36. The use or composition of claim 35, wherein the PEG is a linear N-hydroxysuccinimidyl ester of methoxy poly(ethylene glycol) butanoic acid.

37. The use or composition of claim 35 or claim 36, wherein the PEG has a molecular weight of 30 or about 30 kilodaltons.

38. A composition, comprising a soluble hyaluronidase in an amount sufficient for maintaining a plasma level of the hyaluronidase enzyme in plasma at a
5 level of at least 3 U/mL for at least a week, wherein the soluble hyaluronidase is conjugated to a polymer.

39. The composition of claim 38, wherein the composition is for administration at least twice a week for more than at least one week.

40. The composition of claim 38 or claim 39, wherein:
10 the composition contains at least or about 2.0 mg – 60 mg of the soluble hyaluronidase conjugated to a polymer; and
the soluble hyaluronidase conjugated to a polymer has a specific activity of at least or about 20,000 U/mg, 25,000 U/mg, 30,000 U/mg, 31,000 U/mg, 32,000 U/mg, 33,000 U/mg, 34,000 U/mg, 35,000 U/mg, 36,000 U/mg, 37,000 U/mg, 38, 000
15 U/mg, 39,000 U/mg, 40,000 U/mg, 45,000 U/mg, 50,000 U/mg, 55,000 U/mg, 60,000 U/mg or more.

41. The composition of any of claims 38-40, wherein the composition is at least 10 mL/ per administration.

42. The composition of any of claims 38-41, wherein the composition is
20 ~~formulated for administration orally, intravenously (IV), subcutaneously,~~
intramuscularly, intra-tumorally, radermally, topically, transdermally, rectally or sub-epidermally.

43. The composition of any of claims 38-42, wherein the composition is formulated for intravenous administration.

25 44. The compositon of any of claims 38-43, further comprising histidine and/or NaCl.

45. The compositon of claim 44, wherein the composition is formualted with at or about 10 mM histidine and/or 130 mM NaCl.

46. The composition of any of claims 38-45, wherein the composition has
30 a pH that is or is about 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1 or 7.2.

47. The composition of claim 46 that has a pH of or about 6.5.

48. The composition of any of claims 38-47, wherein the level of hyaluronidase in the plasma is at least or about 3 U/mL- 12 U/mL.

49. The composition of any of claims 38-48, wherein the polymer is a sialation or pegylation moiety.

5 50. A combination, comprising:

a composition containing a soluble hyaluronidase in an amount sufficient for maintaining a plasma level of the hyaluronidase enzyme in plasma at a level of at least 3 U/mL for at least a week, wherein the soluble hyaluronidase is conjugated to a polymer; and

10 a second composition containing a second agent for treating a hyaluronan-associated disease or condition.

51. The combination of claim 50, wherein the composition containing a soluble hyaluronidase is for administration at least twice a week for more than at least one week.

15 52. The combination of claim 50 or claim 51, wherein:

the composition containing a soluble hyaluronidase contains at least or about 2.0 mg – 60 mg of the soluble hyaluronidase conjugated to a polymer; and

the soluble hyaluronidase conjugated to a polymer has a specific activity of at least or about 20,000 U/mg, 25,000 U/mg, 30,000 U/mg, 31,000 U/mg, 32,000 U/mg, 20 33,000 U/mg, 34,000 U/mg, 35,000 U/mg, 36,000 U/mg, 37,000 U/mg, 38, 000 U/mg, 39,000 U/mg, 40,000 U/mg, 45,000 U/mg, 50,000 U/mg, 55,000 U/mg, 60,000 U/mg or more.

53. The combination of any of claims 50-52, wherein the first and second compositions are co-formulated or provided separately.

25 54. The combination of any of claims 50-53, wherein the polymer is a sialation or pegylation moiety.

55. The combination of any of claims 50-54, wherein the second agent is an anti-cancer agent or treatment.

30 56. The combination of claim 55, wherein the anti-cancer agent or treatment is selected from among a chemotherapeutic agent, radiation therapy an antibody, a peptide, a gene therapy vector, a virus and a nucleic acid.

57. The combination of any of claims 50-56, wherein:
the second agent is an anti-cancer agent selected from among Acivicins;
Aclarubicins; Acodazoles; Acronines; Adozelesins; Aldesleukins; Alemtuzumabs;
Alitretinoin (9-Cis-Retinoic Acids); Allopurinols; Altretamines; Alvocidibs;
5 Ambazones; Ambomycins; Ametantrones; Amifostines; Aminoglutethimides;
Amsacrine; Anastrozoles; Anaxirones; Ancitabines; Anthramycins; Apaziquones;
Argimesnas; Arsenic Trioxides; Asparaginases; Asperlins; Atrimustines;
Azacitidines; Azetepas; Azotomycins; Banoxantrones; Batabulins; Batimastats; BCG
Live; Benaxibines; Bendamustines; Benzodepas; Bexarotenes; Bevacizumab;
10 Bicalutamides; Bietaserpines; Biricodars; Bisantrones; Bisantrones; Bisnafide
Dimesylates; Bizelesins; Bleomycins; Bortezomibs; Brequinars; Bropirimines;
Budotitanes; Busulfans; Cactinomycins; Calusterones; Canertinibs; Capecitabines;
Caracemides; Carbetimers; Carboplatins; Carboquones; Carmofurs; Carmustines with
Polifeprosans; Carmustines; Carubicins; Carzelesins; Cedefingols; Celecoxibs;
15 Cemadotins; Chlorambucils; Cioteronels; Cirolemycins; Cisplatins; Cladribines;
Clanfenurs; Clofarabines; Crisnatols; Cyclophosphamides; Cytarabine liposomals;
Cytarabines; Dacarbazines; Dactinomycins; Darbepoetin Alfa; Daunorubicin
liposomals; Daunorubicins/Daunomycins; Daunorubicins; Decitabines; Denileukin
Diftitoxes; Dexniguldipines; Dexonnaplatins; Dexrazoxanes; Dezaguanines;
20 ~~Diaziquones; Dibrospidiums; Dienogests; Dinalins; Disermolides; Docetaxels;~~
Dofequidars; Doxifluridines; Doxorubicin liposomals; Doxorubicin HCL;
Doxorubicin HCL liposome injection; Doxorubicins; Droloxifenes; Dromostanolone
Propionates; Duazomycins; Ecomustines; Edatrexates; Edotecarins; Eflornithines;
Elacridars; Elinafides; Elliott's B Solutions; Elsamitrucins; Emitefurs; Enloplatins;
25 Enpromates; Enzastaurins; Epiropidines; Epirubicins; Epoetin alfa; Eptaloprosts;
Erbulozoles; Esorubicins; Estramustines; Etanidazoles; Etoglucids; Etoposide
phosphates; Etoposide VP-16s; Etoposides; Etoprines; Exemestanes; Exisulinds;
Fadrozoles; Fazarabines; Fenretinides; Filgrastims; Floxuridines; Fludarabines;
Fluorouracils; 5-fluorouracils; Fluoxymesterones; Flurocitabines; Fosquidones;
30 Fostriecins; Fostriecins; Fotretamines; Fulvestrants; Galarubicins; Galocitabines;
Gemcitabines; Gemtuzumabs/Ozogamicins; Geroquinols; Gimatecans; Gimeracils;

- Gloxazones; Glufosfamides; Goserelin acetates; Hydroxyureas;
Ibritumomabs/Tiuxetans; Idarubicins; Ifosfamides; Ilmofofines; Ilomastats; Imatinib
mesylates; Imexons; Improsulfans; Indisulams; Inproquones; Interferon alfa-2as;
Interferon alfa-2bs; Interferon Alfas; Interferon Betas; Interferon Gammas;
5 Interferons; Interleukin-2s and other Interleukins (including recombinant
Interleukins); Intoplicines; Iobenguanes [131-I]; Iproplatins; Irinotecans; Irsogladines;
Ixabepilones; Ketotrexates; L-Alanosines; Lanreotides; Lapatinibs; Ledoxantrones;
Letrozoles; Leucovorins; Leuprolides; Leuprorelins (Leuprorelides); Levamisoles;
Lexacalcitols; Liarozoles; Lobaplatins; Lometrexols; Lomustines/CCNUs;
10 Lomustines; Lonafarnibs; Losoxantrones; Lurtotecans; Mafosfamides; Mannosulfans;
Marimastats; Masoprocals; Maytansines; Mechlorethamines;
Meclorethamines/Nitrogen mustards; Megestrol acetates; Megestrols; Melengestrols;
Melfhalans; MelfhalansIL-PAMs; Menogarils; Mepitiostanes; Mercaptopurines; 6-
Mecaptopurine; Mesnas; Metesinds; Methotrexates; Methoxsalens; Metomidates;
15 Metoprines; Meturedepas; Miboplatins; Miproxifenes; Misonidazoles; Mitindomides;
Mitocarcins; Mitocromins; Mitoflaxones; Mitogillins; Mitoguazones; Mitomalcins;
Mitomycin Cs; Mitomycins; Mitonafides; Mitoquidones; Mitospers; Mitotanes;
Mitoxantrones; Mitozolomides; Mivobulins; Mizoribines; Mofarotenes; Mopidamols;
Mubritinibs; Mycophenolic Acids; Nandrolone Phenpropionates; Nedaplatins;
20 Nelzarabines; Nemorubicins; Nitracines; Nocodazoles; Nofetumomabs;
Nogalamycins; Nolatrexedes; Nortopixantrones; Octreotides; Oprelvekins;
Ormaplatins; Ortataxels; Oteracils; Oxaliplatins; Oxisurans; Oxophenarsines;
Paclitaxels; Pamidronates; Patubilones; Pegademases; Pegaspargases; Pegfilgrastims;
Peldesines; Peliomycins; Pelitrexols; Pemetrexeds; Pentamustines; Pentostatins;
25 Peplomycins; Perfosfamides; Perifosines; Picoplatins; Pinafides; Pipobromans;
Piposulfans; Pirfenidones; Piroxantrones; Pixantrones; Plevitrexeds; Plicamycid
Mithramycins; Plicamycins; Plomestanes; Plomestanes; Porfimer sodiums; Porfimers;
Porfiromycins; Prednimustines; Procarbazines; Propamidines; Prospidium;
Pumitepas; Puromycins; Pyrazofurins; Quinacrine; Ranimustines; Rasburicases;
30 Riboprines; Ritrosulfans; Rituximabs; Rogletimides; Roquinimexs;
Rufocromomycins; Sabarubicins; Safingols; Sargramostims; Satraplatins;

Sebriplatins; Semustines; Simtrazenes; Sizofirans; Sobuzoxanes; Sorafenibs;
Sparfosates; Sparfosic Acids; Sparsomycins; Spirogermaniums; Spiromustines;
Spiroplatins; Spiroplatins; Squalamines; Streptonigrins; Streptovarycins;
Streptozocins; Sufosfamides; Sulofenurs; Sunitinib Malate; 6-thioguanine (6-TG);
5 Tacedinalines; Talcs; Talisomycins; Tallimustines; Tamoxifens; Tariquidars;
Tauromustines; Tecogalans; Tegafurs; Teloxantrones; Temoporfin; Temozolomides;
Teniposides/VM-26s; Teniposides; Teroxirones; Testolactones; Thiamiprines;
Thioguanines; Thiotepas; Tiamiprines; Tiazofurins; Tilomisoles; Tilorones;
Timcodars; Timonacics; Tirapazamines; Topixantrones; Topotecans; Toremifenes;
10 Tositumomabs; Trabectedins (Ecteinascidin 743); Trastuzumabs; Trestolones;
Tretinoin/ATRA; Triciribines; Trilostanes; Trimetrexates; Triplatin Tetranitrates;
Triptorelins; Trofosfamides; Tubulozoles; Ubenimex; Uracil Mustards; Uredepas;
Valrubicins; Valspodars; Vapreotides; Verteporfin; Vinblastines; Vincristines;
Vindesines; Vinepidines; Vinflunines; Vinformides; Vinglycinates; Vinleucinols;
15 Vinleurosines; Vinorelbines; Vinrosidines; Vintriptols; Vinzolidines; Vorozoles;
Xanthomycin As (Guamecyclines); Zeniplatins; Zilascorbs [2-H]; Zinostatin;
Zoledronate; Zorubicins; and Zosuquidars.

58. A method for treating a disease or condition in which a hyaluronidase
substrate accumulates, comprising:
20 ~~administering a soluble hyaluronidase enzyme to a subject more than once a~~
week for a predetermined number of weeks in an amount sufficient to maintain a
pharmacologically active level of the hyaluronidase in the plasma of at least about or
3 U/mL to prevent resynthesis of the substrate to levels prior to treatment, wherein:
the hyaluronidase enzyme is modified by conjugation to a polymer; and
25 the predetermined number of weeks is more than one week.

59. The method of claim 58, wherein the predetermined number of weeks
is at least two weeks.

60. The method of claim 58 or claim 59, wherein the predetermined
number of weeks is two weeks, three weeks, or four weeks.

61. The method of any of claims 58-60, wherein after the predetermined number of weeks, administration is discontinued for a first predetermined period of time, and then resumed for at least one week.

62. The method of claim 61, wherein the first predetermined period of time
5 is at least one week.

63. The method of claim 61 or claim 62, wherein the first predetermined period of time is one week, two weeks, three weeks or four weeks.

64. The method of any of claims 61-63, wherein, after the first
predetermined period of time, further administering the soluble hyaluronidase to the
10 subject more than once a week for a predetermined number of weeks in an amount
sufficient to maintain a pharmacologically active level of the hyaluronidase in the
plasma of at least about or 3 U/mL to prevent resynthesis of the substrate to levels
prior to treatment.

65. The method of claim any of claims 61-64, wherein the cycle of
15 administration and discontinuation of administration is repeated a plurality of times.

66. The method of any of claims 58-65, wherein the hyaluronidase is
administered in an amount sufficient to maintain a pharmacologically active level of
the hyaluronidase in the plasma of at least or about 3 U/mL– 12 U/mL.

67. The method of any of claims 58-65, wherein the hyaluronidase is
20 administered in an amount sufficient to maintain a pharmacologically active level of
the hyaluronidase in the plasma of at least at or about 5 U/mL, 6 U/mL, 7 U/mL, 8
U/mL, 9 U/mL, 10 U/mL, 15 U/mL, 20 U/mL, 30 U/mL, 40 U/mL, 45 U/mL, 50
U/mL or more.

68. The method of any of claims 58-67, wherein the hyaluronidase is
25 administered in an amount sufficient to maintain a pharmacologically active level of
the hyaluronidase in the plasma of at least or about 10 U/mL.

69. The method of any of claims 58-68, wherein the hyaluronidase is
administered twice a week.

70. The method of any of claims 68-69, wherein the hyaluronidase is
30 administered in an amount selected from among 0.02 mg/kg (of the subject), 0.03
mg/kg, 0.04 mg/kg, 0.05 mg/kg, 0.06 mg/kg, 0.07 mg/kg, 0.08 mg/kg, 0.09 mg/kg,

0.1 mg/kg, 0.15 mg/kg, 0.2 mg/kg, 0.25 mg/kg, 0.30 mg/kg, 0.35 mg/kg, 0.40 mg/kg, 0.45 mg/kg, 0.5 mg/kg, 0.55 mg/kg, 0.6 mg/kg, 0.7 mg/kg, 0.8 mg/kg, 0.9 mg/kg, 1.0 mg/kg, 1.1 mg/kg, 1.2 mg/kg, 1.3 mg/kg, 1.4 mg/kg, 1.5 mg/kg or more .

71. The method of any of claims 58-70, wherein the hyaluronidase is
5 administered in an amount selected from among 0.05 mg/kg – 0.8 mg/kg.

72. The method of any of claims 58-71, wherein the hyaluronidase is
administered in an amount that is or is about 50,000 Units (U); 60,000 U; 70,000 U;
80,000 U; 90,000 U; 100,000 U; 200,000 U; 300,000 U; 400,000 U; 500,000 U;
600,000 U; 700,000 U; 800,000 U; 900,000 U; 1,000,000 U; 1,500,000 U; 2,000,000
10 U; 2,500,000 U; 3,000,000 U; 3,500,000 U; 4,000,000 U or more.

73. The method of any of claims 58-72, wherein the hyaluronidase
substrate is hyaluronan.

74. The method of any of claims 58-73, wherein hyaluronan expression in
a sample from the subject is measured prior to treatment.

75. A method for treating a hyaluronan-associated disease or condition in a
15 subject, comprising:

(a) measuring hyaluronan expression or hyaluron in a sample from the subject;
and

(b) if the hyaluronan expression or hyaluron in the sample from the subject is
20 elevated or at a level indicative of the disease or condition, administering a
composition containing a soluble hyaluronidase to the subject, wherein the soluble
hyaluronidase is modified by conjugation to a polymer.

76. The method of claim 74 or claim 75, wherein the sample from the
subject is a tissue or body fluid.

77. The method of any of claims 74-76, wherein the sample from the
25 subject is a blood sample, tumor biopsy, cerebral spinal fluid, urine, sweat, semen or
saliva sample.

78. The method of any of claims 58-77, further comprising:
administering a second and different agent for treating the disease or condition to the
30 subject.

79. The method of claim 78, wherein the second agent and the composition containing the soluble hyaluronidase are administered in a single composition.

80. The method of claim 78, wherein the second agent and the composition containing the soluble hyaluronidase are administered separately.

5 81. The method of claim 80, wherein the second agent and the composition containing the soluble hyaluronidase are administered simultaneously, sequentially or intermittently in any order.

82. The method of claim 81, wherein the second agent is administered after administration of the composition containing the soluble hyaluronidase.

10 83. The method of claim 82, wherein the second agent is administered after the first administration of the soluble hyaluronidase in the cycle of administration, and optionally after one or more subsequent administrations of the soluble hyaluronidase.

15 84. The method of claim 83, wherein the second agent is administered after each subsequent administration of the soluble hyaluronidase.

85. The method of claim 83, wherein the second agent is administered after every other subsequent administration of the soluble hyaluronidase.

86. The method of claim 83, wherein the second agent is administered once a week, once every two weeks, once every three weeks or once a month.

20 87. The method of any of claims 82-86, wherein the second agent is administered at least 0.5 minutes, at least one minute, at least five minutes, at least fifteen minutes, at least thirty minutes at least one hour or more than one hour after the composition containing the soluble hyaluronidase is administered.

25 88. The method of any of claims 82-86, wherein the second agent is administered at least or two hours, four hours, six hours, eight hours, 10 hours, 12 hours, 14 hours, 16 hours, 18 hours, 20 hours, 22 hours or 24 hours after the composition containing the soluble hyaluronidase is administered.

30 89. The method of any of claims 82-86, wherein the second agent is administered at least forty-eight hours after the composition containing the soluble hyaluronidase.

90. The method of any of claims 82-86, wherein second agent is administered at least seventy-two hours after the composition containing the soluble hyaluronidase.

91. The method of any of claims 58-90, wherein the polymer is a sialation or pegylation moiety.

92. The method of any of claims 1-91, wherein the second agent is an anti-cancer agent or treatment.

93. The method of claim 92, wherein the anti-cancer agent or treatment is selected from among a chemotherapeutic agent, radiation therapy an antibody, a peptide, a gene therapy vector, a virus and a nucleic acid.

94. The method of any of any of claims 1-93, wherein:
the second agent is an anti-cancer agent selected from among Acivicins;
Aclarubicins; Acodazoles; Acronines; Adozelesins; Aldesleukins; Alemtuzumabs;
Alitretinoin (9-Cis-Retinoic Acids); Allopurinols; Altretamines; Alvocidibs;
Ambazones; Ambomycins; Ametantrones; Amifostines; Aminoglutethimides;
Amsacrine; Anastrozoles; Anaxirones; Ancitabines; Anthramycins; Apaziquones;
Argimesnas; Arsenic Trioxides; Asparaginases; Asperlins; Atrimustines;
Azacitidines; Azetepas; Azotomycins; Banoxantrones; Batabulins; Batimastats; BCG
Live; Benaxibines; Bendamustines; Benzodepas; Bexarotenes; Bevacizumab;
Bicalutamides; Bietaserpines; Biricodars; Bisantrones; Bisantrones; Bisnafide
Dimesylates; Bizelesins; Bleomycins; Bortezomibs; Brequinars; Bropirimines;
Budotitanes; Busulfans; Cactinomycins; Calusterones; Canertinibs; Capecitabines;
Caracemides; Carbetimers; Carboplatins; Carboquones; Carmofurs; Carmustines with
Polifeprosans; Carmustines; Carubicins; Carzelesins; Cedefingols; Celecoxibs;
Cemadotins; Chlorambucils; Cioterone; Cirolemycins; Cisplatin; Cladribines;
Clanfenurs; Clofarabines; Crisnatols; Cyclophosphamides; Cytarabine liposomals;
Cytarabines; Dacarbazines; Dactinomycins; Darbepoetin Alfa; Daunorubicin
liposomals; Daunorubicins/Daunomycins; Daunorubicins; Decitabines; Denileukin
Diftitoxes; Dexniguldipines; Dexonnaplatins; Dexrazoxanes; Dezaguanines;
Diaziquones; Dibrospidium; Dienogests; Dinalins; Disermolides; Docetaxels;
Dofequidars; Doxifluridines; Doxorubicin liposomals; Doxorubicin HCL;

- Docorubicin HCL liposome injection; Doxorubicins; Droloxifenes; Dromostanolone
 Propionates; Duazomycins; Ecomustines; Edatrexates; Edotecarins; Eflornithines;
 Elacridars; Elinafides; Elliott's B Solutions; Elsamitrucins; Emitefurs; Enloplatins;
 Enpromates; Enzastaurins; Epiropidines; Epirubicins; Epoetin alfas; Eptaloprosts;
 5 Erbulozoles; Esorubicins; Estramustines; Etanidazoles; Etoglucids; Etoposide
 phosphates; Etoposide VP-16s; Etoposides; Etoprines; Exemestanes; Exisulinds;
 Fadrozoles; Fazarabines; Fenretinides; Filgrastims; Floxuridines; Fludarabines;
 Fluorouracils; 5-fluorouracils; Fluoxymesterones; Flurocitabines; Fosquidones;
 Fostriecins; Fostriecins; Fotretamines; Fulvestrants; Galarubicins; Galocitabines;
 10 Gemcitabines; Gemtuzumabs/Ozogamicins; Geroquinols; Gimatecans; Gimeracils;
 Gloxazones; Glufosfamides; Goserelin acetates; Hydroxyureas;
 Ibritumomabs/Tiuxetans; Idarubicins; Ifosfamides; Ilmofosines; Ilomastats; Imatinib
 mesylates; Imexons; Improsulfans; Indisulams; Inproquones; Interferon alfa-2as;
 Interferon alfa-2bs; Interferon Alfas; Interferon Betas; Interferon Gammas;
 15 Interferons; Interleukin-2s and other Interleukins (including recombinant
 Interleukins); Intoplicines; Iobenguanes [131-I]; Iproplatins; Irinotecans; Irsogladines;
 Ixabepilones; Ketotrexates; L-Alanosines; Lanreotides; Lapatinibs; Ledoxantrones;
 Letrozoles; Leucovorins; Leuprolides; Leuprorelins (Leuprorelides); Levamisoles;
 Lexacalcitols; Liarozoles; Lobaplatins; Lometrexols; Lomustines/CCNUs;
 20 Lomustines; Lonafernibs; Losoxantrones; Lurtotecans; Mafosfamides; Mannosulfans;
 Marimastats; Masoprocals; Maytansines; Mechlorethamines;
 Mecllorethamines/Nitrogen mustards; Megestrol acetates; Megestrols; Melengestrols;
 Melphalans; Melphalans/L-PAMs; Menogarils; Mepitiostanes; Mercaptopurines; 6-
 Mecaptopurine; Mesnas; Metesinds; Methotrexates; Methoxsalens; Metomidates;
 25 Metoprines; Meturedepas; Miboplatins; Miproxifenes; Misonidazoles; Mitindomides;
 Mitocarcins; Mitocromins; Mitoflaxones; Mitogillins; Mitoguazones; Mitomalcins;
 Mitomycin Cs; Mitomycins; Mitonafides; Mitoquidones; Mitospers; Mitotanes;
 Mitoxantrones; Mitozolomides; Mivobulins; Mizoribines; Mofarotenes; Mopidamols;
 Mubritinibs; Mycophenolic Acids; Nandrolone Phenpropionates; Nedaplatins;
 30 Nelzarabines; Nemorubicins; Nitracrine; Nocodazoles; Nofetumomabs;
 Nogalamycins; Nolatrexed; Nortopixantrones; Octreotides; Oprelvekins;

- Ormaplatins; Ortataxels; Oteracils; Oxaliplatins; Oxisurans; Oxophenarsines;
Paclitaxels; Pamidronates; Patubilones; Pegademases; Pegaspargases; Pegfilgrastims;
Peldesines; Peliomycins; Pelitrexols; Pemetrexeds; Pentamustines; Pentostatins;
Peplomycins; Perfosfamides; Perifosines; Picoplatins; Pinafides; Pipobromans;
5 Puposulfans; Pirfenidones; Piroxantrones; Pixantrones; Plevitrexeds; Plicamycid
Mithramycins; Plicamycins; Plomestanes; Plomestanes; Porfimer sodiums; Porfimers;
Porfiromycins; Prednimustines; Procarbazines; Propamidines; Prospidium;
Pumitepas; Puromycins; Pyrazofurins; Quinacrines; Ranimustines; Rasburicases;
Riboprines; Ritrosulfans; Rituximabs; Rogletimides; Roquinimexs;
10 Rufocromomycins; Sabarubicins; Safingols; Sargramostims; Satraplatins;
Sebriplatins; Semustines; Simtrazenes; Sizofirans; Sobuzoxanes; Sorafenibs;
Sparfosates; Sparfosic Acids; Sparsomycins; Spirogermaniums; Spiromustines;
Spiroplatins; Spiroplatins; Squalamines; Streptonigrins; Streptovarycins;
Streptozocins; Sufosfamides; Sulofenurs; Sunitinib Malate; 6-thioguanine (6-TG);
15 Tacedinalines; Talcs; Talisomycins; Tallimustines; Tamoxifens; Tariquidars;
Tauromustines; Tecogalans; Tegafurs; Teloxantrones; Temoporphins; Temozolomides;
Teniposides/VM-26s; Teniposides; Teroxirones; Testolactones; Thiamiprines;
Thioguanines; Thiotepas; Tiamiprines; Tiazofurins; Tilomisoles; Tilorones;
Timcodars; Timonacics; Tirapazamines; Topixantrones; Topotecans; Toremifenes;
20 Tositumomabs; Trabectedins (Ecteinascidin 743); Trastuzumabs; Trestolones;
Tretinoin/ATRA; Triciribines; Trilostanes; Trimetrexates; Triplatin Tetranitrates;
Triptorelins; Trofosfamides; Tubulozoles; Ubenimexs; Uracil Mustards; Uredepas;
Valrubicins; Valspodars; Vapreotides; Verteporphins; Vinblastines; Vincristines;
Vindesines; Vinepidines; Vinflunines; Vinformides; Vinglycinates; Vinleucinols;
25 Vinleurosines; Vinorelbines; Vinrosidines; Vintriptols; Vinzolidines; Vorozoles;
Xanthomycin As (Guamecyclines); Zeniplatins; Zilascorbs [2-H]; Zinostatins;
Zoledronate; Zorubicins; and Zosuquidars.

95. The method of any of claims 58-94, wherein the soluble hyaluronidase and/or second agent is administered locally or systemically.

- 30 96. The method of claim 95, wherein the soluble hyaluronidase and/or second agent is administered orally, intravenously (IV), subcutaneously,

intramuscularly, intra-tumorally, radermally, topically, transdermally, rectally or sub-epidermally.

97. The method of claim 96, wherein the soluble hyaluronidase and/or second agent is administered intravenously.

5 98. The method of claim 96, wherein the soluble hyaluronidase and/or second agent is administered intra-tumorally.

99. The method of any of claims 74-98, further comprising comparing the expression of hyaluronan in the sample from the subject to expression in a control sample or a standard.

10 100. The method of any of claims 58-99, wherein the disease or condition in which a hyaluronidase substrate accumulates is associated with high interstitial fluid pressure.

101. The method of any of claims 58-100, wherein the disease or condition is selected from among disc pressure, cancer and edema.

15 102. The method of claim 101, wherein the disease or condition is edema, and the edema is caused by organ transplant, stroke or brain trauma.

103. The method of claim 101, wherein the disease or condition is cancer, and the cancer is a tumor.

20 104. The method of claim 103, wherein the disease or condition is a solid tumor.

105. The method of claim 104, wherein the tumor has increased cellular and/or stromal expression of a hyaluronan, compared to a non-cancerous tissue of the same tissue type or compared to a non-metastatic tumor of the same tumor-type.

25 106. The method of claim 101, wherein:
the disease or condition is selected from among any one or more of a late-stage cancer, a metastatic cancer and an undifferentiated cancer.

107. The method of any of claims 58-106, wherein:
the disease or condition is selected from among any one or more of ovarian cancer, in situ carcinoma (ISC), squamous cell carcinoma (SCC), prostate cancer,
30 pancreatic cancer, non-small cell lung cancer, breast cancer, brain cancer and colon cancer.

108. The method of any of claims 58-107, wherein:
the soluble hyaluronidase is a soluble PH20.

109. The method of claim 108, wherein the PH20 is selected from among an
ovine, mouse, monkey, bovine, bacterial and human PH20.

5 110. The method of claim 108 or claim 109, wherein the soluble form of
PH20 is a soluble PH20 that has been truncated to remove a C-terminal GPI.

111. The method of any of claims 58-110, wherein the soluble
hyaluronidase has a sequence of amino acids included in SEQ ID NO:1 or a sequence
that has at least about 91% amino acid sequence identity with a sequence of amino
10 acids included in SEQ ID NO:1, whereby the soluble hyaluronidase is soluble, N-
glycosylated and neutral active.

112. The method of any of claims 58-111, wherein the soluble
hyaluronidase includes a sequence of amino acids set forth in SEQ ID NO:1 that is
truncated at an amino acid residue that is or is between amino acid residues 467 to
15 483.

113. The method of any of claims 58-112, wherein the soluble
hyaluronidase includes a sequence of amino acids set forth in SEQ ID NO:1 that is
truncated at an amino acid residue selected from among 467, 468, 469, 470, 471, 472,
473, 474, 475, 476, 477, 478, 479, 480, 481, 482 and 483.

20 114. The method of any of claims 58-113, wherein the soluble
hyaluronidase is secreted in CHO cells.

115. The method of any of claims 58-114, wherein the soluble
hyaluronidase has the sequence of amino acids set forth as amino acids 36-467, 36-
468, 36-469, 36-470, 36-471, 36-472, 36-473, 36-474, 36-475, 36-476, 36-477, 36-
25 478, 36-479, 36-480, 36-481, 36-482, or 36-483 of SEQ ID NO:1, or has at least
about 91% amino acid sequence identity with a sequence of amino acids set forth as
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475, 36-476, 36-477, 36-478, 36-479, 36-480, 36-481, 36-482, or 36-483 of SEQ ID
NO:1.

30 116. The method of any claims 58-115, wherein the soluble hyaluronidase
is selected from among polypeptides containing a sequence of amino acids set forth in

any of SEQ ID NOS: 4-9 and 46-48, and allelic variants, species variants and other variants thereof.

117. The method of any of claims 58-116, wherein the soluble hyaluronidase is a polypeptide encoded by a sequence of nucleic acids that encodes a sequence of amino acids set forth in SEQ ID NO:4.

118. The method of any of claims 58-117, wherein the soluble hyaluronidase is selected from among polypeptides encoded by a sequence of nucleic acids that encodes a sequence of amino acids set forth in any of SEQ ID NOS: 4-9.

119. The method of claim 118, wherein the soluble hyaluronidase is produced by expression in CHO cells.

120. The method of claim 119, wherein the soluble hyaluronidase is designated rHuPH20.

121. The method of any of claims 58-120, wherein the soluble hyaluronidase is glycosylated.

122. The method of any of claims 58-121, wherein the polymer conjugated to the soluble hyaluronidase comprises a pegylation moiety (PEG).

123. The method of claim 122, wherein the polymer is selected from among methoxy-poly(ethylene glycol)-succinimidyl butanoate (mPEG-SBA) (5 kDa); methoxy-poly(ethylene glycol)-succinimidyl butanoate (mPEG-SBA) (20 kDa); methoxy-poly(ethylene glycol)-succinimidyl butanoate (mPEG-SBA) (30 kDa);

methoxy-poly(ethylene glycol)-succinimidyl α -methylbutanoate (mPEG-SMB) (20 kDa); methoxy-poly(ethylene glycol)-succinimidyl α -methylbutanoate (mPEG-SMB) (30 kDa); methoxy-poly(ethylene glycol)-butyraldehyde (mPEG-butyraldehyde) (30 kDa); methoxy-poly(ethylene glycol)-succinimidyl propionate (mPEG-SPA) (20 kDa); methoxy-poly(ethylene glycol)-succinimidyl propionate (mPEG-SPA) (30 kDa); (methoxy-poly(ethylene glycol))₂-N-hydroxysuccinimide ester (mPEG₂-NHS) (10 kDa branched); (methoxy-poly(ethylene glycol))₂-N-hydroxysuccinimide ester (mPEG₂-NHS) (20 kDa branched); (methoxy-poly(ethylene glycol))₂-N-hydroxysuccinimide ester (mPEG₂-NHS) (40 kDa branched); (methoxy-poly(ethylene glycol))₂-N-hydroxysuccinimide ester (mPEG₂-NHS) (60 kDa branched); biotin-poly(ethylene glycol)-N-hydroxysuccinimide ester (biotin-PEG-NHS) (5 kDa

biotinylated); poly(ethylene glycol)-p-nitrophenyl carbonate (PEG-p-nitrophenyl-carbonate) (30 kDa); and poly(ethylene glycol)-propionaldehyde (PEG-propionaldehyde) (30 kDa).

5 124. The method of claim 123, wherein the PEG is a branched or linear PEG.

 125. The method of claim 124, wherein the PEG is a linear PEG.

 126. The method of any of claims 122-125, wherein the PEG is a methoxy-PEG (mPEG).

10 127. The method of claim 126, wherein the PEG is a linear N-hydroxysuccinimidyl ester of methoxy poly(ethylene glycol) butanoic acid.

 128. The method of claim 126 or claim 127, wherein the PEG has a molecular weight of 30 or about 30 kilodaltons.

 129. The method of any of claims 58-128, wherein the treatment effects a reduction in the size of a tumor in the subject.

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Frost, Gregory et al.

<120> Modified Hyaluronidases And Uses In Treating Hyaluronan-Associated Diseases And Conditions

<130> 3800020.00102/3066PC

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 Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
 260 265 270
 Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
 275 280 285
 Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
 290 295 300
 Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
 305 310 315 320
 Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
 325 330 335
 Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
 340 345 350
 Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
 355 360 365
 Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
 370 375 380
 Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
 385 390 395 400
 Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
 405 410 415
 Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
 420 425 430
 Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
 435 440 445

<210> 7
 <211> 444
 <212> PRT

<213> Homo sapiens

<220>

<223> soluble rHuPH20 1-444

<400> 7

Leu	Asn	Phe	Arg	Ala	Pro	Pro	Val	Ile	Pro	Asn	Val	Pro	Phe	Leu	Trp
1				5					10					15	
Ala	Trp	Asn	Ala	Pro	Ser	Glu	Phe	Cys	Leu	Gly	Lys	Phe	Asp	Glu	Pro
		20						25					30		
Leu	Asp	Met	Ser	Leu	Phe	Ser	Phe	Ile	Gly	Ser	Pro	Arg	Ile	Asn	Ala
	35						40					45			
Thr	Gly	Gln	Gly	Val	Thr	Ile	Phe	Tyr	Val	Asp	Arg	Leu	Gly	Tyr	Tyr
	50					55					60				
Pro	Tyr	Ile	Asp	Ser	Ile	Thr	Gly	Val	Thr	Val	Asn	Gly	Gly	Ile	Pro
65					70					75				80	
Gln	Lys	Ile	Ser	Leu	Gln	Asp	His	Leu	Asp	Lys	Ala	Lys	Lys	Asp	Ile
				85					90					95	
Thr	Phe	Tyr	Met	Pro	Val	Asp	Asn	Leu	Gly	Met	Ala	Val	Ile	Asp	Trp
			100					105					110		
Glu	Glu	Trp	Arg	Pro	Thr	Trp	Ala	Arg	Asn	Trp	Lys	Pro	Lys	Asp	Val
		115					120					125			
Tyr	Lys	Asn	Arg	Ser	Ile	Glu	Leu	Val	Gln	Gln	Gln	Asn	Val	Gln	Leu
	130					135					140				
Ser	Leu	Thr	Glu	Ala	Thr	Glu	Lys	Ala	Lys	Gln	Glu	Phe	Glu	Lys	Ala
145					150					155					160
Gly	Lys	Asp	Phe	Leu	Val	Glu	Thr	Ile	Lys	Leu	Gly	Lys	Leu	Leu	Arg
				165					170					175	
Pro	Asn	His	Leu	Trp	Gly	Tyr	Tyr	Leu	Phe	Pro	Asp	Cys	Tyr	Asn	His
			180					185					190		
His	Tyr	Lys	Lys	Pro	Gly	Tyr	Asn	Gly	Ser	Cys	Phe	Asn	Val	Glu	Ile
		195					200					205			
Lys	Arg	Asn	Asp	Asp	Leu	Ser	Trp	Leu	Trp	Asn	Glu	Ser	Thr	Ala	Leu
	210					215					220				
Tyr	Pro	Ser	Ile	Tyr	Leu	Asn	Thr	Gln	Gln	Ser	Pro	Val	Ala	Ala	Thr
225					230					235					240
Leu	Tyr	Val	Arg	Asn	Arg	Val	Arg	Glu	Ala	Ile	Arg	Val	Ser	Lys	Ile
				245					250					255	
Pro	Asp	Ala	Lys	Ser	Pro	Leu	Pro	Val	Phe	Ala	Tyr	Thr	Arg	Ile	Val
			260					265					270		
Phe	Thr	Asp	Gln	Val	Leu	Lys	Phe	Leu	Ser	Gln	Asp	Glu	Leu	Val	Tyr
		275					280					285			
Thr	Phe	Gly	Glu	Thr	Val	Ala	Leu	Gly	Ala	Ser	Gly	Ile	Val	Ile	Trp
	290					295					300				
Gly	Thr	Leu	Ser	Ile	Met	Arg	Ser	Met	Lys	Ser	Cys	Leu	Leu	Leu	Asp
305					310					315					320
Asn	Tyr	Met	Glu	Thr	Ile	Leu	Asn	Pro	Tyr	Ile	Ile	Asn	Val	Thr	Leu
				325					330					335	
Ala	Ala	Lys	Met	Cys	Ser	Gln	Val	Leu	Cys	Gln	Glu	Gln	Gly	Val	Cys
			340					345					350		
Ile	Arg	Lys	Asn	Trp	Asn	Ser	Ser	Asp	Tyr	Leu	His	Leu	Asn	Pro	Asp
		355					360					365			
Asn	Phe	Ala	Ile	Gln	Leu	Glu	Lys	Gly	Gly	Lys	Phe	Thr	Val	Arg	Gly
	370					375					380				
Lys	Pro	Thr	Leu	Glu	Asp	Leu	Glu	Gln	Phe	Ser	Glu	Lys	Phe	Tyr	Cys
385					390					395					400
Ser	Cys	Tyr	Ser	Thr	Leu	Ser	Cys	Lys	Glu	Lys	Ala	Asp	Val	Lys	Asp
			405						410					415	
Thr	Asp	Ala	Val	Asp	Val	Cys	Ile	Ala	Asp	Gly	Val	Cys	Ile	Asp	Ala
			420					425					430		
Phe	Leu	Lys	Pro	Pro	Met	Glu	Thr	Glu	Glu	Pro	Gln				
		435					440								

<210> 8

<211> 443

<212> PRT

<213> Homo sapiens

<220>

<223> soluble rHuPH20 1-443

<400> 8

Leu	Asn	Phe	Arg	Ala	Pro	Pro	Val	Ile	Pro	Asn	Val	Pro	Phe	Leu	Trp
1				5					10					15	

3066SEQ PC1.TXT

Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
 20 25 30
 Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
 35 40 45
 Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
 50 55 60
 Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
 65 70 75 80
 Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
 85 90 95
 Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
 100 105 110
 Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
 115 120 125
 Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
 130 135 140
 Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
 145 150 155 160
 Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
 165 170 175
 Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
 180 185 190
 His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
 195 200 205
 Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
 210 215 220
 Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
 225 230 235 240
 Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
 245 250 255
 Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
 260 265 270
 Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
 275 280 285
 Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
 290 295 300
 Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
 305 310 315 320
 Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
 325 330 335
 Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
 340 345 350
 Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
 355 360 365
 Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
 370 375 380
 Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
 385 390 395 400
 Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
 405 410 415
 Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
 420 425 430
 Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro
 435 440

<210> 9
 <211> 442
 <212> PRT
 <213> Homo sapiens

<220>
 <223> soluble rHuPH20 1-442

<400> 9
 Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1 5 10 15
 Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
 20 25 30
 Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
 35 40 45
 Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
 50 55 60
 Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
 65 70 75 80

3066SEQ PC1.TXT

Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
 85 90 95
 Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
 100 105 110
 Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
 115 120 125
 Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
 130 135 140
 Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
 145 150 155 160
 Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
 165 170 175
 Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
 180 185 190
 His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
 195 200 205
 Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
 210 215 220
 Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
 225 230 235 240
 Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
 245 250 255
 Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
 260 265 270
 Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
 275 280 285
 Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
 290 295 300
 Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
 305 310 315 320
 Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
 325 330 335
 Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
 340 345 350
 Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
 355 360 365
 Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
 370 375 380
 Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
 385 390 395 400
 Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
 405 410 415
 Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
 420 425 430
 Phe Leu Lys Pro Pro Met Glu Thr Glu Glu
 435 440

<210> 10
 <211> 450
 <212> PRT
 <213> Bos taurus

<220>
 <223> hyaluronidase

<400> 10
 Met Arg Pro Phe Ser Leu Glu Val Ser Leu His Leu Pro Trp Ala Met
 1 5 10 15
 Ala Ala His Leu Leu Pro Val Cys Thr Leu Phe Leu Asn Leu Leu Ser
 20 25 30
 Met Thr Gln Gly Ser Arg Asp Pro Val Val Pro Asn Gln Pro Phe Thr
 35 40 45
 Thr Ile Trp Asn Ala Asn Thr Glu Trp Cys Met Lys Lys His Gly Val
 50 55 60
 Asp Val Asp Ile Ser Ile Phe Asp Val Val Thr Asn Pro Gly Gln Thr
 65 70 75 80
 Phe Arg Gly Pro Asn Met Thr Ile Phe Tyr Ser Ser Gln Leu Gly Thr
 85 90 95
 Tyr Pro Tyr Tyr Thr Ser Ala Gly Glu Pro Val Phe Gly Gly Leu Pro
 100 105 110
 Gln Asn Ala Ser Leu Asn Ala His Leu Ala Arg Thr Phe Gln Asp Ile
 115 120 125
 Leu Ala Ala Met Pro Glu Pro Arg Phe Ser Gly Leu Ala Val Ile Asp
 130 135 140

3066SEQ PC1.TXT

Trp Glu Ala Trp Arg Pro Arg Trp Ala Phe Asn Trp Asp Thr Lys Asp
 145 150 155 160
 Ile Tyr Arg Gln Arg Ser Arg Ala Leu Val Gln Lys Gln His Pro Asp
 165 170 175
 Trp Leu Ala Pro Arg Val Glu Ala Ala Ala Gln Asp Gln Phe Glu Gly
 180 185 190
 Ala Ala Glu Glu Trp Met Ala Gly Thr Leu Lys Leu Gly Gln Ala Leu
 195 200 205
 Arg Pro Gln Gly Leu Trp Gly Phe Tyr Asn Phe Pro Glu Cys Tyr Asn
 210 215 220
 Tyr Asp Phe Lys Ser Pro Asn Tyr Thr Gly Arg Cys Pro Leu Asn Ile
 225 230 235 240
 Cys Ala Gln Asn Asp Gln Leu Gly Trp Leu Trp Gly Gln Ser Arg Ala
 245 250 255
 Leu Tyr Pro Ser Ile Tyr Leu Pro Ala Ala Leu Glu Gly Thr Lys Lys
 260 265 270
 Thr Gln Met Phe Val Gln His Arg Val Ala Glu Ala Phe Arg Val Ala
 275 280 285
 Ala Gly Ala Gly Asp Pro Lys Leu Pro Val Leu Pro Tyr Met Gln Leu
 290 295 300
 Phe Tyr Asp Met Thr Asn His Phe Leu Pro Ala Glu Glu Leu Glu His
 305 310 315 320
 Ser Leu Gly Glu Ser Ala Ala Gln Gly Ala Ala Gly Val Val Leu Trp
 325 330 335
 Val Ser Trp Leu Ser Thr Ser Thr Lys Glu Ser Cys Gln Ala Ile Lys
 340 345 350
 Glu Tyr Val Asp Thr Thr Leu Gly Pro Ser Ile Leu Asn Val Thr Ser
 355 360 365
 Gly Ala Arg Leu Cys Ser Gln Val Leu Cys Ser Gly His Gly Arg Cys
 370 375 380
 Ala Arg Arg Pro Ser Tyr Pro Lys Ala Arg Leu Ile Leu Asn Ser Thr
 385 390 395 400
 Ser Phe Ser Ile Lys Pro Thr Pro Gly Gly Gly Pro Leu Thr Leu Gln
 405 410 415
 Gly Ala Leu Ser Leu Glu Asp Arg Leu Arg Met Ala Val Glu Phe Glu
 420 425 430
 Cys Arg Cys Tyr Arg Gly Trp Arg Gly Thr Arg Cys Glu Gln Trp Gly
 435 440 445
 Met Trp
 450

<210> 11
 <211> 553
 <212> PRT
 <213> Bos taurus

<220>
 <223> PH20

<400> 11
 Met Arg Met Leu Arg Arg His His Ile Ser Phe Arg Ser Phe Ala Gly
 1 5 10 15
 Ser Ser Gly Thr Pro Gln Ala Val Phe Thr Phe Leu Leu Leu Pro Cys
 20 25 30
 Cys Leu Ala Leu Asp Phe Arg Ala Pro Pro Leu Ile Ser Asn Thr Ser
 35 40 45
 Phe Leu Trp Ala Trp Asn Ala Pro Val Glu Arg Cys Val Asn Arg Arg
 50 55 60
 Phe Gln Leu Pro Pro Asp Leu Arg Leu Phe Ser Val Lys Gly Ser Pro
 65 70 75 80
 Gln Lys Ser Ala Thr Gly Gln Phe Ile Thr Leu Phe Tyr Ala Asp Arg
 85 90 95
 Leu Gly Tyr Tyr Pro His Ile Asp Glu Lys Thr Gly Lys Thr Val Phe
 100 105 110
 Gly Gly Ile Pro Gln Leu Gly Asn Leu Lys Ser His Met Glu Lys Ala
 115 120 125
 Lys Asn Asp Ile Ala Tyr Tyr Ile Pro Asn Asp Ser Val Gly Leu Ala
 130 135 140
 Val Ile Asp Trp Glu Asn Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys
 145 150 155 160
 Pro Lys Asp Val Tyr Arg Asp Glu Ser Val Glu Leu Val Leu Gln Lys
 165 170 175
 Asn Pro Gln Leu Ser Phe Pro Glu Ala Ser Lys Ile Ala Lys Val Asp
 180 185 190

3066SEQ PC1.TXT

Phe Glu Thr Ala Gly Lys Ser Phe Met Gln Glu Thr Leu Lys Leu Gly
 195 200 205
 Lys Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp
 210 215 220
 Cys Tyr Asn His Asn His Asn Gln Pro Thr Tyr Asn Gly Asn Cys Pro
 225 230 235
 Asp Val Glu Lys Arg Arg Asn Asp Asp Leu Glu Trp Leu Trp Lys Glu
 245 250 255
 Ser Thr Ala Leu Phe Pro Ser Val Tyr Leu Asn Ile Arg Leu Lys Ser
 260 265 270
 Thr Gln Asn Ala Ala Leu Tyr Val Arg Asn Arg Val Gln Glu Ala Ile
 275 280 285
 Arg Leu Ser Lys Ile Ala Ser Val Glu Ser Pro Leu Pro Val Phe Val
 290 295 300
 Tyr Ala Arg Pro Val Phe Thr Asp Gly Ser Ser Thr Tyr Leu Ser Gln
 305 310 315 320
 Gly Asp Leu Val Asn Ser Val Gly Glu Ile Val Ser Leu Gly Ala Ser
 325 330 335
 Gly Ile Ile Met Trp Gly Ser Leu Asn Leu Ser Leu Ser Met Gln Ser
 340 345 350
 Cys Met Asn Leu Gly Thr Tyr Leu Asn Thr Thr Leu Asn Pro Tyr Ile
 355 360 365
 Ile Asn Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys His
 370 375 380
 Asn Glu Gly Val Cys Thr Arg Lys His Trp Asn Ser Ser Asp Tyr Leu
 385 390 395 400
 His Leu Asn Pro Met Asn Phe Ala Ile Gln Thr Gly Glu Gly Gly Lys
 405 410 415
 Tyr Thr Val Pro Gly Thr Val Thr Leu Glu Asp Leu Gln Lys Phe Ser
 420 425 430
 Asp Thr Phe Tyr Cys Ser Cys Tyr Ala Asn Ile His Cys Lys Lys Arg
 435 440 445
 Val Asp Ile Lys Asn Val His Ser Val Asn Val Cys Met Ala Glu Asp
 450 455 460
 Ile Cys Ile Asp Ser Pro Val Lys Leu Gln Pro Ser Asp His Ser Ser
 465 470 475 480
 Ser Gln Glu Ala Ser Thr Thr Thr Phe Ser Ser Ile Ser Pro Ser Thr
 485 490 495
 Thr Thr Ala Thr Val Ser Pro Cys Thr Pro Glu Lys His Ser Pro Glu
 500 505 510
 Cys Leu Lys Val Arg Cys Ser Glu Val Ile Pro Asn Val Thr Gln Lys
 515 520 525
 Ala Cys Gln Ser Val Lys Leu Lys Asn Ile Ser Tyr Gln Ser Pro Ile
 530 535 540
 Gln Asn Ile Lys Asn Gln Thr Thr Tyr
 545 550

<210> 12

<211> 331

<212> PRT

<213> *Vesputula vulgaris*

<220>

<223> hyaluronidase A

<400> 12

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

3066SEQ PC1.TXT

Ile	Glu	Leu	Glu	Ala	Ser	Lys	Arg	Phe	Glu	Lys	Tyr	Ala	Arg	Phe	Phe
145					150					155					160
Met	Glu	Glu	Thr	Leu	Lys	Leu	Ala	Lys	Lys	Thr	Arg	Lys	Gln	Ala	Asp
				165					170						175
Trp	Gly	Tyr	Tyr	Gly	Tyr	Pro	Tyr	Cys	Phe	Asn	Met	Ser	Pro	Asn	Asn
			180					185					190		
Leu	Val	Pro	Glu	Cys	Asp	Val	Thr	Ala	Met	His	Glu	Asn	Asp	Lys	Met
		195					200					205			
Ser	Trp	Leu	Phe	Asn	Asn	Gln	Asn	Val	Leu	Leu	Pro	Ser	Val	Tyr	Val
	210				215						220				
Arg	Gln	Glu	Leu	Thr	Pro	Asp	Gln	Arg	Ile	Gly	Leu	Val	Gln	Gly	Arg
225					230					235					240
Val	Lys	Glu	Ala	Val	Arg	Ile	Ser	Asn	Asn	Leu	Lys	His	Ser	Pro	Lys
				245					250					255	
Val	Leu	Ser	Tyr	Trp	Trp	Tyr	Val	Tyr	Gln	Asp	Glu	Thr	Asn	Thr	Phe
			260					265					270		
Leu	Thr	Glu	Thr	Asp	Val	Lys	Lys	Thr	Phe	Gln	Glu	Ile	Val	Ile	Asn
		275					280					285			
Gly	Gly	Asp	Gly	Ile	Ile	Ile	Trp	Gly	Ser	Ser	Ser	Asp	Val	Asn	Ser
	290					295					300				
Leu	Ser	Lys	Cys	Lys	Arg	Leu	Gln	Asp	Tyr	Leu	Leu	Thr	Val	Leu	Gly
305					310					315					320
Pro	Ile	Ala	Ile	Asn	Val	Thr	Glu	Ala	Val	Asn					
				325					330						

<210> 13
 <211> 340
 <212> PRT
 <213> Vesputa vulgaris

<220>
 <223> hyaluronidase B

<400> 13

Asp	Arg	Thr	Ile	Trp	Pro	Lys	Lys	Gly	Phe	Ser	Ile	Tyr	Trp	Asn	Ile
1				5					10					15	
Pro	Thr	His	Phe	Cys	His	Asn	Phe	Gly	Val	Tyr	Phe	Lys	Glu	Leu	Lys
			20					25					30		
Gln	Phe	Asn	Ile	Lys	Tyr	Asn	Ser	Met	Asn	Asn	Phe	Arg	Gly	Glu	Thr
		35					40					45			
Ile	Ser	Leu	Phe	Tyr	Asp	Pro	Gly	Asn	Phe	Pro	Ser	Met	Val	Leu	Leu
	50					55				60					
Lys	Asn	Gly	Thr	Tyr	Glu	Ile	Arg	Asn	Glu	Gly	Val	Pro	Gln	Lys	Gly
65					70					75					80
Asn	Leu	Thr	Ile	His	Leu	Glu	Gln	Phe	Thr	Lys	Glu	Leu	Asp	Glu	Ile
			85						90					95	
Tyr	Pro	Lys	Lys	Ile	Ala	Gly	Gly	Ile	Gly	Val	Ile	His	Phe	His	Asn
			100					105					110		
Trp	Arg	Pro	Ile	Phe	Arg	Arg	Asn	Val	Asp	Asn	Leu	Lys	Ile	Asn	Lys
		115					120					125			
Asp	Ile	Ser	Ile	Asp	Leu	Val	Arg	Lys	Glu	His	Pro	Lys	Trp	Asp	Lys
	130					135					140				
Ser	Met	Ile	Glu	Lys	Glu	Ala	Ser	Asn	Arg	Phe	Glu	Thr	Ser	Ala	Lys
145					150					155					160
Ile	Phe	Met	Glu	Lys	Thr	Leu	Lys	Leu	Ala	Lys	Glu	Ile	Arg	Lys	Lys
			165						170					175	
Thr	Glu	Trp	Gly	Tyr	His	Gly	Tyr	Pro	His	Cys	Leu	Ser	Gly	Ser	Thr
			180					185					190		
Asp	Lys	Pro	Ser	Phe	Asp	Cys	Asp	Ala	Leu	Ser	Met	Ser	Glu	Asn	Asp
		195					200					205			
Lys	Met	Ser	Trp	Leu	Phe	Asn	Asn	Gln	Asn	Val	Leu	Leu	Pro	Ser	Ile
	210					215					220				
Tyr	Leu	Lys	Asn	Val	Leu	Lys	Pro	Asp	Glu	Lys	Ile	His	Leu	Val	Gln
225					230					235					240
Glu	Arg	Leu	Lys	Glu	Ala	Ile	Arg	Ile	Ser	Lys	Asn	Phe	Lys	His	Leu
			245						250					255	
Pro	Lys	Val	Leu	Pro	Tyr	Trp	Trp	Tyr	Thr	Tyr	Gln	Asp	Lys	Glu	Ser
		260						265					270		
Ile	Phe	Leu	Thr	Glu	Ala	Asp	Val	Lys	Asn	Thr	Phe	Lys	Glu	Ile	Leu
		275					280					285			
Thr	Asn	Gly	Ala	Asp	Gly	Ile	Ile	Ile	Trp	Gly	Val	Ser	Tyr	Glu	Leu
	290					295					300				
Thr	Asp	Arg	Lys	Arg	Cys	Glu	Lys	Leu	Lys	Glu	Tyr	Leu	Met	Lys	Ile
305					310					315					320

Leu Gly Pro Ile Ala Phe Lys Val Thr Lys Ala Val Lys Glu Asn Thr
 325 330 335
 Pro Leu Asn Phe
 340

<210> 14
 <211> 382
 <212> PRT
 <213> *Apis mellifera*

<220>
 <223> hyaluronidase

<400> 14
 Met Ser Arg Pro Leu Val Ile Thr Glu Gly Met Met Ile Gly Val Leu
 1 5 10 15
 Leu Met Leu Ala Pro Ile Asn Ala Leu Leu Gly Phe Val Gln Ser
 20 25 30
 Thr Pro Asp Asn Asn Lys Thr Val Arg Glu Phe Asn Val Tyr Trp Asn
 35 40 45
 Val Pro Thr Phe Met Cys His Lys Tyr Gly Leu Arg Phe Glu Glu Val
 50 55 60
 Ser Glu Lys Tyr Gly Ile Leu Gln Asn Trp Met Asp Lys Phe Arg Gly
 65 70 75 80
 Glu Glu Ile Ala Ile Leu Tyr Asp Pro Gly Met Phe Pro Ala Leu Leu
 85 90 95
 Lys Asp Pro Asn Gly Asn Val Val Ala Arg Asn Gly Gly Val Pro Gln
 100 105 110
 Leu Gly Asn Leu Thr Lys His Leu Gln Val Phe Arg Asp His Leu Ile
 115 120 125
 Asn Gln Ile Pro Asp Lys Ser Phe Pro Gly Val Gly Val Ile Asp Phe
 130 135 140
 Glu Ser Trp Arg Pro Ile Phe Arg Gln Asn Trp Ala Ser Leu Gln Pro
 145 150 155 160
 Tyr Lys Lys Leu Ser Val Glu Val Val Arg Arg Glu His Pro Phe Trp
 165 170 175
 Asp Asp Gln Arg Val Glu Gln Glu Ala Lys Arg Arg Phe Glu Lys Tyr
 180 185 190
 Gly Gln Leu Phe Met Glu Glu Thr Leu Lys Ala Ala Lys Arg Met Arg
 195 200 205
 Pro Ala Ala Asn Trp Gly Tyr Tyr Ala Tyr Pro Tyr Cys Tyr Asn Leu
 210 215 220
 Thr Pro Asn Gln Pro Ser Ala Gln Cys Glu Ala Thr Thr Met Gln Glu
 225 230 235 240
 Asn Asp Lys Met Ser Trp Leu Phe Glu Ser Glu Asp Val Leu Leu Pro
 245 250 255

 Ser Val Tyr Leu Arg Trp Asn Leu Thr Ser Gly Glu Arg Val Gly Leu
 260 265 270
 Val Gly Gly Arg Val Lys Glu Ala Leu Arg Ile Ala Arg Gln Met Thr
 275 280 285
 Thr Ser Arg Lys Lys Val Leu Pro Tyr Tyr Trp Tyr Lys Tyr Gln Asp
 290 295 300
 Arg Arg Asp Thr Asp Leu Ser Arg Ala Asp Leu Glu Ala Thr Leu Arg
 305 310 315 320
 Lys Ile Thr Asp Leu Gly Ala Asp Gly Phe Ile Ile Trp Gly Ser Ser
 325 330 335
 Asp Asp Ile Asn Thr Lys Ala Lys Cys Leu Gln Phe Arg Glu Tyr Leu
 340 345 350
 Asn Asn Glu Leu Gly Pro Ala Val Lys Arg Ile Ala Leu Asn Asn Asn
 355 360 365
 Ala Asn Asp Arg Leu Thr Val Asp Val Ser Val Asp Gln Val
 370 375 380

<210> 15
 <211> 331
 <212> PRT
 <213> *Dolichovespula maculata*

<220>
 <223> hyaluronidase

<400> 15
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 Page 13

3066SEQ PC1.TXT

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				20					25						30			
Asn	Ile	Lys	His	Asn	Ser	Lys	Asp	Asp	Asp	Phe	Gln	Gly	Asp	Lys	Ile	Ser		
		35					40						45					
Ile	Phe	Tyr	Asp	Pro	Gly	Glu	Phe	Pro	Ala	Leu	Leu	Pro	Leu	Lys	Glu			
		50				55						60						
Gly	Asn	Tyr	Lys	Ile	Arg	Asn	Gly	Gly	Val	Pro	Gln	Glu	Gly	Asn	Ile			
65				70					75						80			
Thr	Ile	His	Leu	Gln	Arg	Phe	Ile	Glu	Asn	Leu	Asp	Lys	Thr	Tyr	Pro			
				85					90					95				
Asn	Arg	Asn	Phe	Asn	Gly	Ile	Gly	Val	Ile	Asp	Phe	Glu	Arg	Trp	Arg			
			100					105					110					
Pro	Ile	Phe	Arg	Gln	Asn	Trp	Gly	Asn	Met	Met	Ile	His	Lys	Lys	Phe			
		115					120					125						
Ser	Ile	Asp	Leu	Val	Arg	Asn	Glu	His	Pro	Phe	Trp	Asp	Lys	Lys	Met			
	130					135					140							
Ile	Glu	Leu	Glu	Ala	Ser	Lys	Arg	Phe	Glu	Lys	Tyr	Ala	Arg	Leu	Phe			
145				150					155					160				
Met	Glu	Glu	Thr	Leu	Lys	Leu	Ala	Lys	Lys	Thr	Arg	Lys	Gln	Ala	Asp			
			165						170					175				
Trp	Gly	Tyr	Tyr	Gly	Tyr	Pro	Tyr	Cys	Phe	Asn	Met	Ser	Pro	Asn	Asn			
		180						185					190					
Leu	Val	Pro	Asp	Cys	Asp	Ala	Thr	Ala	Met	Leu	Glu	Asn	Asp	Lys	Met			
		195				200						205						
Ser	Trp	Leu	Phe	Asn	Asn	Gln	Asn	Val	Leu	Leu	Pro	Ser	Val	Tyr	Ile			
	210			215				220										
Arg	His	Glu	Leu	Thr	Pro	Asp	Gln	Arg	Val	Gly	Leu	Val	Gln	Gly	Arg			
225				230					235					240				
Val	Lys	Glu	Ala	Val	Arg	Ile	Ser	Asn	Asn	Leu	Lys	His	Ser	Pro	Lys			
			245					250					255					
Val	Leu	Ser	Tyr	Trp	Trp	Tyr	Val	Tyr	Gln	Asp	Asp	Thr	Asn	Thr	Phe			
		260					265					270						
Leu	Thr	Glu	Thr	Asp	Val	Lys	Lys	Thr	Phe	Gln	Glu	Ile	Ala	Ile	Asn			
	275					280						285						
Gly	Gly	Asp	Gly	Ile	Ile	Ile	Trp	Gly	Ser	Ser	Ser	Asp	Val	Asn	Ser			
	290				295						300							
Leu	Ser	Lys	Cys	Lys	Arg	Leu	Arg	Glu	Tyr	Leu	Leu	Thr	Val	Leu	Gly			
305				310					315					320				
Pro	Ile	Thr	Val	Asn	Val	Thr	Glu	Thr	Val	Asn								
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<211> 367

<212> PRT

<213> Polistes annularis

<220>

<223> hyaluronidase

<400> 16

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

3066SEQ PC1.TXT

Thr	Leu	Lys	180	Leu	Ala	Lys	Lys	Thr	185	Arg	Lys	Arg	Ala	Lys	190	Trp	Gly	Tyr
Tyr	Gly	195	Phe	Pro	Tyr	Cys	Tyr	200	Asn	Val	Thr	Pro	Asn	205	Asn	Pro	Gly	Pro
Asp	210	Cys	Asp	Ala	Lys	Ala	Thr	215	Ile	Glu	Asn	Asp	220	Arg	Leu	Ser	Trp	Met
225	Tyr	Asn	Asn	Gln	Glu	Ile	Leu	230	Phe	Pro	Ser	Val	235	Tyr	Val	Arg	His	Glu
Gln	Lys	Pro	Glu	Glu	Arg	Val	Tyr	245	Leu	Val	Gln	Gly	250	Arg	Ile	Lys	Glu	
Ala	Val	Arg	260	Ile	Ser	Asn	Asn	265	Leu	Glu	His	Ser	Pro	270	Ser	Val	Leu	Ala
Tyr	Trp	Trp	Tyr	Val	Tyr	Gln	Asp	275	Lys	Met	Asp	Ile	Tyr	285	Leu	Ser	Glu	
Thr	305	Asp	Val	Glu	Lys	Thr	Phe	295	Gln	Glu	Ile	Val	Thr	300	Asn	Gly	Gly	Asp
Gly	Ile	Ile	Ile	Trp	Gly	Ser	Ser	310	Ser	Ser	Asp	Val	Asn	315	Ser	Leu	Ser	Lys
Cys	Lys	Arg	Leu	Arg	Glu	Tyr	Leu	325	Leu	Leu	Asn	Thr	Leu	330	Gly	Pro	Phe	Ala
Val	Asn	Val	340	Thr	Glu	Thr	Val	345	Asn	Gly	Arg	Ser	Ser	350	Leu	Asn	Phe	
		355						360						365				

<210> 17
 <211> 462
 <212> PRT
 <213> Mus musculus

<220>
 <223> hyaluronidase

Met	Leu	Gly	Leu	Thr	Gln	His	Ala	Gln	Lys	Val	Trp	Arg	Met	Lys	Pro
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			20						25					30	
Leu	Leu	Arg	Ile	Ser	Thr	Leu	Phe	Leu	Thr	Leu	Leu	Glu	Leu	Ala	Gln
		35					40					45			
Val	Cys	Arg	Gly	Ser	Val	Val	Ser	Asn	Arg	Pro	Phe	Ile	Thr	Val	Trp
	50					55					60				
Asn	Gly	Asp	Thr	His	Trp	Cys	Leu	Thr	Glu	Tyr	Gly	Val	Asp	Val	Asp
65					70					75					80
Val	Ser	Val	Phe	Asp	Val	Val	Ala	Asn	Lys	Glu	Gln	Ser	Phe	Gln	Gly
			85						90					95	
Ser	Asn	Met	Thr	Ile	Phe	Tyr	Arg	Glu	Glu	Leu	Gly	Thr	Tyr	Pro	Tyr
			100					105					110		
Tyr	Thr	Pro	Thr	Gly	Glu	Pro	Val	Phe	Gly	Gly	Leu	Pro	Gln	Asn	Ala
		115					120					125			
Ser	Leu	Val	Thr	His	Leu	Ala	His	Thr	Phe	Gln	Asp	Ile	Lys	Ala	Ala
	130					135					140				
Met	Pro	Glu	Pro	Asp	Phe	Ser	Gly	Leu	Ala	Val	Ile	Asp	Trp	Glu	Ala
145					150					155					160
Trp	Arg	Pro	Arg	Trp	Ala	Phe	Asn	Trp	Asp	Ser	Lys	Asp	Ile	Tyr	Arg
			165						170					175	
Gln	Arg	Ser	Met	Glu	Leu	Val	Gln	Ala	Glu	His	Pro	Asp	Trp	Pro	Glu
			180					185					190		
Thr	Leu	Val	Glu	Ala	Ala	Ala	Lys	Asn	Gln	Phe	Gln	Glu	Ala	Ala	Glu
	195						200					205			
Ala	Trp	Met	Ala	Gly	Thr	Leu	Gln	Leu	Gly	Gln	Val	Leu	Arg	Pro	Arg
	210					215					220				
Gly	Leu	Trp	Gly	Tyr	Tyr	Gly	Phe	Pro	Asp	Cys	Tyr	Asn	Asn	Asp	Phe
225					230					235					240
Leu	Ser	Leu	Asn	Tyr	Thr	Gly	Gln	Cys	Pro	Val	Phe	Val	Arg	Asp	Gln
			245						250					255	
Asn	Asp	Gln	Leu	Gly	Trp	Leu	Trp	Asn	Gln	Ser	Tyr	Ala	Leu	Tyr	Pro
			260					265					270		
Ser	Ile	Tyr	Leu	Pro	Ala	Ala	Leu	Met	Gly	Thr	Gly	Lys	Ser	Gln	Met
	275						280					285			
Tyr	Val	Arg	His	Arg	Val	Gln	Glu	Ala	Leu	Arg	Val	Ala	Ile	Val	Ser
	290					295					300				
Arg	Asp	Pro	His	Val	Pro	Val	Met	Pro	Tyr	Val	Gln	Ile	Phe	Tyr	Glu
305					310						315				320
Met	Thr	Asp	Tyr	Leu	Leu	Pro	Leu	Glu	Glu	Leu	Glu	His	Ser	Leu	Gly

				325					330					335			
Glu	Ser	Ala	Ala	Gln	Gly	Val	Ala	Gly	Ala	Val	Leu	Trp	Leu	Ser	Ser		
			340					345					350				
Asp	Lys	Thr	Ser	Thr	Lys	Glu	Ser	Cys	Gln	Ala	Ile	Lys	Ala	Tyr	Met		
		355					360					365					
Asp	Ser	Thr	Leu	Gly	Pro	Phe	Ile	Val	Asn	Val	Thr	Ser	Ala	Ala	Leu		
		370				375					380						
Leu	Cys	Ser	Glu	Ala	Leu	Cys	Ser	Gly	His	Gly	Arg	Cys	Val	Arg	His		
385				390				395							400		
Pro	Ser	Tyr	Pro	Glu	Ala	Leu	Leu	Thr	Leu	Asn	Pro	Ala	Ser	Phe	Ser		
			405					410						415			
Ile	Glu	Leu	Thr	His	Asp	Gly	Arg	Pro	Ser	Leu	Lys	Gly	Thr	Leu			
			420					425				430					
Ser	Leu	Lys	Asp	Arg	Ala	Gln	Met	Ala	Met	Lys	Phe	Arg	Cys	Arg	Cys		
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Tyr	Arg	Gly	Trp	Arg	Gly	Lys	Trp	Cys	Asp	Lys	Arg	Gly	Met				
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<210> 18

<211> 473

<212> PRT

<213> Mus musculus

<220>

<223> Hyaluronidase 2

<400> 18

Met	Arg	Ala	Gly	Leu	Gly	Pro	Ile	Ile	Thr	Leu	Ala	Leu	Val	Leu	Glu		
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			20					25					30				
Gly	Arg	Pro	Phe	Val	Val	Ala	Trp	Asn	Val	Pro	Thr	Gln	Glu	Cys	Ala		
		35				40						45					
Pro	Arg	His	Lys	Val	Pro	Leu	Asp	Leu	Arg	Ala	Phe	Asp	Val	Lys	Ala		
		50				55					60						
Thr	Pro	Asn	Glu	Gly	Phe	Phe	Asn	Gln	Asn	Ile	Thr	Thr	Phe	Tyr	Tyr		
65				70						75					80		
Asp	Arg	Leu	Gly	Leu	Tyr	Pro	Arg	Phe	Asp	Ala	Ala	Gly	Thr	Ser	Val		
			85					90						95			
His	Gly	Gly	Val	Pro	Gln	Asn	Gly	Ser	Leu	Cys	Ala	His	Leu	Pro	Met		
			100					105					110				
Leu	Lys	Glu	Ser	Val	Glu	Arg	Tyr	Ile	Gln	Thr	Gln	Glu	Pro	Gly	Gly		
		115					120					125					
Leu	Ala	Val	Ile	Asp	Trp	Glu	Glu	Trp	Arg	Pro	Val	Trp	Val	Arg	Asn		
		130				135					140						
Trp	Gln	Glu	Lys	Asp	Val	Tyr	Arg	Gln	Ser	Ser	Arg	Gln	Leu	Val	Ala		
145				150						155					160		
Ser	Arg	His	Pro	Asp	Trp	Pro	Ser	Asp	Arg	Val	Met	Lys	Gln	Ala	Gln		
				165					170					175			
Tyr	Glu	Phe	Glu	Phe	Ala	Ala	Arg	Gln	Phe	Met	Leu	Asn	Thr	Leu	Arg		
		180						185					190				
Tyr	Val	Lys	Ala	Val	Arg	Pro	Gln	His	Leu	Trp	Gly	Phe	Tyr	Leu	Phe		
		195					200					205					
Pro	Asp	Cys	Tyr	Asn	His	Asp	Tyr	Val	Gln	Asn	Trp	Glu	Ser	Tyr	Thr		
		210				215					220						
Gly	Arg	Cys	Pro	Asp	Val	Glu	Val	Ala	Arg	Asn	Asp	Gln	Leu	Ala	Trp		
225				230					235					240			
Leu	Trp	Ala	Glu	Ser	Thr	Ala	Leu	Phe	Pro	Ser	Val	Tyr	Leu	Asp	Glu		
			245					250					255				
Thr	Leu	Ala	Ser	Ser	Val	His	Ser	Arg	Asn	Phe	Val	Ser	Phe	Arg	Val		
		260						265					270				
Arg	Glu	Ala	Leu	Arg	Val	Ala	His	Thr	His	His	Ala	Asn	His	Ala	Leu		
		275					280					285					
Pro	Val	Tyr	Val	Phe	Thr	Arg	Pro	Thr	Tyr	Thr	Arg	Gly	Leu	Thr	Gly		
		290				295					300						
Leu	Ser	Gln	Val	Asp	Leu	Ile	Ser	Thr	Ile	Gly	Glu	Ser	Ala	Ala	Leu		
305				310						315					320		
Gly	Ser	Ala	Gly	Val	Ile	Phe	Trp	Gly	Asp	Ser	Glu	Asp	Ala	Ser	Ser		
			325					330					335				
Met	Glu	Thr	Cys	Gln	Tyr	Leu	Lys	Asn	Tyr	Leu	Thr	Gln	Leu	Leu	Val		
			340					345					350				
Pro	Tyr	Ile	Val	Asn	Val	Ser	Trp	Ala	Thr	Gln	Tyr	Cys	Ser	Trp	Thr		
		355				360					365						
Gln	Cys	His	Gly	His	Gly	Arg	Cys	Val	Arg	Arg	Asn	Pro	Ser	Ala	Asn		

370	375	380															
Thr	Phe	Leu	His	Leu	Asn	Ala	Ser	Ser	Phe	Arg	Leu	Val	Pro	Gly	His		
385					390					395					400		
Thr	Pro	Ser	Glu	Pro	Gln	Leu	Arg	Pro	Glu	Gly	Gln	Leu	Ser	Glu	Ala		
				405					410					415			
Asp	Leu	Asn	Tyr	Leu	Gln	Lys	His	Phe	Arg	Cys	Gln	Cys	Tyr	Leu	Gly		
			420					425					430				
Trp	Gly	Gly	Glu	Gln	Cys	Gln	Arg	Asn	Tyr	Lys	Gly	Ala	Ala	Gly	Asn		
		435					440					445					
Ala	Ser	Arg	Ala	Trp	Ala	Gly	Ser	His	Leu	Thr	Ser	Leu	Leu	Gly	Leu		
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Val	Ala	Val	Ala	Leu	Thr	Trp	Thr	Leu									
465					470												

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 <212> PRT
 <213> Mus musculus

<220>
 <223> hyaluronidase 3

<400> 19																	
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Leu	Met	His	Gly	Gln	Ala	Leu	Leu	Gln	Val	Pro	Glu	His	Pro	Phe	Ser		
			20					25					30				
Val	Val	Trp	Asn	Val	Pro	Ser	Ala	Arg	Cys	Lys	Ala	His	Phe	Gly	Val		
		35					40					45					
His	Leu	Pro	Leu	Asp	Ala	Leu	Gly	Ile	Val	Ala	Asn	His	Gly	Gln	His		
	50					55					60						
Phe	His	Gly	Gln	Asn	Ile	Ser	Ile	Phe	Tyr	Lys	Asn	Gln	Phe	Gly	Leu		
65				70					75					80			
Tyr	Pro	Tyr	Phe	Gly	Pro	Arg	Gly	Thr	Ala	His	Asn	Gly	Gly	Ile	Pro		
				85					90					95			
Gln	Ala	Val	Ser	Leu	Asp	His	His	Leu	Ala	Arg	Ala	Ala	His	Gln	Ile		
			100					105					110				
Leu	His	Ser	Leu	Gly	Ser	Ser	Phe	Ala	Gly	Leu	Ala	Val	Leu	Asp	Trp		
		115					120					125					
Glu	Glu	Trp	Tyr	Pro	Leu	Trp	Ala	Gly	Asn	Trp	Gly	Pro	His	Arg	Gln		
	130					135					140						
Val	Tyr	Leu	Ala	Ala	Ser	Trp	Val	Trp	Thr	Gln	Gln	Met	Phe	Pro	Gly		
145					150					155				160			
Leu	Asp	Pro	Gln	Glu	Gln	Leu	His	Lys	Ala	His	Thr	Ser	Phe	Glu	Gln		
				165					170					175			
Ala	Ala	Arg	Ala	Leu	Met	Glu	Tyr	Thr	Leu	Gln	Leu	Gly	Arg	Thr	Leu		
			180					185					190				
Arg	Pro	Ser	Gly	Leu	Trp	Gly	Phe	Tyr	Arg	Tyr	Pro	Ala	Cys	Gly	Asn		
		195					200					205					
Gly	Trp	His	Lys	Met	Ala	Ser	Asn	Tyr	Thr	Gly	His	Cys	His	Ala	Ala		
	210					215					220						
Ile	Thr	Thr	Gln	Asn	Thr	Gln	Leu	Arg	Trp	Leu	Trp	Ala	Ala	Ser	Ser		
225				230						235				240			
Ala	Leu	Phe	Pro	Ser	Ile	Tyr	Leu	Pro	Pro	Arg	Leu	Pro	Leu	Ala	Tyr		
				245					250					255			
Arg	Gln	Ala	Phe	Val	Arg	His	Arg	Leu	Glu	Glu	Ala	Phe	Arg	Val	Ala		
		260						265					270				
Leu	Leu	Glu	His	Ser	His	Pro	Leu	Pro	Val	Leu	Ala	Tyr	Ser	Arg	Leu		
		275					280					285					
Thr	His	Arg	Ser	Ser	Gly	Arg	Phe	Leu	Ser	Leu	Asp	Asp	Leu	Met	Gln		
	290					295					300						
Thr	Ile	Gly	Val	Ser	Ala	Ala	Leu	Gly	Thr	Ala	Gly	Val	Val	Leu	Trp		
305					310					315				320			
Gly	Asp	Leu	Ser	Phe	Ser	Ser	Ser	Glu	Glu	Lys	Cys	Trp	Arg	Leu	His		
				325					330					335			
Asp	Tyr	Leu	Val	Gly	Thr	Leu	Gly	Pro	Tyr	Val	Ile	Asn	Val	Thr	Lys		
			340					345					350				
Ala	Asp	Met	Ala	Cys	Ser	His	Gln	Arg	Cys	His	Gly	His	Gly	Arg	Cys		
		355					360					365					
Ala	Arg	Lys	Asp	Pro	Gly	Gln	Met	Glu	Ala	Phe	Leu	His	Leu	Gln	Pro		
	370					375					380						
Asp	Asp	Ser	Leu	Gly	Ala	Trp	Asn	Ser	Phe	Arg	Cys	His	Cys	Tyr	Ser		
385					390					395				400			
Gly	Trp	Ala	Gly	Pro	Thr	Cys	Leu	Glu	Pro	Lys	Pro						

<210> 20
 <211> 435
 <212> PRT
 <213> Sus scrofa

<220>
 <223> hyaluronidase

<400> 20

Met	Ala	Ala	His	Leu	Leu	Pro	Ile	Cys	Thr	Leu	Phe	Leu	Asn	Leu	Leu
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Ser	Val	Ala	Gln	Gly	Ser	Arg	Asp	Pro	Val	Val	Leu	Asn	Arg	Pro	Phe
		20					25						30		
Thr	Thr	Ile	Trp	Asn	Ala	Asn	Thr	Gln	Trp	Cys	Leu	Lys	Arg	His	Gly
		35					40					45			
Val	Asp	Val	Asp	Val	Ser	Val	Phe	Glu	Val	Val	Val	Asn	Pro	Gly	Gln
	50					55					60				
Thr	Phe	Arg	Gly	Pro	Asn	Met	Thr	Ile	Phe	Tyr	Ser	Ser	Gln	Leu	Gly
65					70				75					80	
Thr	Tyr	Pro	Tyr	Tyr	Thr	Ser	Ala	Gly	Glu	Pro	Val	Phe	Gly	Gly	Leu
				85					90					95	
Pro	Gln	Asn	Ala	Ser	Leu	Asp	Val	His	Leu	Asn	Arg	Thr	Phe	Lys	Asp
			100					105					110		
Ile	Leu	Ala	Ala	Met	Pro	Glu	Ser	Asn	Phe	Ser	Gly	Leu	Ala	Val	Ile
		115					120					125			
Asp	Trp	Glu	Ala	Trp	Arg	Pro	Arg	Trp	Ala	Phe	Asn	Trp	Asp	Ala	Lys
	130					135					140				
Asp	Ile	Tyr	Arg	Gln	Arg	Ser	Arg	Ala	Leu	Val	Gln	Lys	Gln	His	Pro
145					150					155					160
Asp	Trp	Pro	Ala	Pro	Trp	Val	Glu	Ala	Ala	Ala	Gln	Asp	Gln	Phe	Gln
				165				170						175	
Glu	Ala	Ala	Gln	Thr	Trp	Met	Ala	Gly	Thr	Leu	Lys	Leu	Gly	Gln	Thr
			180					185					190		
Leu	Arg	Pro	His	Gly	Leu	Trp	Gly	Phe	Tyr	Gly	Phe	Pro	Asp	Cys	Tyr
		195					200					205			
Asn	Tyr	Asp	Phe	Gln	Ser	Ser	Asn	Tyr	Thr	Gly	Gln	Cys	Pro	Pro	Gly
	210					215					220				
Val	Ser	Ala	Gln	Asn	Asp	Gln	Leu	Gly	Trp	Leu	Trp	Gly	Gln	Ser	Arg
225					230					235					240
Ala	Leu	Tyr	Pro	Ser	Ile	Tyr	Leu	Pro	Ser	Ala	Leu	Glu	Gly	Thr	Asn
				245					250					255	
Lys	Thr	Gln	Leu	Tyr	Val	Gln	His	Arg	Val	Asn	Glu	Ala	Phe	Arg	Val
			260					265					270		
Ala	Ala	Ala	Ala	Gly	Asp	Pro	Asn	Leu	Pro	Val	Leu	Pro	Tyr	Ala	Gln
		275					280					285			
Ile	Phe	His	Asp	Met	Thr	Asn	Arg	Leu	Leu	Ser	Arg	Glu	Glu	Leu	Glu
	290					295					300				
His	Ser	Leu	Gly	Glu	Ser	Ala	Ala	Gln	Gly	Ala	Ala	Gly	Val	Val	Leu
305					310					315					320
Trp	Val	Ser	Trp	Glu	Asn	Thr	Arg	Thr	Lys	Glu	Ser	Cys	Gln	Ser	Ile
				325					330					335	
Lys	Glu	Tyr	Val	Asp	Thr	Thr	Leu	Gly	Pro	Phe	Ile	Leu	Asn	Val	Thr
			340					345					350		
Ser	Gly	Ala	Leu	Leu	Cys	Ser	Gln	Ala	Val	Cys	Ser	Gly	His	Gly	Arg
		355					360					365			
Cys	Val	Arg	Arg	Pro	Ser	His	Thr	Glu	Ala	Leu	Pro	Ile	Leu	Asn	Pro
	370					375					380				
Ser	Ser	Phe	Ser	Ile	Lys	Pro	Thr	Pro	Gly	Gly	Gly	Pro	Leu	Thr	Leu
385					390					395					400
Gln	Gly	Ala	Leu	Ser	Leu	Lys	Asp	Arg	Val	Gln	Met	Ala	Glu	Glu	Phe
				405					410					415	
Gln	Cys	Arg	Cys	Tyr	Pro	Gly	Trp	Arg	Gly	Thr	Trp	Cys	Glu	Gln	Gln
			420					425					430		
Gly	Thr	Arg													
	435														

<210> 21
 <211> 419
 <212> PRT
 <213> Sus scrofa

<220>

<223> hyaluronidase 3

<400> 21

Met Thr Met Gln Leu Gly Leu Ala Leu Val Leu Gly Val Ala Met Cys
 1 5 10 15
 Leu Gly Cys Gly Gln Pro Leu Leu Arg Ala Pro Glu Arg Pro Phe Cys
 20 25 30
 Val Leu Trp Asn Val Pro Ser Ala Arg Cys Lys Ala Arg Phe Gly Val
 35 40 45
 His Leu Pro Leu Glu Ala Leu Gly Ile Thr Ala Asn His Gly Gln Arg
 50 55 60
 Phe His Gly Gln Asn Ile Thr Ile Phe Tyr Lys Ser Gln Leu Gly Leu
 65 70 75 80
 Tyr Pro Tyr Phe Gly Pro Arg Gly Thr Ala His Asn Gly Gly Ile Pro
 85 90 95
 Gln Ala Val Ser Leu Asp His His Leu Ala Arg Ala Ala Tyr Gln Ile
 100 105 110
 His Arg Ser Leu Arg Pro Gly Phe Thr Gly Leu Ala Val Leu Asp Trp
 115 120 125
 Glu Glu Trp Cys Pro Leu Trp Ala Gly Asn Trp Gly Arg Arg Gln Ala
 130 135 140
 Tyr Gln Ala Ala Ser Cys Ala Trp Ala Gln Arg Val Tyr Pro Asn Leu
 145 150 155 160
 Asp Pro Gln Glu Gln Leu Cys Lys Ala Arg Ala Gly Phe Glu Glu Ala
 165 170 175
 Ala Arg Ala Leu Met Glu Asp Thr Leu Arg Leu Gly Arg Met Leu Arg
 180 185 190
 Pro His Gly Leu Trp Gly Phe Tyr His Tyr Pro Ala Cys Gly Asn Gly
 195 200 205
 Trp His Gly Thr Ala Ser Asn Tyr Thr Gly His Cys His Ala Ala Ala
 210 215 220
 Leu Ala Arg Asn Thr Gln Leu Tyr Trp Leu Trp Ala Ala Ser Ser Ala
 225 230 235 240
 Leu Phe Pro Ser Ile Tyr Leu Pro Pro Gly Leu Pro Pro Ala Tyr His
 245 250 255
 Gln Ala Phe Val Arg Tyr Arg Leu Glu Ala Phe Arg Val Ala Leu
 260 265 270
 Val Gly His Pro His Pro Leu Pro Val Leu Ala Tyr Ala Arg Leu Thr
 275 280 285
 His Arg Asn Ser Gly Arg Phe Leu Ser Gln Asp Glu Leu Val Gln Thr
 290 295 300
 Ile Gly Val Ser Ala Ala Leu Gly Ala Ser Gly Val Val Leu Trp Gly
 305 310 315 320
 Asp Leu Ser Phe Ser Ser Ser Glu Glu Glu Cys Trp His Leu Arg Gly
 325 330 335
 Tyr Leu Val Gly Thr Leu Gly Pro Tyr Val Ile Asn Val Thr Arg Ala
 340 345 350
 Ala Met Ala Cys Ser His Gln Arg Cys His Gly His Gly Arg Cys Ala
 355 360 365
 Trp Gln Asp Pro Gly Gln Leu Lys Val Phe Leu His Leu His Pro Gly
 370 375 380
 Gly Ser Pro Gly Ala Trp Glu Ser Phe Ser Cys Arg Cys Tyr Trp Gly
 385 390 395 400
 Trp Ala Gly Pro Thr Cys Gln Glu Pro Arg Pro Glu Leu Gly Pro Glu
 405 410 415
 Glu Ala Thr

<210> 22

<211> 449

<212> PRT

<213> Rattus norvegicus

<220>

<223> hyaluronidase 1

<400> 22

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

3066SEQ PC1.TXT

Thr Val Trp Asn Ala Asp Thr His Trp Cys Leu Lys Asp His Gly Val
 50 55 60
 Asp Val Asp Val Ser Val Phe Asp Val Val Ala Asn Lys Glu Gln Asn
 65 70 75 80
 Phe Gln Gly Pro Asn Met Thr Ile Phe Tyr Arg Glu Glu Leu Gly Thr
 85 90 95
 Tyr Pro Tyr Tyr Thr Pro Thr Gly Glu Pro Val Phe Gly Gly Leu Pro
 100 105 110
 Gln Asn Ala Ser Leu Val Thr His Leu Ala His Ala Phe Gln Asp Ile
 115 120 125
 Lys Ala Ala Met Pro Glu Pro Asp Phe Ser Gly Leu Ala Val Ile Asp
 130 135 140
 Trp Glu Ala Trp Arg Pro Arg Trp Ala Phe Asn Trp Asp Ser Lys Asp
 145 150 155 160
 Ile Tyr Gln Gln Arg Ser Met Glu Leu Val Arg Ala Glu His Pro Asp
 165 170 175
 Trp Pro Glu Thr Leu Val Glu Ala Glu Ala Gln Gly Gln Phe Gln Glu
 180 185 190
 Ala Ala Glu Ala Trp Met Ala Gly Thr Leu Gln Leu Gly Gln Val Leu
 195 200 205
 Arg Pro Arg Gly Leu Trp Gly Tyr Tyr Gly Phe Pro Asp Cys Tyr Asn
 210 215 220
 Tyr Asp Phe Leu Ser Pro Asn Tyr Thr Gly Gln Cys Ser Leu Ser Ile
 225 230 235 240
 His Asp Gln Asn Asp Gln Leu Gly Trp Leu Trp Asn Gln Ser Tyr Ala
 245 250 255
 Leu Tyr Pro Ser Ile Tyr Leu Pro Ala Ala Leu Met Gly Thr Gly Lys
 260 265 270
 Ser Gln Met Tyr Val Arg Tyr Arg Val Gln Glu Ala Phe Arg Leu Ala
 275 280 285
 Leu Val Ser Arg Asp Pro His Val Pro Ile Met Pro Tyr Val Gln Ile
 290 295 300
 Phe Tyr Glu Lys Thr Asp Tyr Leu Leu Pro Leu Glu Glu Leu Glu His
 305 310 315 320
 Ser Leu Gly Glu Ser Ala Ala Gln Gly Ala Ala Gly Ala Val Leu Trp
 325 330 335
 Ile Ser Ser Glu Lys Thr Ser Thr Lys Glu Ser Cys Gln Ala Ile Lys
 340 345 350
 Ala Tyr Met Asp Ser Thr Leu Gly Pro Phe Ile Leu Asn Val Thr Ser
 355 360 365
 Ala Ala Leu Leu Cys Ser Glu Ala Leu Cys Ser Gly Arg Gly Arg Cys
 370 375 380
 Val Arg His Pro Ser Tyr Pro Glu Ala Leu Leu Thr Leu Ser Pro Ala
 385 390 395 400
 Ser Phe Ser Ile Glu Pro Thr His Asp Gly Arg Pro Leu Ser Leu Lys
 405 410 415
 Gly Thr Leu Ser Leu Lys Asp Arg Ala Gln Met Ala Met Lys Phe Lys
 420 425 430
 Cys Arg Cys Tyr Arg Gly Trp Ser Gly Glu Trp Cys Lys Lys Gln Asp
 435 440 445
 Met

<210> 23
 <211> 473
 <212> PRT
 <213> Rattus norvegicus

<220>
 <223> hyaluronidase 2

<400> 23
 Met Arg Ala Gly Leu Gly Pro Ile Ile Thr Leu Ala Leu Val Leu Glu
 1 5 10 15
 Val Ala Trp Ala Ser Glu Leu Lys Pro Thr Ala Pro Pro Ile Phe Thr
 20 25 30
 Gly Arg Pro Phe Val Val Ala Trp Asn Val Pro Thr Gln Glu Cys Ala
 35 40 45
 Pro Arg His Lys Val Pro Leu Asp Leu Arg Ala Phe Asp Val Glu Ala
 50 55 60
 Thr Pro Asn Glu Gly Phe Phe Asn Gln Asn Ile Thr Thr Phe Tyr Tyr
 65 70 75 80
 Asp Arg Leu Gly Leu Tyr Pro Arg Phe Asp Ala Ala Gly Met Ser Val
 85 90 95

3066SEQ PC1.TXT

His	Gly	Gly	Val	Pro	Gln	Asn	Gly	Ser	Leu	Cys	Ala	His	Leu	Pro	Met
			100					105					110		
Leu	Lys	Glu	Ala	Val	Glu	Arg	Tyr	Ile	Gln	Thr	Gln	Glu	Pro	Ala	Gly
		115					120					125			
Leu	Ala	Val	Ile	Asp	Trp	Glu	Glu	Trp	Arg	Pro	Val	Trp	Val	Arg	Asn
		130				135					140				
Trp	Gln	Glu	Lys	Asp	Val	Tyr	Arg	Gln	Ser	Ser	Arg	Gln	Leu	Val	Ala
		145			150				155						160
Ser	Arg	His	Pro	Asp	Trp	Pro	Ser	Asp	Arg	Ile	Val	Lys	Gln	Ala	Gln
				165					170					175	
Tyr	Glu	Phe	Glu	Phe	Ala	Ala	Arg	Gln	Phe	Met	Leu	Asn	Thr	Leu	Arg
			180					185					190		
Tyr	Val	Lys	Ala	Val	Arg	Pro	Gln	His	Leu	Trp	Gly	Phe	Tyr	Leu	Phe
		195					200					205			
Pro	Asp	Cys	Tyr	Asn	His	Asp	Tyr	Val	Gln	Asn	Trp	Asp	Ser	Tyr	Thr
		210				215					220				
Gly	Arg	Cys	Pro	Asp	Val	Glu	Val	Ala	Gln	Asn	Asp	Gln	Leu	Ala	Trp
		225			230					235					240
Leu	Trp	Ala	Glu	Asn	Thr	Ala	Leu	Phe	Pro	Ser	Val	Tyr	Leu	Asp	Lys
				245					250					255	
Thr	Leu	Ala	Ser	Ser	Lys	His	Ser	Arg	Asn	Phe	Val	Ser	Phe	Arg	Val
			260					265					270		
Gln	Glu	Ala	Leu	Arg	Val	Ala	His	Thr	His	His	Ala	Asn	His	Ala	Leu
		275					280					285			
Pro	Val	Tyr	Val	Phe	Thr	Arg	Pro	Thr	Tyr	Thr	Arg	Arg	Leu	Thr	Glu
		290				295					300				
Leu	Asn	Gln	Met	Asp	Leu	Ile	Ser	Thr	Ile	Gly	Glu	Ser	Ala	Ala	Leu
		305			310					315					320
Gly	Ser	Ala	Gly	Val	Ile	Phe	Trp	Gly	Asp	Ser	Val	Tyr	Ala	Ser	Ser
				325					330					335	
Met	Glu	Asn	Cys	Gln	Asn	Leu	Lys	Lys	Tyr	Leu	Thr	Gln	Thr	Leu	Val
			340					345					350		
Pro	Tyr	Ile	Val	Asn	Val	Ser	Trp	Ala	Thr	Gln	Tyr	Cys	Ser	Trp	Thr
		355					360					365			
Gln	Cys	His	Gly	His	Gly	Arg	Cys	Val	Arg	Arg	Asn	Pro	Ser	Ala	Ser
		370				375					380				
Thr	Phe	Leu	His	Leu	Ser	Pro	Ser	Ser	Phe	Arg	Leu	Val	Pro	Gly	Arg
		385			390				395						400
Thr	Pro	Ser	Glu	Pro	Gln	Leu	Arg	Pro	Glu	Gly	Glu	Leu	Ser	Glu	Asp
				405					410					415	
Asp	Leu	Ser	Tyr	Leu	Gln	Met	His	Phe	Arg	Cys	His	Cys	Tyr	Leu	Gly
			420					425					430		
Trp	Gly	Gly	Glu	Gln	Cys	Gln	Trp	Asn	His	Lys	Arg	Ala	Ala	Gly	Asp
		435				440						445			
Ala	Ser	Arg	Ala	Trp	Ala	Gly	Ala	His	Leu	Ala	Ser	Leu	Leu	Gly	Leu
		450				455					460				
Val	Ala	Met	Thr	Leu	Thr	Trp	Thr	Leu							

<210> 24
 <211> 412
 <212> PRT
 <213> Rattus norvegicus

<220>
 <223> hyaluronidase 3

<400> 24

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

3066SEQ PC1.TXT

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

<210> 25

<211> 545

<212> PRT

<213> Oryctolagus cuniculus

<220>

<223> PH20

<400> 25

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

Tyr Asn His His Tyr Asp Lys Pro Asn Leu Tyr Lys Gly Ser Cys Phe
 225 230 235 240
 Asp Ile Glu Lys Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Lys Glu
 245 250 255
 Ser Thr Ala Leu Phe Pro Ser Val Tyr Leu Thr Ser Arg Ala Arg Ser
 260 265 270
 Ala Thr Ala Leu Ser Lys Leu Tyr Val Val Arg Asn Arg Val His Glu
 275 280 285
 Ala Ile Arg Val Ser Lys Ile Pro Asp Asp Lys Ser Pro Leu Pro Asn
 290 295 300
 Phe Val Tyr Thr Arg Leu Val Phe Thr Asp Gln Ile Phe Gln Phe Leu
 305 310 315 320
 Ser His His Asp Leu Val Tyr Thr Ile Gly Glu Ile Val Ala Leu Gly
 325 330 335
 Ala Ser Gly Ile Val Val Trp Gly Ser Gln Ser Leu Ala Arg Ser Met
 340 345 350
 Lys Ser Cys Leu His Leu Asp Asn Tyr Met Lys Thr Ile Leu Asn Pro
 355 360 365
 Tyr Leu Ile Asn Val Thr Leu Ala Ala Lys Met Cys Asn Gln Val Leu
 370 375 380
 Cys Gln Glu Gln Gly Val Cys Thr Arg Lys Asn Trp Asn Pro Asn Asp
 385 390 395 400
 Tyr Leu His Leu Asn Pro Gly Asn Phe Ala Ile Gln Leu Gly Ser Asn
 405 410 415
 Gly Thr Tyr Lys Val Asp Gly Lys Pro Thr Leu Thr Asp Leu Glu Gln
 420 425 430
 Phe Ser Lys Asn Phe Gln Cys Ser Cys Tyr Thr Asn Leu Asn Cys Lys
 435 440 445
 Glu Arg Thr Asp Met Asn Asn Val Arg Thr Val Asn Val Cys Ala Val
 450 455 460
 Glu Asn Val Cys Ile Asp Thr Asn Val Gly Pro Gln Ala Val Thr Tyr
 465 470 475 480
 Ala Pro Lys Glu Lys Lys Asp Val Ala His Ile Leu Ser Asn Thr Thr
 485 490 495
 Ser Ile Asn Ser Thr Thr Met Ser Leu Pro Phe Pro Arg Lys His
 500 505 510
 Val Ser Gly Cys Leu Leu Val Leu Cys Met Tyr Ser Gln Tyr Leu Asn
 515 520 525
 Ile Cys Tyr Arg Leu Val Ala Ile Gly Ile Gln His Gly Tyr Tyr Leu
 530 535 540
 Lys
 545

<210> 26

<211> 476

<212> PRT

<213> Ovis aries

<220>

<223> hyaluronidase 2

<400> 26

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

3066SEQ PC1.TXT

Glu Ala Gln Tyr Glu Phe Glu Phe Ala Ala Arg Gln Phe Met Leu Glu
 180 185 190
 Thr Leu Arg Phe Val Lys Ala Phe Arg Pro Arg His Leu Trp Gly Phe
 195 200 205
 Tyr Leu Phe Pro Asp Cys Tyr Asn His Asp Tyr Val Gln Asn Trp Glu
 210 215 220
 Thr Tyr Thr Gly Arg Cys Pro Asp Val Glu Val Ser Arg Asn Asp Gln
 225 230 235 240
 Leu Ser Trp Leu Trp Ala Glu Ser Thr Ala Leu Phe Pro Ser Val Tyr
 245 250 255
 Leu Glu Glu Thr Leu Ala Ser Ser Thr His Gly Arg Asn Phe Val Ser
 260 265 270
 Phe Arg Val Gln Glu Ala Leu Arg Val Ala Asp Val His His Ala Asn
 275 280 285
 His Ala Leu Pro Val Tyr Val Phe Thr Arg Pro Thr Tyr Ser Arg Gly
 290 295 300
 Leu Thr Gly Leu Ser Glu Met Asp Leu Ile Ser Thr Ile Gly Glu Ser
 305 310 315 320
 Ala Ala Leu Gly Ala Glu Val Ile Leu Trp Gly Asp Ala Gly Phe
 325 330 335
 Thr Thr Ser Asn Glu Thr Cys Arg Arg Leu Lys Asp Tyr Leu Thr Arg
 340 345 350
 Ser Leu Val Pro Tyr Val Val Asn Val Ser Trp Ala Ala Gln Tyr Cys
 355 360 365
 Ser Trp Ala Gln Cys His Gly His Gly Arg Cys Val Arg Arg Asp Pro
 370 375 380
 Asn Ala His Thr Phe Leu His Leu Ser Ala Ser Ser Phe Arg Leu Val
 385 390 395 400
 Pro Ser His Ala Pro Asp Glu Pro Arg Leu Arg Pro Glu Gly Glu Leu
 405 410 415
 Ser Trp Ala Asp Arg Asn His Leu Gln Thr His Phe Arg Cys Gln Cys
 420 425 430
 Tyr Leu Gly Trp Gly Gly Glu Gln Cys Gln Trp Asp Arg Arg Arg Ala
 435 440 445
 Ala Gly Gly Ala Ser Gly Ala Trp Ala Gly Ser His Leu Thr Gly Leu
 450 455 460
 Leu Ala Val Ala Val Leu Ala Phe Thr Trp Thr Ser
 465 470 475

<210> 27
 <211> 114
 <212> PRT
 <213> Ovis aries

<220>
 <223> PH20 partial sequence

<400> 27
 Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Leu Ser Lys Ile
 1 5 10 15
 Ala Ser Val Glu Ser Pro Leu Pro Val Phe Val Tyr His Arg Pro Val
 20 25 30
 Phe Thr Asp Gly Ser Ser Thr Tyr Leu Ser Gln Gly Asp Leu Val Asn
 35 40 45
 Ser Val Gly Glu Ile Val Ala Leu Gly Ala Ser Gly Ile Ile Met Trp
 50 55 60
 Gly Ser Leu Asn Leu Ser Leu Thr Met Gln Ser Cys Met Asn Leu Gly
 65 70 75 80
 Asn Tyr Leu Asn Thr Thr Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
 85 90 95
 Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
 100 105 110
 Ile Arg

<210> 28
 <211> 414
 <212> PRT
 <213> Pongo pygmaeus

<220>
 <223> hyaluronidase 3

3066SEQ PC1.TXT

<400> 28

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Met Thr Thr Arg Leu Gly Pro Ala Leu Val Leu Gly Val Ala Leu Cys
 1      5      10      15
Leu Gly Cys Gly Gln Pro Leu Pro Gln Val Pro Glu Arg Pro Phe Ser
 20      25      30
Val Leu Trp Asn Val Pro Ser Ala His Cys Lys Ser Arg Phe Gly Val
 35      40      45
His Leu Pro Leu Asn Ala Leu Gly Ile Ile Ala Asn Arg Gly Gln His
 50      55      60
Phe His Gly Gln Asn Met Thr Ile Phe Tyr Lys Asn Gln Leu Gly Leu
 65      70      75
Tyr Pro Tyr Phe Gly Pro Lys Gly Thr Ala His Asn Gly Gly Ile Pro
 85      90      95
Gln Ala Leu Pro Leu Asp Arg His Leu Ala Leu Ala Ala Tyr Gln Ile
100      105      110
His His Ser Leu Arg Pro Gly Phe Ala Gly Pro Ala Val Leu Asp Trp
115      120      125
Glu Glu Trp Cys Pro Leu Trp Ala Gly Asn Trp Gly Arg Arg Arg Ala
130      135      140
Tyr Gln Ala Ala Ser Trp Ala Trp Ala Gln Gln Val Phe Pro Asp Leu
145      150      155
Asp Pro Gln Glu Gln Leu Tyr Lys Ala Tyr Thr Gly Phe Glu Gln Ala
165      170      175
Ala Arg Ala Leu Met Glu Asp Thr Leu Arg Val Ala Gln Ala Leu Arg
180      185      190
Pro His Gly Leu Trp Gly Phe Tyr His Tyr Pro Ala Cys Gly Asn Gly
195      200      205
Trp His Ser Met Ala Ser Asn Tyr Thr Gly Arg Cys His Ala Ala Thr
210      215      220
Leu Ala Arg Asn Thr Gln Leu His Trp Leu Trp Ala Ala Ser Ser Ala
225      230      235
Leu Phe Pro Ser Ile Tyr Leu Pro Pro Arg Leu Pro Pro Ala His His
245      250      255
Gln Ala Phe Val Arg His Arg Leu Glu Glu Ala Phe Arg Val Ala Leu
260      265      270
Val Gly His Leu Pro Val Leu Ala Tyr Val Arg Leu Thr His Arg Arg
275      280      285
Ser Gly Arg Phe Leu Ser Gln Asp Asp Leu Val Gln Thr Ile Gly Val
290      295      300
Ser Ala Ala Leu Gly Ala Ala Gly Val Val Leu Trp Gly Asp Leu Ser
305      310      315
Leu Ser Ser Ser Glu Glu Cys Trp His Leu His Asp Tyr Leu Val
325      330      335
Asp Thr Leu Gly Pro Tyr Gly Ile Asn Val Thr Arg Ala Ala Met Ala
340      345      350
Cys Ser His Gln Arg Cys His Gly His Gly Arg Cys Ala Arg Arg Asp
355      360      365
Pro Gly Gln Met Glu Ala Phe Leu His Leu Trp Pro Asp Gly Ser Leu
370      375      380
Gly Asp Trp Lys Ser Phe Ser Cys His Cys Tyr Trp Gly Trp Ala Gly
385      390      395
Pro Thr Cys Gln Glu Pro Arg Leu Gly Pro Lys Glu Ala Val
405      410

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<210> 29

<211> 510

<212> PRT

<213> Macaca fascicularis

<220>

<223> PH20

<400> 29

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

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3066SEQ PC1.TXT

				85					90				95				
Gly	Tyr	Tyr	Pro	Tyr	Ile	Asp	Leu	Thr	Thr	Gly	Val	Thr	Val	His	Gly		
			100					105					110				
Gly	Ile	Pro	Gln	Lys	Val	Ser	Leu	Gln	Asp	His	Leu	Asp	Lys	Ser	Lys		
		115					120					125					
Gln	Asp	Ile	Leu	Phe	Tyr	Met	Pro	Val	Asp	Asn	Leu	Gly	Met	Ala	Val		
		130				135					140						
Ile	Asp	Trp	Glu	Glu	Trp	Arg	Pro	Thr	Trp	Ala	Arg	Asn	Trp	Lys	Pro		
					150					155					160		
Lys	Asp	Val	Tyr	Lys	Asn	Arg	Ser	Ile	Glu	Leu	Val	Gln	Gln	Gln	Asn		
				165					170					175			
Val	Gln	Leu	Ser	Leu	Pro	Gln	Ala	Thr	Asp	Lys	Ala	Lys	Gln	Glu	Phe		
			180					185					190				
Glu	Lys	Ala	Gly	Lys	Asp	Phe	Met	Leu	Glu	Thr	Ile	Lys	Leu	Gly	Arg		
		195				200						205					
Ser	Leu	Arg	Pro	Asn	His	Leu	Trp	Gly	Tyr	Tyr	Leu	Phe	Pro	Asp	Cys		
		210				215					220						
Tyr	Asn	His	His	Tyr	Arg	Lys	Pro	Gly	Tyr	Asn	Gly	Ser	Cys	Phe	Asp		
					230					235					240		
Val	Glu	Ile	Lys	Arg	Asn	Asp	Asp	Leu	Ser	Trp	Leu	Trp	Asn	Glu	Ser		
				245					250					255			
Thr	Ala	Leu	Tyr	Pro	Ser	Ile	Tyr	Leu	Asn	Thr	Gln	Gln	Ser	Val	Val		
			260					265					270				
Val	Ala	Thr	Leu	Tyr	Val	Arg	Asn	Arg	Val	Arg	Glu	Ala	Ile	Arg	Val		
		275					280					285					
Ser	Lys	Ile	Pro	Asp	Ala	Lys	Asn	Pro	Leu	Pro	Val	Phe	Val	Tyr	Ala		
		290				295					300						
Arg	Leu	Val	Phe	Thr	Asp	Gln	Val	Leu	Lys	Phe	Leu	Ser	Arg	Glu	Glu		
					310					315					320		
Leu	Val	Ser	Thr	Leu	Gly	Glu	Thr	Val	Ala	Leu	Gly	Ala	Ser	Gly	Ile		
				325					330					335			
Val	Ile	Trp	Gly	Ser	Leu	Ser	Ile	Thr	Arg	Ser	Met	Lys	Ser	Cys	Leu		
			340					345					350				
Leu	Leu	Asp	Thr	Tyr	Met	Glu	Thr	Ile	Leu	Asn	Pro	Tyr	Ile	Ile	Asn		
		355					360					365					
Val	Thr	Leu	Ala	Ala	Lys	Met	Cys	Ser	Gln	Val	Leu	Cys	Gln	Glu	Gln		
		370				375					380						
Gly	Val	Cys	Ile	Arg	Lys	Asp	Trp	Asn	Ser	Ser	Asp	Tyr	Leu	His	Leu		
					390				395						400		
Asn	Pro	Asp	Asn	Phe	Asp	Ile	Arg	Leu	Glu	Lys	Gly	Gly	Lys	Phe	Thr		
				405					410					415			
Val	His	Gly	Lys	Pro	Thr	Val	Glu	Asp	Leu	Glu	Glu	Phe	Ser	Glu	Lys		
			420					425					430				
Phe	Tyr	Cys	Ser	Cys	Tyr	Thr	Asn	Leu	Ser	Cys	Lys	Glu	Lys	Ala	Asp		
		435					440					445					
Val	Lys	Asp	Thr	Asp	Ala	Val	Asp	Val	Cys	Ile	Ala	Asp	Gly	Val	Cys		
		450				455					460						

Ile	Asp	Ala	Ser	Leu	Lys	Pro	Pro	Val	Glu	Thr	Glu	Gly	Ser	Pro	Pro		
					470				475						480		
Ile	Phe	Tyr	Asn	Thr	Ser	Ser	Ser	Thr	Val	Ser	Thr	Thr	Met	Phe	Ile		
				485					490					495			
Val	Asn	Ile	Leu	Phe	Leu	Ile	Ile	Ser	Ser	Val	Ala	Ser	Leu				
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<210> 30
 <211> 529
 <212> PRT
 <213> Cavia porcellus

<220>
 <223> PH20

<400> 30
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 Cys Leu Ala Asp Lys Arg Ala Pro Pro Leu Ile Pro Asn Val Pro Leu
 35 40 45
 Leu Trp Val Trp Asn Ala Pro Thr Glu Phe Cys Ile Gly Gly Thr Asn
 50 55 60
 Gln Pro Leu Asp Met Ser Phe Phe Ser Ile Val Gly Thr Pro Arg Lys
 65 70 75 80
 Asn Ile Thr Gly Gln Ser Ile Thr Leu Tyr Tyr Val Asp Arg Leu Gly

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Tyr	Tyr	Pro	Tyr	Ile	Asp	Pro	His	Thr	Gly	Ala	Ile	Val	His	Gly	Gly		
			100					105					110				
Leu	Pro	Gln	Leu	Met	Asn	Leu	Gln	Gln	His	Leu	Arg	Lys	Ser	Arg	Gln		
		115					120					125					
Asp	Ile	Leu	Phe	Tyr	Met	Pro	Thr	Asp	Ser	Val	Gly	Leu	Ala	Val	Ile		
		130				135					140						
Asp	Trp	Glu	Glu	Trp	Arg	Pro	Thr	Trp	Thr	Arg	Asn	Trp	Arg	Pro	Lys		
		145			150					155					160		
Asp	Ile	Tyr	Arg	Asn	Lys	Ser	Ile	Glu	Leu	Val	Lys	Ser	Gln	His	Pro		
				165					170					175			
Gln	Tyr	Asn	His	Ser	Tyr	Ala	Val	Ala	Val	Ala	Lys	Arg	Asp	Phe	Glu		
			180					185					190				
Arg	Thr	Gly	Lys	Ala	Phe	Met	Leu	Glu	Thr	Leu	Lys	Leu	Gly	Lys	Ser		
		195				200						205					
Leu	Arg	Pro	Ser	Ser	Leu	Trp	Gly	Tyr	Tyr	Leu	Phe	Pro	Asp	Cys	Tyr		
		210				215					220						
Asn	Thr	His	Phe	Thr	Lys	Pro	Asn	Tyr	Asp	Gly	His	Cys	Pro	Pro	Ile		
		225			230					235					240		
Glu	Leu	Gln	Arg	Asn	Asn	Asp	Leu	Gln	Trp	Leu	Trp	Asn	Asp	Ser	Thr		
				245					250					255			
Ala	Leu	Tyr	Pro	Ser	Val	Tyr	Leu	Thr	Ser	Arg	Val	Arg	Ser	Ser	Gln		
			260					265					270				
Asn	Gly	Ala	Leu	Tyr	Val	Arg	Asn	Arg	Val	His	Glu	Ser	Ile	Arg	Val		
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Ser	Lys	Leu	Met	Asp	Asp	Lys	Asn	Pro	Leu	Pro	Ile	Tyr	Val	Tyr	Ile		
		290			295					300							
Arg	Leu	Val	Phe	Thr	Asp	Gln	Thr	Thr	Thr	Phe	Leu	Glu	Leu	Asp	Asp		
		305			310					315				320			
Leu	Val	His	Ser	Val	Gly	Glu	Ile	Val	Pro	Leu	Gly	Val	Ser	Gly	Ile		
				325					330					335			
Ile	Ile	Trp	Gly	Ser	Leu	Ser	Leu	Thr	Arg	Ser	Leu	Val	Ser	Cys	Ile		
			340					345					350				
Gly	Leu	Glu	Asn	Tyr	Met	Lys	Gly	Thr	Leu	Leu	Pro	Tyr	Leu	Ile	Asn		
		355					360					365					
Val	Thr	Leu	Ala	Ala	Lys	Met	Cys	Gly	Gln	Val	Leu	Cys	Lys	Asn	Gln		
		370				375					380						
Gly	Ile	Cys	Thr	Arg	Lys	Asp	Trp	Asn	Thr	Asn	Thr	Tyr	Leu	His	Leu		
		385			390				395					400			
Asn	Ala	Thr	Asn	Phe	Asp	Ile	Glu	Leu	Gln	Gln	Asn	Gly	Lys	Phe	Val		
				405					410					415			
Val	His	Gly	Lys	Pro	Ser	Leu	Glu	Asp	Leu	Gln	Glu	Phe	Ser	Lys	Asn		
			420					425					430				
Phe	His	Cys	Ser	Cys	Tyr	Thr	Asn	Val	Ala	Cys	Lys	Asp	Arg	Leu	Asp		
		435				440						445					
Val	His	Asn	Val	Arg	Ser	Val	Asn	Val	Cys	Thr	Ala	Asn	Asn	Ile	Cys		
		450				455					460						

Ile	Asp	Ala	Val	Leu	Asn	Phe	Pro	Ser	Leu	Asp	Asp	Asp	Glu	Pro			
				470					475				480				
Pro	Ile	Thr	Asp	Asp	Thr	Ser	Gln	Asn	Gln	Asp	Ser	Ile	Ser	Asp	Ile		
				485					490					495			
Thr	Ser	Ser	Ala	Pro	Pro	Ser	Ser	His	Ile	Leu	Pro	Lys	Asp	Leu	Ser		
			500					505					510				
Trp	Cys	Leu	Phe	Leu	Leu	Ser	Ile	Phe	Ser	Gln	His	Trp	Lys	Tyr	Leu		
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Leu

<210> 31
 <211> 512
 <212> PRT
 <213> Rattus norvegicus

<220>
 <223> PH20

<400> 31
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 Ser Leu Thr Val Asp Tyr Arg Ala Thr Pro Val Leu Ser Asp Thr Thr
 35 40 45
 Phe Val Trp Val Trp Asn Val Pro Thr Glu Ala Cys Val Glu Asn Val

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

<210> 32
 <211> 512
 <212> PRT
 <213> Mus musculus

<220>
 <223> PH20

<400> 32
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 Ser Leu Thr Val Asp Tyr Arg Ala Ala Pro Ile Leu Ser Asn Thr Thr
 35 40 45
 Phe Leu Trp Ile Trp Asn Val Pro Thr Glu Arg Cys Val Gly Asn Val

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Asn Asp Pro Ile Asp Leu Ser Phe Phe Ser Leu Ile Gly Ser Pro Arg
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Lys Thr Ala Thr Gly Gln Pro Val Thr Leu Phe Tyr Val Asp Arg Leu
      85      90      95
Gly Leu Tyr Pro His Ile Asp Ala Asn Gln Ala Glu His Tyr Gly Gly
100      105      110
Ile Pro Gln Arg Gly Asp Tyr Gln Ala His Leu Arg Lys Ala Lys Thr
115      120      125
Asp Ile Glu His Tyr Ile Pro Asp Asp Lys Leu Gly Leu Ala Ile Ile
130      135      140
Asp Trp Glu Glu Trp Arg Pro Thr Trp Leu Arg Asn Trp Lys Pro Lys
145      150      155      160
Asp Asn Tyr Arg Asn Lys Ser Ile Glu Leu Val Gln Ser Thr Asn Pro
165      170      175
Gly Leu Ser Ile Thr Glu Ala Thr Gln Lys Ala Ile Gln Gln Phe Glu
180      185      190
Glu Ala Gly Arg Lys Phe Met Glu Gly Thr Leu His Leu Gly Lys Phe
195      200      205
Leu Arg Pro Asn Gln Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr
210      215      220
Asn Asn Lys Phe Gln Asp Pro Lys Tyr Asp Gly Gln Cys Pro Ala Val
225      230      235      240
Glu Lys Lys Arg Asn Asp Asn Leu Lys Trp Leu Trp Lys Ala Ser Thr
245      250      255
Gly Leu Tyr Pro Ser Val Tyr Leu Lys Lys Asp Leu Lys Ser Asn Arg
260      265      270
Gln Ala Thr Leu Tyr Val Arg Tyr Arg Val Val Glu Ala Ile Arg Val
275      280      285
Ser Lys Val Gly Asn Ala Ser Asp Pro Val Pro Ile Phe Val Tyr Ile
290      300
Arg Leu Val Phe Thr Asp Arg Thr Ser Glu Tyr Leu Leu Glu Asp Asp
305      310      315      320
Leu Val Asn Thr Ile Gly Glu Ile Val Ala Leu Gly Thr Ser Gly Ile
325      330      335
Ile Ile Trp Asp Ala Met Ser Leu Ala Gln Arg Ala Ala Gly Cys Pro
340      345      350
Ile Leu His Lys Tyr Met Gln Thr Thr Leu Asn Pro Tyr Ile Val Asn
355      360      365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Thr Leu Cys Asn Glu Lys
370      375      380
Gly Met Cys Ser Arg Arg Lys Glu Ser Ser Asp Val Tyr Leu His Leu
385      390      395      400
Asn Pro Ser His Phe Asp Ile Met Leu Thr Glu Thr Gly Lys Tyr Glu
405      410      415
Val Leu Gly Asn Pro Arg Val Gly Asp Leu Glu Tyr Phe Ser Glu His
420      425      430
Phe Lys Cys Ser Cys Phe Ser Arg Met Thr Cys Lys Glu Thr Ser Asp
435      440      445
Val Lys Asn Val Gln Asp Val Asn Val Cys Val Gly Asp Asn Val Cys
450      455      460
Ile Lys Ala Lys Val Glu Pro Asn Pro Ala Phe Tyr Leu Leu Pro Gly
465      470      475      480
Lys Ser Leu Leu Phe Met Thr Thr Leu Gly His Val Leu Tyr His Leu
485      490      495
Pro Gln Asp Ile Phe Val Phe Pro Arg Lys Thr Leu Val Ser Thr Pro
500      505      510

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<210> 33

<211> 807

<212> PRT

<213> Staphylococcus aureus

<220>

<223> hyaluronidase

<400> 33

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

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3066SEQ PC1.TXT

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Glu Lys Glu Ala Thr Asn Leu Leu Lys Glu Met Lys Thr Glu Ser Gly															
85	90	95													
Arg Lys Tyr Leu Trp Ser Gly Ala Glu Thr Leu Glu Thr Asn Ser Ser															
100	105	110													
His Met Thr Arg Thr Tyr Arg Asn Ile Glu Lys Ile Ala Glu Ala Met															
115	120	125													
Arg Asn Pro Lys Thr Thr Leu Asn Thr Asp Glu Asn Lys Lys Lys Val															
130	135	140													
Arg Asp Ala Leu Glu Trp Leu His Lys Asn Ala Tyr Gly Lys Glu Pro															
145	150	155													
Asp Lys Lys Val Lys Glu Leu Ser Glu Asn Phe Thr Lys Thr Thr Gly															
165	170	175													
Lys Asn Thr Asn Leu Asn Trp Trp Asp Tyr Glu Ile Gly Thr Pro Lys															
180	185	190													
Ser Leu Thr Asn Thr Leu Ile Leu Asn Asp Gln Phe Ser Asn Glu															
195	200	205													
Glu Lys Lys Lys Phe Thr Ala Pro Ile Lys Thr Phe Ala Pro Asp Ser															
210	215	220													
Asp Lys Ile Leu Ser Ser Val Gly Lys Ala Glu Leu Ala Lys Gly Gly															
225	230	235													
Asn Leu Val Asp Ile Ser Lys Val Lys Leu Leu Glu Cys Ile Ile Glu															
245	250	255													
Glu Asp Lys Asp Met Met Lys Lys Ser Ile Asp Ser Phe Asn Lys Val															
260	265	270													
Phe Thr Tyr Val Gln Asp Ser Ala Thr Gly Lys Glu Arg Asn Gly Phe															
275	280	285													
Tyr Lys Asp Gly Ser Tyr Ile Asp His Gln Asp Val Pro Tyr Thr Gly															
290	295	300													
Ala Tyr Gly Val Val Leu Leu Glu Gly Ile Ser Gln Met Met Pro Met															
305	310	315													
Ile Lys Glu Thr Pro Phe Asn Asp Lys Thr Gln Asn Asp Thr Thr Leu															
325	330	335													
Lys Ser Trp Ile Asp Asp Gly Phe Met Pro Leu Ile Tyr Lys Gly Glu															
340	345	350													
Met Met Asp Leu Ser Arg Gly Arg Ala Ile Ser Arg Glu Asn Glu Thr															
355	360	365													
Ser His Ser Ala Ser Ala Thr Val Met Lys Ser Leu Leu Arg Leu Ser															
370	375	380													
Asp Ala Met Asp Asp Ser Thr Lys Ala Lys Tyr Lys Lys Ile Val Lys															
385	390	395													
Ser Ser Val Glu Ser Asp Ser Ser Tyr Lys Gln Asn Asp Tyr Leu Asn															
405	410	415													
Ser Tyr Ser Asp Ile Asp Lys Met Lys Ser Leu Met Thr Asp Asn Ser															
420	425	430													
Ile Ser Lys Asn Gly Leu Thr Gln Gln Leu Lys Ile Tyr Asn Asp Met															
435	440	445													
Asp Arg Val Thr Tyr His Asn Lys Asp Leu Asp Phe Ala Phe Gly Leu															
450	455	460													
Ser Met Thr Ser Lys Asn Val Ala Arg Tyr Glu Ser Ile Asn Gly Glu															
465	470	475													
Asn Leu Lys Gly Trp His Thr Gly Ala Gly Met Ser Tyr Leu Tyr Asn															
485	490	495													
Ser Asp Val Lys His Tyr His Asp Asn Phe Trp Val Thr Ala Asp Met															
500	505	510													
Lys Arg Leu Ser Gly Thr Thr Thr Leu Asp Asn Glu Ile Leu Lys Asp															
515	520	525													
Thr Asp Asp Lys Lys Ser Ser Lys Thr Phe Val Gly Gly Thr Lys Val															
530	535	540													
Asp Asp Gln His Ala Ser Ile Gly Met Asp Phe Glu Asn Gln Asp Lys															
545	550	555													
Thr Leu Thr Ala Lys Lys Ser Tyr Phe Ile Leu Asn Asp Lys Ile Val															
565	570	575													
Phe Leu Gly Thr Gly Ile Lys Ser Thr Asp Ser Ser Lys Asn Pro Val															
580	585	590													
Thr Thr Ile Glu Asn Arg Lys Ala Asn Gly Tyr Thr Leu Tyr Thr Asp															
595	600	605													
Asp Lys Gln Thr Thr Asn Ser Asp Asn Gln Glu Asn Asn Ser Val Phe															
610	615	620													
Leu Glu Ser Thr Asp Thr Lys Lys Asn Ile Gly Tyr His Phe Leu Asn															
625	630	635													
Lys Pro Lys Ile Thr Val Lys Lys Glu Ser His Thr Gly Lys Trp Lys															
645	650	655													

Glu Ile Asn Lys Ser Gln Lys Asp Thr 660 Gln Lys Thr Asp 670 Tyr Tyr
 Glu Val Thr Gln Lys His Ser Asn Ser Asp Asn Lys Tyr Gly Tyr Val
 Leu Tyr Pro Gly Leu Ser Lys Asp Val Phe Lys Thr Lys Lys Asp Glu
 Val Thr Val Val Lys Gln Glu Asp Asp Phe His Val Val Lys Asp Asn
 Glu Ser Val Trp Ala Gly Val Asn Tyr Ser Asn Ser Thr Gln Thr Phe
 Asp Ile Asn Asn Thr Lys Val Glu Val Lys Ala Lys Gly Met Phe Ile
 Leu Lys Lys Lys Asp Asp Asn Thr Tyr Glu Cys Ser Phe Tyr Asn Pro
 Glu Ser Thr Asn Ser Ala Ser Asp Ile Glu Ser Lys Ile Ser Met Thr
 Gly Tyr Ser Ile Thr Asn Lys Asn Thr Ser Thr Ser Asn Glu Ser Gly
 Val His Phe Glu Leu Thr Lys

<210> 34

<211> 371

<212> PRT

<213> Streptococcus pyogenes bacteriophage H4489A

<220>

<223> hyaluronidase

<400> 34

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

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

Thr Lys Asp Tyr Val Asp Glu Lys Ile Ala Glu Leu Lys Lys Leu Ile
 355 360 365
 Leu Lys Lys
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<210> 35
 <211> 1628
 <212> PRT
 <213> Clostridium perfringens

<220>
 <223> hyaluronidase

<400> 35
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 35 40 45
 Ser Tyr Ser Gly Gly Glu Phe Gln Ile Ser Asp Glu Ile Asn Ile Val
 50 55 60
 Tyr Asp Asp Gly Ile Asp Thr Tyr Thr Lys Lys Arg Val Asp Glu Val
 65 70 75 80
 Leu Glu Ala Ser Asn Leu Glu Ala Thr Val Ser Asn Glu Ile Val Pro
 85 90 95
 Gly Lys Thr Asn Phe Leu Val Gly Ile Asn Glu Ser Gly Gly Val Val
 100 105 110
 Asp Asn Tyr Phe Asn Lys Asn Ile Pro His Asp Glu Ser Phe Phe Asp
 115 120 125
 Glu Lys Met Asp Ala Asn Ile Val Ser Val Lys Asp Gly Val Ile Gly
 130 135 140
 Val Ile Gly Glu Asp Thr Asp Ser Ala Phe Tyr Gly Val Thr Thr Leu
 145 150 155 160
 Lys His Val Phe Asn Gln Leu Glu Glu Gly Asn Lys Ile Gln Ser Phe
 165 170 175
 Arg Ala Asp Asp Tyr Ala Glu Val Ala His Arg Gly Phe Ile Glu Gly
 180 185 190
 Tyr Tyr Gly Asn Pro Trp Ser Asn Glu Asp Arg Ala Glu Leu Met Lys
 195 200 205
 Phe Gly Gly Asp Tyr Lys Leu Asn Gln Tyr Val Phe Ala Pro Lys Asp
 210 215 220
 Asp Pro Tyr His Asn Ser Lys Trp Arg Asp Leu Tyr Pro Glu Glu Lys
 225 230 235 240
 Leu Ser Glu Ile Lys Lys Leu Ala Gln Val Gly Asn Glu Thr Lys Asn
 245 250 255

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

Lys	Tyr	Met	Asp	His	Gly	Thr	Ala	Glu	485	Glu	Thr	Asn	Ser	Ser	Leu	Ala
Leu	Arg	Glu	Ile	Ser	Lys	His	Met	Ile	500	Asn	Gln	Asn	Met	Asp	Gly	Arg
Val	Arg	Pro	Leu	Gln	Glu	Ser	Val	Glu	515	Leu	Ala	Pro	Lys	Leu	Glu	Ala
Phe	Lys	Gln	Lys	Tyr	Asp	Ser	Val	Gly	520	Ala	Ser	Ile	Lys	Glu	Asp	Ala
Glu	Leu	Ile	Ala	Glu	Phe	Thr	Asn	Leu	535	Gln	Lys	Ala	Ala	Asp	Tyr	Tyr
Lys	Asn	Asn	Pro	Gly	Asn	Glu	Arg	Thr	550	Arg	Asp	Gln	Ile	Ile	Tyr	Trp
Leu	Asn	Cys	Trp	Glu	Asp	Thr	Met	Asp	565	Ala	Ala	Ile	Gly	Tyr	Leu	Lys
Ser	Ala	Ile	Ala	Ile	Glu	Glu	Gly	Asp	580	Asp	Glu	Ala	Ala	Trp	Ala	Asn
Tyr	Ser	Glu	Ala	Gln	Gly	Ala	Phe	Glu	595	Lys	Ser	Lys	Thr	Tyr	Gly	Phe
His	Tyr	Val	Asp	His	Thr	Glu	Tyr	Ala	610	Val	Gly	Val	Gln	His	Ile	
Val	Pro	Phe	Ile	Lys	Ser	Met	Gly	Gln	625	Asn	Leu	Ser	Val	Val	Ile	Gly
Ser	Ile	Val	Asp	Pro	Asn	Arg	Ile	Ile	635	Ala	Thr	Tyr	Ile	Ser	Asn	Arg
Gln	Asp	Ala	Pro	Thr	Gly	Asn	Pro	Asp	645	Asn	Ile	Phe	Asp	Asn	Asn	Ala
Ser	Thr	Glu	Leu	Val	Tyr	Lys	Asn	Pro	660	Asn	Arg	Ile	Asp	Val	Gly	Thr
Tyr	Val	Gly	Val	Lys	Tyr	Ser	Asn	Pro	675	Ile	Thr	Leu	Asn	Asn	Val	Glu
Phe	Leu	Met	Gly	Ala	Asn	Ser	Asn	Pro	690	Asp	Thr	Met	Gln	Lys	Ala	
Lys	Ile	Gln	Tyr	Thr	Val	Asp	Gly	Arg	705	Glu	Trp	Ile	Asp	Leu	Glu	Glu
Gly	Val	Glu	Tyr	Thr	Met	Pro	Gly	Ala	725	Ile	Lys	Val	Glu	Asn	Leu	Asp
Leu	Lys	Val	Arg	Gly	Val	Arg	Leu	Ile	740	Ala	Thr	Glu	Ala	Arg	Glu	Asn
Thr	Trp	Leu	Gly	Val	Arg	Asp	Ile	Asn	755	Val	Asn	Lys	Lys	Glu	Asp	Ser
Asn	Ser	Gly	Val	Glu	Phe	Asn	Pro	Ser	770	Ile	Arg	Ser	Glu	Ser	Trp	
Gln	Val	Tyr	Glu	Gly	Asn	Glu	Ala	Asn	785	Leu	Leu	Asp	Gly	Asp	Asp	Asn
Thr	Gly	Val	Trp	Tyr	Lys	Thr	Leu	Asn	800	Gly	Asp	Thr	Ser	Leu	Ala	Gly
Glu	Phe	Ile	Gly	Leu	Asp	Leu	Gly	Lys	815	Ile	Lys	Leu	Asp	Gly	Ile	
Arg	Phe	Val	Ile	Gly	Lys	Asn	Gly	Gly	835	Ser	Ser	Asp	Lys	Trp	Asn	
Lys	Phe	Lys	Leu	Glu	Tyr	Ser	Leu	Asp	850	Asn	Glu	Ser	Trp	Thr	Thr	Ile
Lys	Glu	Tyr	Asp	Lys	Thr	Gly	Ala	Pro	865	Ala	Gly	Lys	Asp	Val	Ile	Glu
Glu	Ser	Phe	Glu	Thr	Pro	Ile	Ser	Ala	885	Lys	Tyr	Ile	Arg	Leu	Thr	Asn
Met	Glu	Asn	Ile	Asn	Lys	Trp	Leu	Thr	900	Phe	Ser	Glu	Phe	Ala	Ile	Ile
Ser	Asp	Glu	Leu	Glu	Asn	Ala	Gly	Asn	915	Lys	Glu	Asn	Val	Tyr	Thr	Asn
Thr	Glu	Leu	Asp	Leu	Leu	Ser	Leu	Ala	930	Lys	Glu	Asp	Val	Thr	Lys	Leu
Ile	Pro	Thr	Asp	Asp	Ile	Ser	Leu	Asn	945	His	Gly	Glu	Tyr	Ile	Gly	Val
Lys	Leu	Asn	Arg	Ile	Lys	Asp	Leu	Ser	965	Asn	Ile	Asn	Leu	Glu	Ile	Ser
Asn	Asp	Thr	Gly	Leu	Lys	Leu	Gln	Ser	980	Met	Asn	Gly	Val	Glu	Trp	
Thr	Glu	Ile	Thr	Asp	Lys	Asn	Thr	Leu	995	Asp	Gly	Arg	Tyr	Val	Arg	
Leu	Ile	Asn	Thr	Ser	Asn	Glu	Ala	Val	1010	Phe	Asn	Leu	Thr	Lys	Phe	
Glu	Val	Asn	Ser	Asn	Glu	Val	Tyr	Glu	1025	Pro	Ser	Leu	Val	Asp	Ala	Tyr
Val	Gly	Asp	Asp	Gly	Ala	Lys	Lys	Ala	1045	Val	Asp	Gly	Asp	Leu	Lys	Thr

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1075 1080 1085
 Arg Val Lys Phe Leu Gly Ala Pro Ser Thr Gly Asp Thr Ile Val Tyr
 1090 1095 1100
 Asp Leu Gly Gln Glu Ile Leu Val Asp Asn Leu Lys Tyr Val Val Leu
 1105 1110 1115 1120
 Asp Thr Glu Val Asp His Val Arg Asp Gly Lys Ile Gln Leu Ser Leu
 1125 1130 1135
 Asp Gly Glu Thr Trp Thr Asp Ala Ile Thr Ile Gly Asp Gly Val Glu
 1140 1145 1150
 Asn Gly Val Asp Asp Met Phe Ser Thr Pro Leu Lys Asn Gly Tyr Lys
 1155 1160 1165
 His Gly Asn Gln Ser Gly Gly Ile Val Pro Ile Asp Ser Ala Tyr Val
 1170 1175 1180
 Glu Gly Asp Asn Leu Asn Gln Lys Ala Arg Tyr Val Arg Ile Leu Phe
 1185 1190 1195 1200
 Thr Ala Pro Tyr Arg His Arg Trp Thr Val Ile Asn Glu Leu Met Ile
 1205 1210 1215
 Asn Asn Gly Glu Tyr Ile Ser Thr Val Asn Asp Pro Thr Tyr Ile Ser
 1220 1225 1230
 Asn Pro Ile Glu Glu Arg Gly Phe Ala Pro Ser Asn Leu Arg Asp Gly
 1235 1240 1245
 Asn Leu Thr Thr Ser Tyr Lys Pro Asn Thr Asn Asn Gly Glu Ile Ser
 1250 1255 1260
 Glu Gly Ser Ile Thr Tyr Arg Leu Ser Glu Lys Thr Asp Val Arg Lys
 1265 1270 1275 1280
 Val Thr Ile Val Gln Ser Gly Ser Ser Ile Ser Asn Ala Lys Val Met
 1285 1290 1295
 Ala Arg Val Gly Asp Gly Ser Glu Asn Val Thr Asp Gln Trp Val Gln
 1300 1305 1310
 Leu Gly Thr Leu Ser Asn Ser Leu Asn Glu Phe Ile Asn Arg Asp Tyr
 1315 1320 1325
 Asn Asn Ile Tyr Glu Ile Lys Ile Glu Trp Thr Asp Val Ala Pro Asn
 1330 1335 1340
 Ile Tyr Glu Ile Ile Thr Leu Asn Gln Glu Phe Glu Phe Pro Val Asn
 1345 1350 1355 1360
 Asp Ser Leu Lys Ala Lys Tyr Asp Glu Leu Ile Asn Leu Ser Gly Asp
 1365 1370 1375
 Glu Tyr Thr Leu Ser Ser Phe Glu Thr Leu Lys Glu Ala Leu Asn Glu
 1380 1385 1390
 Ala Lys Ser Ile Leu Asp Asp Ser Asn Ser Ser Gln Lys Lys Ile Asp
 1395 1400 1405
 Lys Ala Leu Glu Lys Leu Asn Lys Ala Glu Glu Arg Leu Asp Leu Arg
 1410 1415 1420
 Ala Thr Asp Phe Glu Asp Phe Asn Lys Val Leu Thr Leu Gly Asn Ser
 1425 1430 1435 1440
 Leu Val Glu Glu Glu Tyr Thr Ala Glu Ser Trp Ala Leu Phe Ser Glu
 1445 1450 1455
 Val Leu Glu Ala Ala Asn Glu Ala Asn Lys Asn Lys Ala Asp Tyr Thr
 1460 1465 1470
 Gln Asp Gln Ile Asn Gln Ile Val Ile Asp Leu Asp Ala Ser Ile Lys
 1475 1480 1485
 Ala Leu Val Lys Glu Thr Pro Glu Val Asp Lys Thr Asn Leu Gly Glu
 1490 1495 1500
 Leu Ile Asn Gln Gly Lys Ser Leu Leu Asp Glu Ser Val Glu Gly Phe
 1505 1510 1515 1520
 Asn Val Gly Glu Tyr His Lys Gly Ala Lys Asp Gly Leu Thr Val Glu
 1525 1530 1535
 Ile Asn Lys Ala Glu Glu Val Phe Asn Lys Glu Asp Ala Thr Glu Glu
 1540 1545 1550
 Glu Ile Asn Leu Ala Lys Glu Ser Leu Glu Gly Ala Ile Ala Arg Phe
 1555 1560 1565
 Asn Ser Leu Leu Ile Glu Glu Ser Thr Gly Asp Phe Asn Gly Asn Gly
 1570 1575 1580
 Lys Ile Asp Ile Gly Asp Leu Ala Met Val Ser Lys Asn Ile Gly Ser
 1585 1590 1595 1600
 Thr Thr Asn Thr Ser Leu Asp Leu Asn Lys Asp Gly Ser Ile Asp Glu
 1605 1610 1615
 Tyr Glu Ile Ser Phe Ile Asn His Arg Ile Leu Asn
 1620 1625

<210> 36
 <211> 435
 <212> PRT
 <213> Homo sapiens

<220>

<223> Hyaluronidase-1 [Precursor]

<400> 36

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Met Ala Ala His Leu Leu Pro Ile Cys Ala Leu Phe Leu Thr Leu Leu
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Asp Met Ala Gln Gly Phe Arg Gly Pro Leu Leu Pro Asn Arg Pro Phe
20      25      30
Thr Thr Val Trp Asn Ala Asn Thr Gln Trp Cys Leu Glu Arg His Gly
35      40      45
Val Asp Val Asp Val Ser Val Phe Asp Val Val Ala Asn Pro Gly Gln
50      55      60
Thr Phe Arg Gly Pro Asp Met Thr Ile Phe Tyr Ser Ser Gln Leu Gly
65      70      75      80
Thr Tyr Pro Tyr Tyr Thr Pro Thr Gly Glu Pro Val Phe Gly Gly Leu
85      90      95
Pro Gln Asn Ala Ser Leu Ile Ala His Leu Ala Arg Thr Phe Gln Asp
100     105     110
Ile Leu Ala Ala Ile Pro Ala Pro Asp Phe Ser Gly Leu Ala Val Ile
115     120     125
Asp Trp Glu Ala Trp Arg Pro Arg Trp Ala Phe Asn Trp Asp Thr Lys
130     135     140
Asp Ile Tyr Arg Gln Arg Ser Arg Ala Leu Val Gln Ala Gln His Pro
145     150     155     160
Asp Trp Pro Ala Pro Gln Val Glu Ala Val Ala Gln Asp Gln Phe Gln
165     170     175
Gly Ala Ala Arg Ala Trp Met Ala Gly Thr Leu Gln Leu Gly Arg Ala
180     185     190
Leu Arg Pro Arg Gly Leu Trp Gly Phe Tyr Gly Phe Pro Asp Cys Tyr
195     200     205
Asn Tyr Asp Phe Leu Ser Pro Asn Tyr Thr Gly Gln Cys Pro Ser Gly
210     215     220
Ile Arg Ala Gln Asn Asp Gln Leu Gly Trp Leu Trp Gly Gln Ser Arg
225     230     235     240
Ala Leu Tyr Pro Ser Ile Tyr Met Pro Ala Val Leu Glu Gly Thr Gly
245     250     255
Lys Ser Gln Met Tyr Val Gln His Arg Val Ala Glu Ala Phe Arg Val
260     265     270
Ala Val Ala Ala Gly Asp Pro Asn Leu Pro Val Leu Pro Tyr Val Gln
275     280     285
Ile Phe Tyr Asp Thr Thr Asn His Phe Leu Pro Leu Asp Glu Leu Glu
290     295     300
His Ser Leu Gly Glu Ser Ala Ala Gln Gly Ala Ala Gly Val Val Leu
305     310     315     320
Trp Val Ser Trp Glu Asn Thr Arg Thr Lys Glu Ser Cys Gln Ala Ile
325     330     335
Lys Glu Tyr Met Asp Thr Thr Leu Gly Pro Phe Ile Leu Asn Val Thr
340     345     350
Ser Gly Ala Leu Leu Cys Ser Gln Ala Leu Cys Ser Gly His Gly Arg
355     360     365
Cys Val Arg Arg Thr Ser His Pro Lys Ala Leu Leu Leu Asn Pro
370     375     380
Ala Ser Phe Ser Ile Gln Leu Thr Pro Gly Gly Gly Pro Leu Ser Leu
385     390     395     400
Arg Gly Ala Leu Ser Leu Glu Asp Gln Ala Gln Met Ala Val Glu Phe
405     410     415
Lys Cys Arg Cys Tyr Pro Gly Trp Gln Ala Pro Trp Cys Glu Arg Lys
420     425     430
Ser Met Trp
435

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<210> 37

<211> 473

<212> PRT

<213> Homo sapiens

<220>

<223> Hyaluronidase-2 [Precursor]

<400> 37

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Met Arg Ala Gly Pro Gly Pro Thr Val Thr Leu Ala Leu Val Leu Ala
1      5      10      15
Val Ala Trp Ala Met Glu Leu Lys Pro Thr Ala Pro Pro Ile Phe Thr

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			20						25						30			
Gly	Arg	Pro	Phe	Val	Val	Ala	Trp	Asp	Val	Pro	Thr	Gln	Asp	Cys	Gly			
35			Lys	Val	Pro	Leu	Asp	Leu	Asn	Ala	Phe	Asp	Val	Gln	Ala			
Pro	Arg	Leu				55												
50	Pro	Asn	Glu	Gly	Phe	Val	Asn	Gln	Asn	Ile	Thr	Ile	Phe	Tyr	Arg			
65	Arg	Leu	Gly	Leu	Tyr	Pro	Arg	Phe	Asp	Ser	Ala	Gly	Arg	Ser	Val			
85			Val	Pro	Gln	Asn	Val	Ser	Leu	Trp	Ala	His	Arg	Lys	Met			
100	Gly	Gly	Val	Arg	Val	Glu	His	Tyr	Ile	Arg	Thr	Gln	Glu	Ser	Ala			
115			Val	Arg	Val	Glu	His	Tyr	Ile	Arg	Thr	Gln	Glu	Ser	Ala			
130	Ala	Val	Ile	Asp	Trp	Glu	Asp	Trp	Arg	Pro	Val	Trp	Val	Arg	Asn			
145	Gln	Asp	Lys	Asp	Val	Tyr	Arg	Arg	Leu	Ser	Arg	Gln	Leu	Val	Ala			
165			Pro	Asp	Trp	Pro	Pro	Asp	Arg	Ile	Val	Lys	Gln	Ala	Gln			
180	Glu	Phe	Glu	Phe	Ala	Ala	Gln	Gln	Phe	Met	Leu	Glu	Thr	Leu	Arg			
195			Ala	Val	Arg	Pro	Arg	His	Leu	Trp	Gly	Phe	Tyr	Leu	Phe			
210	Asp	Cys	Tyr	Asn	His	Asp	Tyr	Val	Gln	Asn	Trp	Glu	Ser	Tyr	Thr			
225	Arg	Cys	Pro	Asp	Val	Glu	Val	Ala	Arg	Asn	Asp	Gln	Leu	Ala	Trp			
240	Leu	Trp	Ala	Glu	Ser	Thr	Ala	Leu	Phe	Pro	Ser	Val	Tyr	Leu	Glu			
255			Ser	Ser	Arg	His	Gly	Arg	Asn	Phe	Val	Ser	Phe	Arg	Val			
270	Gln	Glu	Ala	Leu	Arg	Val	Ala	Arg	Thr	His	His	Ala	Asn	His	Ala			
285			Val	Phe	Thr	Arg	Pro	Thr	Tyr	Ser	Arg	Arg	Leu	Thr	Gly			
290	Val	Tyr	Val	Phe	Thr	Arg	295	Thr	Tyr	Ser	Arg	300	Arg	Leu	Thr			
305	Ser	Glu	Met	Asp	Leu	Ile	Ser	Thr	Ile	Gly	Glu	Ser	Ala	Ala	Leu			
320			Ala	Gly	Val	Ile	Leu	Trp	Gly	Asp	Ala	Gly	Tyr	Thr	Thr			
335	Ala	Ala	Gly	Val	Ile	Leu	Trp	Gly	Asp	Ala	Gly	Tyr	Thr	Thr	Ser			
340			Gln	Tyr	Leu	Lys	Asp	Thr	Leu	Thr	Arg	Leu	Ser	Ala	Val			
355	Glu	Thr	Cys	Val	Asn	Val	Ser	Trp	Ala	Thr	Gln	Tyr	Cys	Ser	Arg			
360			Val	Asn	Val	Ser	Trp	Ala	Thr	Gln	Tyr	Cys	Ser	Arg	Ala			
370	Cys	His	Gly	His	Gly	Arg	Cys	Val	Arg	Arg	Asn	Pro	Ser	Ala	Ser			
385	Phe	Leu	His	Leu	Ser	Thr	Asn	Ser	Phe	Arg	Leu	Val	Pro	Gly	His			
390			Gln	Leu	Arg	Pro	Val	Gly	Glu	Leu	Ser	Trp	Ala	Gly	His			
405	Pro	Gly	Glu	Pro	Gln	Leu	Arg	Pro	Val	Gly	Glu	Leu	Ser	Trp	Ala			
415			His	Leu	Gln	Thr	His	Phe	Arg	Cys	Gln	Cys	Tyr	Leu	Gly			
420	Ile	Asp	His	Leu	Gln	Thr	His	Phe	Arg	Cys	Gln	Cys	Tyr	Leu	Gly			
435			Glu	Gln	Cys	Gln	Trp	Asp	His	Arg	Gln	Ala	Ala	Gly	Gly			
445	Ser	Glu	Ala	Trp	Ala	Gly	Ser	His	Leu	Thr	Ser	Leu	Leu	Ala	Leu			
455			Ala	Trp	Thr	Trp	Thr	Leu										
465	Ala	Leu	Ala	Phe	Thr	470												

<400> 38
Met Thr Thr Gln Leu Gly Pro Ala Leu Val Leu Gly Val Ala Leu Cys
1 5 10 15
Leu Gly Cys Gly Gln Pro Leu Pro Gln Val Pro Glu Arg Pro Phe Ser
20 25 30
Val Leu Trp Asn Val Pro Ser Ala His Cys Glu Ala Arg Phe Gly Val
35 40 45
His Leu Pro Leu Asn Ala Leu Gly Ile Ile Ala Asn Arg Gly Gln His

3066SEQ PC1.TXT

[illegible]

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<210> 39
<211> 481
<212> PRT
<213> Homo sapiens
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<220>
<223> Hyaluronidase-4

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

130	Asp	Trp	Glu	Tyr	Trp	Arg	Pro	Gln	Trp	Ala	Arg	Asn	Trp	Asn	Ser	Lys
145	Asp	Val	Tyr	Arg	Gln	Lys	Ser	Arg	Lys	Leu	Ile	Ser	Asp	Met	Gly	Lys
	Asn	Val	Ser	Ala	Thr	Asp	Ile	Glu	Tyr	Leu	Ala	Lys	Val	Thr	Phe	Glu
	Glu	Ser	Ala	Lys	Ala	Phe	Met	Lys	Glu	Thr	Ile	Lys	Leu	Gly	Ile	Lys
	Ser	Arg	Pro	Lys	Gly	Leu	Trp	Gly	Tyr	Tyr	Leu	Tyr	Pro	Asp	Cys	His
	Asn	Tyr	Asn	Val	Tyr	Ala	Pro	Asn	Tyr	Ser	Gly	Ser	Cys	Pro	Glu	Asp
	Glu	Val	Leu	Arg	Asn	Asn	Glu	Leu	Ser	Trp	Leu	Trp	Asn	Ser	Ser	Ala
	Ala	Leu	Tyr	Pro	Ser	Ile	Gly	Val	Trp	Lys	Ser	Leu	Gly	Asp	Ser	Glu
	Asn	Ile	Leu	Arg	Phe	Ser	Lys	Phe	Arg	Val	His	Glu	Ser	Met	Arg	Ile
	Ser	Thr	Met	Thr	Ser	His	Asp	Tyr	Ala	Leu	Pro	Val	Phe	Val	Tyr	Thr
	Arg	Leu	Gly	Tyr	Arg	Asp	Glu	Pro	Leu	Phe	Phe	Leu	Ser	Lys	Gln	Asp
	Leu	Val	Ser	Thr	Ile	Gly	Glu	Ser	Ala	Ala	Leu	Gly	Ala	Ala	Gly	Ile
	Val	Ile	Trp	Gly	Asp	Met	Asn	Leu	Thr	Ala	Ser	Lys	Ala	Asn	Cys	Thr
	Lys	Val	Lys	Gln	Phe	Val	Ser	Ser	Asp	Leu	Gly	Ser	Tyr	Ile	Ala	Asn
	Val	Thr	Arg	Ala	Ala	Glu	Val	Cys	Ser	Leu	His	Leu	Cys	Arg	Asn	Asn
	Gly	Arg	Cys	Ile	Arg	Lys	Met	Trp	Asn	Ala	Pro	Ser	Tyr	Leu	His	Leu
	Asn	Pro	Ala	Ser	Tyr	His	Ile	Glu	Ala	Ser	Glu	Asp	Gly	Glu	Phe	Thr
	Val	Lys	Gly	Lys	Ala	Ser	Asp	Thr	Asp	Leu	Ala	Val	Met	Ala	Asp	Thr
	Phe	Ser	Cys	His	Cys	Tyr	Gln	Gly	Tyr	Glu	Gly	Ala	Asp	Cys	Arg	Glu
	Ile	Lys	Thr	Ala	Asp	Gly	Cys	Ser	Gly	Val	Ser	Pro	Ser	Pro	Gly	Ser
	Leu	Met	Thr	Leu	Cys	Leu	Leu	Leu	Ala	Ser	Tyr	Arg	Ser	Ile	Gln	
465	Leu															

<210> 40
 <211> 467
 <212> PRT
 <213> Homo sapiens

<220>
 <223> sHuPH20 precursor 1-467

<400> 40	Met	Gly	Val	Leu	Lys	Phe	Lys	His	Ile	Phe	Phe	Arg	Ser	Phe	Val	Lys
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	Cys	Leu	Thr	Leu	Asn	Phe	Arg	Ala	Pro	Pro	Val	Ile	Pro	Asn	Val	Pro
	Phe	Leu	Trp	Ala	Trp	Asn	Ala	Pro	Ser	Glu	Phe	Cys	Leu	Gly	Lys	Phe
	Asp	Glu	Pro	Leu	Asp	Met	Ser	Leu	Phe	Ser	Phe	Ile	Gly	Ser	Pro	Arg
	Ile	Asn	Ala	Thr	Gly	Gln	Gly	Val	Thr	Ile	Phe	Tyr	Val	Asp	Arg	Leu
	Gly	Tyr	Tyr	Pro	Tyr	Ile	Asp	Ser	Ile	Thr	Gly	Val	Thr	Val	Asn	Gly
	Gly	Ile	Pro	Gln	Lys	Ile	Ser	Leu	Gln	Asp	His	Leu	Asp	Lys	Ala	Lys
	Lys	Asp	Ile	Thr	Phe	Tyr	Met	Pro	Val	Asp	Asn	Leu	Gly	Met	Ala	Val
	Ile	Asp	Trp	Glu	Glu	Trp	Arg	Pro	Thr	Trp	Ala	Arg	Asn	Trp	Lys	Pro

[illegible]

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<210> 41
<211> 477
<212> PRT
<213> Homo sapiens
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<220>
<223> sHuPH20 precursor 1-477

<400>	41															
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Ser	Ser	Gly	Val	Ser	Gln	Ile	Val	Phe	Thr	Phe	Leu	Leu	Ile	Pro	Cys	
			20					25					30			
Cys	Leu	Thr	Leu	Asn	Phe	Arg	Ala	Pro	Pro	Val	Ile	Pro	Asn	Val	Pro	
		35					40					45				
Phe	Leu	Trp	Ala	Trp	Asn	Ala	Pro	Ser	Glu	Phe	Cys	Leu	Gly	Lys	Phe	
	50				55						60					
Asp	Glu	Pro	Leu	Asp	Met	Ser	Leu	Phe	Ser	Phe	Ile	Gly	Ser	Pro	Arg	
65					70					75					80	
Ile	Asn	Ala	Thr	Gly	Gln	Gly	Val	Thr	Ile	Phe	Tyr	Val	Asp	Arg	Leu	
				85					90					95		
Gly	Tyr	Tyr	Pro	Tyr	Ile	Asp	Ser	Ile	Thr	Gly	Val	Thr	Val	Asn	Gly	
			100					105					110			
Gly	Ile	Pro	Gln	Lys	Ile	Ser	Leu	Gln	Asp	His	Leu	Asp	Lys	Ala	Lys	
		115					120					125				
Lys	Asp	Ile	Thr	Phe	Tyr	Met	Pro	Val	Asp	Asn	Leu	Gly	Met	Ala	Val	
	130					135					140					
Ile	Asp	Trp	Glu	Glu	Trp	Arg	Pro	Thr	Trp	Ala	Arg	Asn	Trp	Lys	Pro	
145					150					155					160	
Lys	Asp	Val	Tyr	Lys	Asn	Arg	Ser	Ile	Glu	Leu	Val	Gln	Gln	Gln	Asn	
				165					170					175		

Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
 180 185 190
 Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
 195 200 205
 Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
 210 215 220
 Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
 225 230 235 240
 Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
 245 250 255
 Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
 260 265 270
 Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
 275 280 285
 Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
 290 295 300
 Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
 305 310 315 320
 Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
 325 330 335
 Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
 340 345 350
 Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
 355 360 365
 Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
 370 375 380
 Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
 385 390 395 400
 Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
 405 410 415
 Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
 420 425 430
 Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
 435 440 445
 Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
 450 455 460
 Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu
 465 470 475

<210> 42
 <211> 478
 <212> PRT
 <213> Homo sapiens

<220>
 <223> sHuPH20 precursor 1-478

<400> 42
 Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1 5 10 15
 Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
 20 25 30
 Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
 35 40 45
 Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
 50 55 60
 Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
 65 70 75 80
 Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
 85 90 95
 Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
 100 105 110
 Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
 115 120 125
 Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
 130 135 140
 Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
 145 150 155 160
 Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
 165 170 175
 Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
 180 185 190
 Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys

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

<210> 43

<211> 479

<212> PRT

<213> Homo sapiens

<220>

<223> sHuPH20 precursor 1-479

<400> 43

Met Gly Val	Leu Lys Phe	Lys His Ile	Phe Phe Arg	Ser Phe Val	Lys
1	5	10	15	20	25
Ser Ser Gly	Val Ser Gln	Ile Val Phe	Thr Phe Leu	Leu Ile Pro	Cys
30	35	40	45	50	55
Cys Leu Thr	Leu Asn Phe	Arg Ala Pro	Pro Val Ile	Pro Asn Val	Pro
60	65	70	75	80	85
Phe Leu Trp	Ala Trp Asn	Ala Pro Ser	Glu Phe Cys	Leu Gly Lys	Phe
90	95	100	105	110	115
Asp Glu Pro	Leu Asp Met	Ser Leu Phe	Ser Phe Ile	Gly Ser Pro	Arg
120	125	130	135	140	145
Ile Asn Ala	Thr Gly Gln	Gly Val Thr	Ile Phe Tyr	Val Asp Arg	Leu
150	155	160	165	170	175
Gly Tyr Tyr	Pro Tyr Ile	Asp Ser Ile	Thr Gly Val	Thr Val Asn	Gly
180	185	190	195	200	205
Gly Ile Pro	Gln Lys Ile	Ser Leu Gln	Asp His Leu	Asp Lys Ala	Lys
210	215	220	225	230	235
Lys Asp Ile	Thr Phe Tyr	Met Pro Val	Asp Asn Leu	Gly Met Ala	Val
240	245	250	255	260	265
Ile Asp Trp	Glu Glu Trp	Arg Pro Thr	Trp Ala Arg	Asn Trp Lys	Pro
270	275	280	285	290	295
Lys Asp Val	Tyr Lys Asn	Arg Ser Ile	Glu Leu Val	Gln Gln Gln	Asn
300	305	310	315	320	325
Val Gln Leu	Ser Leu Thr	Glu Ala Thr	Glu Lys Ala	Lys Gln Glu	Phe
330	335	340	345	350	355
Glu Lys Ala	Gly Lys Asp	Phe Leu Val	Glu Thr Ile	Lys Leu Gly	Lys
360	365	370	375	380	385
Leu Leu Arg	Pro Asn His	Leu Trp Gly	Tyr Tyr Leu	Phe Pro Asp	Cys
390	395	400	405	410	415

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Tyr	Asn	His	His	Tyr	Lys	Lys	Pro	Gly	Tyr	Asn	Gly	Ser	Cys	Phe	Asn
225					230				235						240
Val	Glu	Ile	Lys	Arg	Asn	Asp	Asp	Leu	Ser	Trp	Leu	Trp	Asn	Glu	Ser
				245					250						255
Thr	Ala	Leu	Tyr	Pro	Ser	Ile	Tyr	Leu	Asn	Thr	Gln	Gln	Ser	Pro	Val
				260					265						270
Ala	Ala	Thr	Leu	Tyr	Val	Arg	Asn	Arg	Val	Arg	Glu	Ala	Ile	Arg	Val
				275											285
Ser	Lys	Ile	Pro	Asp	Ala	Lys	Ser	Pro	Leu	Pro	Val	Phe	Ala	Tyr	Thr
															290
Arg	Ile	Val	Phe	Thr	Asp	Gln	Val	Leu	Lys	Phe	Leu	Ser	Gln	Asp	Glu
305					310					315					320
Leu	Val	Tyr	Thr	Phe	Gly	Glu	Thr	Val	Ala	Leu	Gly	Ala	Ser	Gly	Ile
				325					330						335
Val	Ile	Trp	Gly	Thr	Leu	Ser	Ile	Met	Arg	Ser	Met	Lys	Ser	Cys	Leu
				340					345						350
Leu	Leu	Asp	Asn	Tyr	Met	Glu	Thr	Ile	Leu	Asn	Pro	Tyr	Ile	Ile	Asn
				355					360						365
Val	Thr	Leu	Ala	Ala	Lys	Met	Cys	Ser	Gln	Val	Leu	Cys	Gln	Glu	Gln
															370
Gly	Val	Cys	Ile	Arg	Lys	Asn	Trp	Asn	Ser	Ser	Asp	Tyr	Leu	His	Leu
385					390					395					400
Asn	Pro	Asp	Asn	Phe	Ala	Ile	Gln	Leu	Glu	Lys	Gly	Gly	Lys	Phe	Thr
				405					410						415
Val	Arg	Gly	Lys	Pro	Thr	Leu	Glu	Asp	Leu	Glu	Gln	Phe	Ser	Glu	Lys
				420					425						430
Phe	Tyr	Cys	Ser	Cys	Tyr	Ser	Thr	Leu	Ser	Cys	Lys	Glu	Lys	Ala	Asp
															435
Val	Lys	Asp	Thr	Asp	Ala	Val	Asp	Val	Cys	Ile	Ala	Asp	Gly	Val	Cys
															440
Ile	Asp	Ala	Phe	Leu	Lys	Pro	Pro	Met	Glu	Thr	Glu	Glu	Pro	Gln	
465					470				475						

<210> 44
 <211> 480
 <212> PRT
 <213> Homo sapiens

<220>
 <223> sHuPH20 precursor 1-480

<400> 44	Met	Gly	Val	Leu	Lys	Phe	Lys	His	Ile	Phe	Phe	Arg	Ser	Phe	Val	Lys
	1				5					10					15	
	Ser	Ser	Gly	Val	Ser	Gln	Ile	Val	Phe	Thr	Phe	Leu	Leu	Ile	Pro	Cys
				20					25					30		
	Cys	Leu	Thr	Leu	Asn	Phe	Arg	Ala	Pro	Pro	Val	Ile	Pro	Asn	Val	Pro
				35				40					45			
	Phe	Leu	Trp	Ala	Trp	Asn	Ala	Pro	Ser	Glu	Phe	Cys	Leu	Gly	Lys	Phe
				50				55				60				
	Asp	Glu	Pro	Leu	Asp	Met	Ser	Leu	Phe	Ser	Phe	Ile	Gly	Ser	Pro	Arg
65								70				75				80
	Ile	Asn	Ala	Thr	Gly	Gln	Gly	Val	Thr	Ile	Phe	Tyr	Val	Asp	Arg	Leu
				85						90					95	
	Gly	Tyr	Tyr	Pro	Tyr	Ile	Asp	Ser	Ile	Thr	Gly	Val	Thr	Val	Asn	Gly
				100					105					110		
	Gly	Ile	Pro	Gln	Lys	Ile	Ser	Leu	Gln	Asp	His	Leu	Asp	Lys	Ala	Lys
				115				120					125			
	Lys	Asp	Ile	Thr	Phe	Tyr	Met	Pro	Val	Asp	Asn	Leu	Gly	Met	Ala	Val
				130				135				140				
	Ile	Asp	Trp	Glu	Glu	Trp	Arg	Pro	Thr	Trp	Ala	Arg	Asn	Trp	Lys	Pro
145								150			155					160
	Lys	Asp	Val	Tyr	Lys	Asn	Arg	Ser	Ile	Glu	Leu	Val	Gln	Gln	Gln	Asn
					165					170					175	
	Val	Gln	Leu	Ser	Leu	Thr	Glu	Ala	Thr	Glu	Lys	Ala	Lys	Gln	Glu	Phe
				180					185					190		
	Glu	Lys	Ala	Gly	Lys	Asp	Phe	Leu	Val	Glu	Thr	Ile	Lys	Leu	Gly	Lys
				195				200					205			
	Leu	Leu	Arg	Pro	Asn	His	Leu	Trp	Gly	Tyr	Tyr	Leu	Phe	Pro	Asp	Cys
				210			215					220				
	Tyr	Asn	His	His	Tyr	Lys	Lys	Pro	Gly	Tyr	Asn	Gly	Ser	Cys	Phe	Asn
225						230					235					240
	Val	Glu	Ile	Lys	Arg	Asn	Asp	Asp	Leu	Ser	Trp	Leu	Trp	Asn	Glu	Ser

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

<210> 45

<211> 481

<212> PRT

<213> Homo sapiens

<220>

<223> sHuPH20 precursor 1-481

<400> 45

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

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

<210> 46
 <211> 483
 <212> PRT
 <213> Homo sapiens

<220>
 <223> sHuPH20 precursor 1-483

<400> 46

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

3066SEQ PC1.TXT

275	Ser	Lys	Ile	Pro	Asp	Ala	Lys	280	Ser	Pro	Leu	Pro	Val	285	Phe	Ala	Tyr	Thr
290	Arg	Ile	Val	Phe	Thr	Asp	Gln	295	Val	Leu	Lys	Phe	Leu	300	Ser	Gln	Asp	Glu
305	Leu	Val	Tyr	Thr	Phe	310	Gly	Glu	Thr	Val	Ala	Leu	Gly	315	Ala	Ser	Gly	Ile
	Val	Ile	Trp	Gly	Thr	Leu	Ser	Ile	Met	Arg	Ser	Met	Lys	330	Ser	Cys	Leu	
	Leu	Leu	Asp	Asn	Tyr	Met	Glu	Thr	Ile	Leu	Asn	Pro	Tyr	340	Ile	Ile	Asn	
	Val	Thr	Leu	Ala	Ala	Lys	Met	Cys	Ser	Gln	Val	Leu	Cys	350	Gln	Glu	Gln	
	Gly	Val	Cys	Ile	Arg	Lys	Asn	Trp	Asn	Ser	Ser	Asp	Tyr	360	Leu	His	Leu	
385	Asn	Pro	Asp	Asn	Phe	Ala	Ile	Gln	Leu	Glu	Lys	Gly	Gly	370	Lys	Phe	Thr	
	Val	Arg	Gly	Lys	Pro	Thr	Leu	Glu	Asp	Leu	Glu	Gln	Phe	400	Ser	Glu	Lys	
	Phe	Tyr	Cys	Ser	Cys	Tyr	Ser	Thr	Leu	Ser	Cys	Lys	Glu	410	Lys	Ala	Asp	
	Val	Lys	Asp	Thr	Asp	Ala	Val	Asp	Val	Cys	Ile	Ala	Asp	420	Gly	Val	Cys	
465	Ile	Asp	Ala	Phe	Leu	Lys	Pro	Pro	Met	Glu	Thr	Glu	Glu	430	Pro	Gln	Ile	
	Phe	Tyr	Asn											440				

<210> 47
 <211> 432
 <212> PRT
 <213> Homo sapiens

<220>
 <223> SHuPH20 mature 36-467

<400> 47	Leu	Asn	Phe	Arg	Ala	Pro	Pro	Val	Ile	Pro	Asn	Val	Pro	Phe	Leu	Trp
1	Ala	Trp	Asn	Ala	5	Pro	Ser	Glu	Phe	10	Cys	Leu	Gly	Lys	Phe	15
	Leu	Asp	Met	Ser	Leu	Phe	Ser	Phe	Ile	20	Gly	Ser	Pro	Arg	Ile	25
	Thr	Gly	Gln	Gly	Val	Thr	Ile	Phe	Tyr	30	Val	Asp	Arg	Leu	Gly	35
50	Pro	Tyr	Ile	Asp	Ser	Ile	Thr	Gly	Val	40	Thr	Val	Asn	Gly	Gly	45
65	Gln	Lys	Ile	Ser	Leu	Gln	Asp	His	Leu	50	Asp	Lys	Ala	Lys	Lys	55
	Thr	Phe	Tyr	Met	Pro	Val	Asp	Asn	Leu	60	Gly	Met	Ala	Val	Ile	65
	Glu	Glu	Trp	Arg	Pro	Thr	Trp	Ala	Arg	70	Asn	Trp	Lys	Pro	Lys	75
	Tyr	Lys	Asn	Arg	Ser	Ile	Glu	Leu	Val	80	Gln	Gln	Gln	Asn	Val	85
	Ser	Leu	Thr	Glu	Ala	Thr	Glu	Lys	Ala	90	Lys	Gln	Glu	Phe	Glu	95
145	Gly	Lys	Asp	Phe	Leu	Val	Glu	Thr	Ile	100	Lys	Leu	Gly	Lys	Leu	105
	Pro	Asn	His	Leu	Trp	Gly	Tyr	Tyr	Leu	110	Phe	Pro	Asp	Cys	Tyr	115
	His	Tyr	Lys	Lys	Pro	Gly	Tyr	Asn	Gly	120	Ser	Cys	Phe	Asn	Val	125
	Lys	Arg	Asn	Asp	Asp	Leu	Ser	Trp	Leu	130	Trp	Asn	Glu	Ser	Thr	135
225	Tyr	Pro	Ser	Ile	Tyr	Leu	Asn	Thr	Gln	140	Gln	Ser	Pro	Val	Ala	145
	Leu	Tyr	Val	Arg	Asn	Arg	Val	Arg	Glu	150	Ala	Ile	Arg	Val	Ser	155
	Pro	Asp	Ala	Lys	Ser	Pro	Leu	Pro	Val	160	Phe	Ala	Tyr	Thr	Arg	165
	Phe	Thr	Asp	Gln	Val	Leu	Lys	Phe	Leu	170	Ser	Gln	Asp	Glu	Leu	175

3066SEQ PC1.TXT

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290						295					300				
Gly	Thr	Leu	Ser	Ile	Met	Arg	Ser	Met	Lys	Ser	Cys	Leu	Leu	Leu	Asp
305					310					315					320
Asn	Tyr	Met	Glu	Thr	Ile	Leu	Asn	Pro	Tyr	Ile	Ile	Asn	Val	Thr	Leu
				325					330					335	
Ala	Ala	Lys	Met	Cys	Ser	Gln	Val	Leu	Cys	Gln	Glu	Gln	Gly	Val	Cys
			340					345					350		
Ile	Arg	Lys	Asn	Trp	Asn	Ser	Ser	Asp	Tyr	Leu	His	Leu	Asn	Pro	Asp
		355					360					365			
Asn	Phe	Ala	Ile	Gln	Leu	Glu	Lys	Gly	Gly	Lys	Phe	Thr	Val	Arg	Gly
370						375					380				
Lys	Pro	Thr	Leu	Glu	Asp	Leu	Glu	Gln	Phe	Ser	Glu	Lys	Phe	Tyr	Cys
385					390					395					400
Ser	Cys	Tyr	Ser	Thr	Leu	Ser	Cys	Lys	Glu	Lys	Ala	Asp	Val	Lys	Asp
				405					410					415	
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 <212> PRT
 <213> Homo sapiens

<220>
 <223> sHuPH20 mature 36-483

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Thr	Phe	Tyr	Met	Pro	Val	Asp	Asn	Leu	Gly	Met	Ala	Val	Ile	Asp	Trp
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225					230					235					240
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				245					250					255	
Pro	Asp	Ala	Lys	Ser	Pro	Leu	Pro	Val	Phe	Ala	Tyr	Thr	Arg	Ile	Val
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			340					345					350		
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3066SEQ PC1.TXT

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Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385      390      395      400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
      405      410      415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
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<211> 1446

<212> DNA

<213> Homo sapiens

<220>

<223> DNA encoding soluble rHuPH20 "precursor"

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<211> 509

<212> PRT

<213> Homo sapiens

<220>

<223> PH20 variant P48A

<400> 50

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Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Ala
      35      40      45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
      50      55      60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65      70      75      80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
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Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
      100      105      110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
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Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val

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3066SEQ PC1.TXT

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	Val	Gln	Leu	Ser	Leu	Thr	Glu	Ala	Thr	Glu	Lys	Ala	Lys	Gln	Glu	Phe
	Glu	Lys	Ala	Gly	Lys	Asp	Phe	Leu	Val	Glu	Thr	Ile	Lys	Leu	Gly	Lys
	Leu	Leu	Arg	Pro	Asn	His	Leu	Trp	Gly	Tyr	Tyr	Leu	Phe	Pro	Asp	Cys
	Tyr	Asn	His	His	Tyr	Lys	Lys	Pro	Gly	Tyr	Asn	Gly	Ser	Cys	Phe	Asn
	Val	Glu	Ile	Lys	Arg	Asn	Asp	Asp	Leu	Ser	Trp	Leu	Trp	Asn	Glu	Ser
	Thr	Ala	Leu	Tyr	Pro	Ser	Ile	Tyr	Leu	Asn	Thr	Gln	Gln	Ser	Pro	Val
	Ala	Ala	Thr	Leu	Tyr	Val	Arg	Asn	Arg	Val	Arg	Glu	Ala	Ile	Arg	Val
	Ser	Lys	Ile	Pro	Asp	Ala	Lys	Ser	Pro	Leu	Pro	Val	Phe	Ala	Tyr	Thr
	Arg	Ile	Val	Phe	Thr	Asp	Gln	Val	Leu	Lys	Phe	Leu	Ser	Gln	Asp	Glu
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	Val	Ile	Trp	Gly	Thr	Leu	Ser	Ile	Met	Arg	Ser	Met	Lys	Ser	Cys	Leu
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	Val	Thr	Leu	Ala	Ala	Lys	Met	Cys	Ser	Gln	Val	Leu	Cys	Gln	Glu	Gln
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	Val	Arg	Gly	Lys	Pro	Thr	Leu	Glu	Asp	Leu	Glu	Gln	Phe	Ser	Glu	Lys
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	Ile	Asp	Ala	Phe	Leu	Lys	Pro	Pro	Met	Glu	Thr	Glu	Glu	Pro	Gln	Ile
	Phe	Tyr	Asn	Ala	Ser	Pro	Ser	Thr	Leu	Ser	Ala	Thr	Met	Phe	Ile	Val
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 <213> Homo sapiens

<220>
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	Ile	Asn	Ala	Thr	Gly	Gln	Gly	Val	Thr	Ile	Phe	Tyr	Val	Asp	Arg	Leu
	Gly	Tyr	Tyr	Pro	Tyr	Ile	Asp	Ser	Ile	Thr	Gly	Val	Thr	Val	Asn	Gly
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3066SEQ PC1.TXT

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Lys	Asp	Val	Tyr	Lys	Asn	Arg	Ser	Ile	Glu	Leu	Val	Gln	Gln	Gln	Asn
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210					215						220				
Tyr	Asn	His	His	Tyr	Lys	Lys	Pro	Gly	Tyr	Asn	Gly	Ser	Cys	Phe	Asn
225				230						235					240
Val	Glu	Ile	Lys	Arg	Asn	Asp	Asp	Leu	Ser	Trp	Leu	Trp	Asn	Glu	Ser
			245					250						255	
Thr	Ala	Leu	Tyr	Pro	Ser	Ile	Tyr	Leu	Asn	Thr	Gln	Gln	Ser	Pro	Val
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Ala	Ala	Thr	Leu	Tyr	Val	Arg	Asn	Arg	Val	Arg	Glu	Ala	Ile	Arg	Val
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Val	Arg	Gly	Lys	Pro	Thr	Leu	Glu	Asp	Leu	Glu	Gln	Phe	Ser	Glu	Lys
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Phe	Tyr	Cys	Ser	Cys	Tyr	Ser	Thr	Leu	Ser	Cys	Lys	Glu	Lys	Ala	Asp
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Val	Lys	Asp	Thr	Asp	Ala	Val	Asp	Val	Cys	Ile	Ala	Asp	Gly	Val	Cys
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Ile	Asp	Ala	Phe	Leu	Lys	Pro	Pro	Met	Glu	Thr	Glu	Glu	Pro	Gln	Ile
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Phe	Tyr	Asn	Ala	Ser	Pro	Ser	Thr	Leu	Ser	Ala	Thr	Met	Phe	Ile	Val
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<212> PRT

<213> Mus musculus

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<400> 53

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Arg Pro Leu Lys Asp Arg Ile Asn Ile Val Leu Ser Arg Glu Leu Lys
 65          70          75          80
Glu Pro Pro Arg Gly Ala His Phe Leu Ala Lys Ser Leu Asp Asp Ala
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Leu Arg Leu Ile Glu Gln Pro Glu Leu Ala Ser Lys Val Asp Met Val
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Trp Ile Val Gly Gly Ser Ser Val Tyr Gln Glu Ala Met Asn Gln Pro
115          120          125
Gly His Leu Arg Leu Phe Val Thr Arg Ile Met Gln Glu Phe Glu Ser
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<223> His tag

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<210> 55

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<223> Flag tag

<400> 55

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<223> Gen2 mRNA sequence

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<212> PRT

<213> Homo sapiens

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<400> 57

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<212> PRT

<213> Homo sapiens

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<400> 58

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 <213> Homo sapiens

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 431-442

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 <212> PRT
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 Gly Gly Ile Pro Gln Leu Gly Asn Leu Lys Ser His Leu Glu Lys Ala
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 Tyr Ala Arg Pro Val Phe Thr Asp Gly Ser Ser Thr Tyr Leu Ser Gln
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 Tyr Thr Val Pro Gly Thr Leu Thr Leu Glu Asp Leu Gln Lys Phe Ser
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<220>
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 Gly Gln Phe Ile Thr Leu Phe Tyr Ala Asp Arg Leu Gly Tyr Tyr Pro
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Asn	Phe	Ala	Ile	Gln	Thr	Gly	Lys	Gly	Gly	Lys	Tyr	Thr	Val	Pro	Gly	380
Lys	Val	Thr	Leu	Glu	Asp	Leu	Gln	Thr	Phe	Ser	Asp	Lys	Phe	Tyr	Cys	400
Ser	Cys	Tyr	Ala	Asn	Ile	Asn	Cys	Lys	Lys	Arg	Val	Asp	Ile	Lys	Asn	415
Val	His	Ser	Val	Asn	Val	Cys	Met	Ala	Glu	Asp	Ile	Cys	Ile	Glu	Gly	430
Pro	Val	Lys	Leu	Gln	Pro	Ser	Asp	His	Ser	Ser	Ser	Gln	Asn	Glu	Ala	445
Ser	Thr	Thr	Thr	Val	Ser	Ser	Ile	Ser	Pro	Ser	Thr	Thr	Ala	Thr	Thr	460
Val	Ser	Pro	Cys	Thr	Pro	Glu	Lys	Gln	Ser	Pro	Glu	Cys	Leu	Lys	Val	480
Arg	Cys	Leu	Glu	Ala	Ile	Ala	Asn	Val	Thr	Gln	Thr	Gly	Cys	Gln	Gly	495
Val	Lys	Trp	Lys	Asn	Thr	Ser	Ser	Gln	Ser	Ser	Ile	Gln	Asn	Ile	Lys	510
Asn	Gln	Thr	Thr	Tyr												515

<210> 66
 <211> 1620
 <212> DNA
 <213> Ovis aries

<220>
 <223> encoding sheep PH20

<400> 66
 ttttccgtgt tgggttggtc tggacttcag agcaccctt ctcatttcaa acacttcttt 60
 cctctgggccc tggaatgccc cagctgaacg ttgtattaaa atctttaaac tacctccaga 120
 tctgagactc ttctctgtaa aaggaagccc ccaaaaaagt gctacgggac aattttattac 180
 attattttat gctgatagac ttggctacta tcctcatata gatgaaaaaa caggcaacac 240
 tgtatatgga ggaattcccc agttgggaaa cttaaaaaat catttgaaa aagccaaaaa 300
 agacattgcc tattacatac caaatgacag cgtgggcttg gcggtcattg actgggaaaa 360

3066SEQ PC1.TXT

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ctggaggcct acctgggcaa gaaactggaa acctaaagat gtttacaggg atgagtctgt 420
tgagttgggt ctgcaaaaaa atccacaact cagtttccca gaggcttcca agattgcaaa 480
agtggatttt gagacagcag gaaagagttt catgcaagag actttaaaac tgggaaaatt 540
acttcggcca aatcacttat ggggttatta tctttttcct gattgttaca atcataatta 600
taaccagcct acttacaatg gaaattgctc tgatttagaa aaaaggagaa atgatgatct 660
cgactggttg tggaaggaaa gcaactgccct tttcccttct gtttatttga atatcaagtt 720
aaaatctact ccaaaaagctg ccttctatgt tcgtaatcgt gtccaggaag ccattcggtt 780
gtctaaaata gcgagtgttg aaagtccact tcccgttttt gtatatcacc gtccagtttt 840
tactgatggg tcttcaacat acctttctca gggtgacctt gtgaattcgg ttggtgagat 900
cgttgctcta ggtgcctctg ggattataat gtggggcagt ctcaatctaa gcctaactat 960
gcaatcttgc atgaacctag gcaattactt gaacactaca ctgaatcctt acataatcaa 1020
cgtcacccta gcagccaaaa tgtgcagcca agtgctttgc cacgatgaag gagtgtgtac 1080
aaggaaaaca tggaattcaa gcgactatct tcacctgaac ccaatgaatt ttgctattca 1140
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gttttctgat aaattttatt gcagttgtta tgccaacatc aactgtaaga agagagttga 1260
tataaaaaat gttcatagtg itaatgtatg tatggcagaa gacatttgta tagagggccc 1320
tgtgaagtta caaccagtg atcattcctc tagccagaat gaggcattca ctaccaccgt 1380
cagcagtatc tcaccctcta ctacagccac cacagtatct ccattgtact ctgagaaaaca 1440
gtcccctgag tgccctcaaag tcagggtgtt ggaagccatc gccaacgtca cccaaacggg 1500
gtgtcaaggt gttaaatgga agaacacttc cagtcagtca agtattcaaa atattaaaaa 1560
tcaacaacc tattaaaata taaattcagt gcttataaaa aaaaaaaaaa aaaaaaaaaa 1620

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<210> 67

<211> 793

<212> PRT

<213> *Arthrobacter* sp.

<220>

<223> hyaluronan lyase (strain FB24)

<400> 67

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Met Thr Arg Glu Phe Ser Arg Arg Thr Ala Leu Lys Gly Ala Ala Leu
 1          5          10          15
Ser Gly Leu Leu Ala Met Val His Gly Pro Ala His Ala Ala Ala
 20          25          30
Thr Ala Asn Ala Thr Leu Thr Pro Ala Asp Phe Ala Gly Leu Arg Gln
 35          40          45
Arg Trp Val Asp Gln Ile Thr Gly Arg Lys Val Leu Val Ala Gly Asp
 50          55          60
Asn Asp Phe Val Thr Ala Leu Ala Ala Leu Asp Lys Lys Ala Arg Thr
 65          70          75          80
Ala Ile Asp Leu Leu Glu Arg Ser Ala Gly Arg Leu Thr Val Phe Ser
 85          90          95
Asp Leu Ser Leu Ala Lys Asp Thr Asp Leu Val Thr Thr His Thr Arg
100          105          110
Leu Ala Thr Met Ala Thr Ala Trp Ala Thr Pro Gly Ser Glu His Phe
115          120          125
Ala Asp Ala Gly Leu Leu Ala Ala Ile Arg Ala Gly Leu Ala Asp Ala
130          135          140
Asn Ser Leu Cys Tyr Asn Ala Ser Lys Glu Glu Gln Gly Asn Trp Trp
145          150          155          160
Ser Trp Glu Ile Gly Thr Pro Lys Ala Leu Ala Asp Thr Met Val Leu
165          170          175
Leu His Ala Glu Leu Thr Ala Ala Glu Arg Ala Ala Tyr Cys Ala Ala
180          185          190
Ile Asp His Phe Val Pro Asp Pro Trp Gln Gln Phe Pro Pro Lys Arg
195          200          205
Gly Lys Ile Thr Ser Val Gly Ala Asn Arg Val Asp Leu Cys Gln Ala
210          215          220
Val Thr Ile Arg Ser Leu Val Asp Glu Asp Ala Glu Lys Leu Thr His
225          230          235          240
Ala Val Ala Gly Leu Ser Glu Val Trp Gln Tyr Val Ser Ala Gly Asn
245          250          255
Gly Phe Phe Thr Asp Gly Ser Phe Ile Gln His Ser Thr Thr Pro Tyr
260          265          270
Thr Gly Ser Tyr Gly Val Val Leu Leu Thr Gly Leu Ser Lys Leu Phe
275          280          285
Ala Leu Leu Gly Gly Thr Gly Ala Glu Val Ser Asp Pro Ser Arg Asp
290          295          300
Ile Leu Phe Lys Thr Val Glu Gly Ser Phe Ala Pro Phe Met Val Ala
305          310          315          320
Gly Ala Met Ala Asp Ser Val Arg Gly Arg Ser Ile Ser Arg Glu Ser
325          330          335
Asn Thr Gly Phe Asp Leu Gly Ala Ser Thr Ile Glu Ser Ile Leu Leu

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3066SEQ PC1.TXT

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      340      345      350
Leu Ala Arg Ala Val Asp Pro Val Thr Ala Arg Arg Trp Arg Ser Leu
      355      360      365
Cys Leu Ala Trp Ile Asn Gln Asn Arg Lys Ala Pro Ile Leu Ala Asp
      370      375      380
Ala Gly Val Gly Arg Thr Ala Leu Val Lys Glu Leu Leu Ala Met Gly
      385      390      395      400
Leu Thr Glu Thr Asp Leu Pro Gly Gly His Tyr Leu Phe Pro Ala Met
      405      410      415
Asp Arg Thr Met His His Ser Gln Gly Trp Thr Leu Ser Thr Ala Met
      420      425      430
Ala Ser Ser Arg Ile Ala Trp Tyr Glu Cys Gly Asn Gly Glu Asn Asn
      435      440      445
Arg Gly Tyr His Thr Gly Ser Gly Met Thr Tyr Val Tyr Asp Gly Asp
      450      455      460
Leu Gly Gln Tyr Asp Asp Ala Phe Trp Ala Thr Ala Asn His Cys Arg
      465      470      475      480
Leu Pro Gly Ile Thr Val Asp Thr Ser Ser Leu Pro Asp Lys Val Glu
      485      490      495
Gly Glu Trp Gly Ala Ala Thr Pro Ala Asn Glu Trp Thr Gly Ser Thr
      500      505      510
Ala Tyr Gly Asp Val Ala Ala Val Gly Gln His Leu Ile Gly Pro Gly
      515      520      525
Gly Thr Gly Leu Thr Ala Arg Lys Ser Trp Phe Val Ser Lys Asp Val
      530      535      540
Ile Val Cys Leu Gly Ala Asp Ile Arg Thr Gly Ser Gly Ser Arg Ile
      545      550      555      560
Glu Thr Val Val Asp His Arg Asn Leu His Ala Gly Phe Asn Ala Met
      565      570      575
Gly Thr Ala Ala Gly Thr Val Ala Ala Thr Pro Gly His Pro Glu Val
      580      585      590
Leu Thr Val Asp Arg Trp Val His Leu Glu Gly Phe Gly Gly Tyr Val
      595      600      605
Val Leu Asp Ala Ala Pro Leu Gln Val Leu Arg Glu Gln Arg Glu Gly
      610      615      620
Ser Trp Ser Glu Val Asn Val Lys Gly Ser Ala Ala Arg Gln Thr Arg
      625      630      635      640
Asn Tyr Ala Thr Leu Tyr Phe Asp His Gly His Glu Pro Glu Ala Ala
      645      650      655
Ser Tyr Ala Tyr Leu Val Ala Pro Gly Ala Ser Ala Ser Met Thr Ser
      660      665      670
Ser Leu Ser Gly Gln Ser Phe His Thr Val Leu Arg Asn Asp Glu Val
      675      680      685
Ala Gln Ala Val Lys Phe Lys Lys Glu Lys Thr Thr Ala Ala Thr Phe
      690      695      700
Trp Arg Pro Gly Thr Val Gly Asp Leu Ala Leu Ser Gly Pro Ala Cys
      705      710      715      720
Val Val Val Lys Glu Val Gly Asp Arg Leu Ser Ile Ala Val Ser Asp
      725      730      735
Pro Thr Gln Asn Ala Ser Thr Leu Thr Leu Arg Leu Lys Thr Lys Arg
      740      745      750
Phe Phe Arg Ile Ile Glu Gly Gln Gly Ala Ser Leu Ser His Gly Ala
      755      760      765
Asp Gly Phe Thr Val Leu Glu Val Asp Ile Ala Asn His Ala Gly Arg
      770      775      780
Thr Lys Gln Ile Glu Leu Ser Ala Glu
      785      790

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<210> 68

<211> 557

<212> PRT

<213> Bdellovibrio bacteriovorus

<220>

<223> hyaluronan lyase

<400> 68

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

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3066SEQ PC1.TXT

50	Gly	Met	Leu	Pro	Pro	Tyr	55	Glu	Pro	Val	Arg	Arg	60	Leu	Ile	Glu	Glu	Lys	
65	Asn	Ser	Asn	Pro	Leu	Arg	70	Lys	Trp	Ile	Gln	Ser	75	Leu	Met	Lys	Gly	80	Leu
	Ser	Gly	Phe	Arg	85	Phe	Glu	Asp	Val	100	Leu	Lys	Asn	Leu	Gly	95	Leu	His	
	Lys	Tyr	Pro	Leu	Glu	Asn	Glu	Arg	Gly	105	Val	Tyr	Ala	Val	Pro	Tyr	Pro		
	Asn	Glu	Ile	Arg	Pro	Asp	Thr	Leu	Met	Gly	Phe	Gly	Leu	Ile	Glu	Arg			
	Asn	Gly	Ala	Glu	Gly	Phe	Thr	Phe	Ser	Cys	Ala	Ala	Cys	His	Ser	Ser			
145	Asn	Leu	Phe	Gly	Lys	Thr	Val	Leu	Gly	Met	Thr	Asn	Arg	Phe	Pro	Arg			
	Ala	Asn	Glu	Phe	Phe	Ile	Lys	Ala	Lys	Lys	Val	Met	Pro	Leu	Met	Asp			
	Pro	His	Ile	Phe	Gln	Ala	Tyr	Thr	Arg	Ala	Thr	Asp	Ala	Glu	Thr	Ala			
	Leu	Leu	Val	Glu	Ser	Lys	Glu	Arg	Leu	Lys	Ser	Val	Ala	Leu	Lys	Gln			
225	Pro	Ile	Ala	Leu	Gly	Leu	Asp	Thr	Ser	Leu	Ala	Gln	Val	Ser	Leu	Ser			
	Leu	Asn	Arg	Arg	Ala	Lys	Asp	Gly	Tyr	Ala	Asn	Tyr	Ser	Asp	Lys	Ala			
	Ala	Arg	Ser	Pro	Arg	Ala	Asp	Ala	Tyr	Leu	Asp	Asn	Lys	Pro	Ala	Asp			
	Ser	Lys	Pro	Ala	Val	Trp	Trp	Asn	Val	Lys	Tyr	Lys	Asn	Arg	Trp	Leu			
	Ser	Asp	Gly	Ser	Val	Leu	Ser	Gly	Asn	Pro	Ile	Phe	Thr	Asn	Leu	Ile			
305	Trp	Asn	Glu	Ile	Gly	Arg	Gly	Ala	Asp	Leu	His	Glu	Leu	Glu	Gln	Trp			
	Leu	Ala	Asp	Asn	Asp	His	Ile	Ile	Lys	Glu	Leu	Thr	Thr	Ala	Val	Phe			
	Ala	Ser	Glu	Ala	Pro	His	Ile	Thr	Asp	Phe	Tyr	Pro	Ala	Glu	Lys	Ile			
	Asp	Leu	Gly	Arg	Ala	Lys	Ala	Gly	Glu	Gln	Ile	Phe	Lys	Asn	Thr	Cys			
	Ala	Lys	Cys	His	Gly	His	Tyr	Glu	Lys	Ala	Trp	Asn	Leu	Pro	Gln	Ala			
385	Leu	Val	Leu	Ser	Ala	Ala	Glu	Arg	Leu	Lys	Thr	Val	Glu	Val	Arg	Tyr			
	Lys	Glu	Lys	Thr	Pro	Val	Val	Asn	Val	Gly	Thr	Asp	Pro	Phe	Arg	Arg			
	Gln	Gly	Met	Lys	Ser	Leu	Glu	Gln	Leu	Asn	Asp	Leu	Glu	Ile	Ser	Lys			
	Lys	Asn	Gly	Ile	Val	Ile	Lys	Ala	Gln	Glu	Gly	Tyr	Val	Pro	Pro	Pro			
	Leu	Val	Gly	Ile	Trp	Ala	Arg	Trp	Pro	Tyr	Met	His	Asn	Asn	Ser	Ile			
465	Pro	Asn	Leu	Cys	Val	Leu	Leu	Thr	Pro	Ala	Lys	Lys	Arg	Pro	Ser	Ile			
	Tyr	Tyr	Ser	Gly	Glu	Ala	Leu	Asn	Lys	Asp	Thr	Asp	Tyr	Asp	Phe	Ser			
	Cys	Gly	Gly	Tyr	Pro	Ile	Gly	Asp	Lys	Thr	Pro	Lys	Ala	Trp	Lys	Thr			
	Arg	Glu	His	Leu	Tyr	Asp	Thr	Arg	Asn	Pro	Gly	Met	Gly	Asn	Met	Gly			
	His	Asp	Glu	Gly	Ile	Phe	Ile	Lys	Asp	Gly	Lys	Glu	Ile	Leu	Ser	Ala			
545	Glu	Asp	Lys	Tyr	Asn	Leu	Ile	Gln	Phe	Leu	Gln	Thr	Leu						

<210> 69

<211> 813

<212> PRT

<213> Propionibacterium acnes

<220>

<223> hyaluronan lyase

<400> 69

Met Phe Gly Thr Pro Ser Arg Arg Thr Phe Leu Thr Ala Ser Ala Leu

3066SEQ PC1.TXT

1	5	10	15
Ser Ala Met	Ala Leu Ala Ala Ser	Pro Thr Val Thr Asp	Ala Ile Ala
Ala Pro Gly	Pro Asp Ser Trp Ser	Ala Leu Cys Glu Arg	Trp Ile Asp
Ile Ile Thr	Gly Arg Arg Ala Ala	Arg Thr Ser Asp Pro	Arg Ala Arg
Ala Ile Ile	Ala Lys Thr Asp Arg	Lys Val Ala Glu Ile	Leu Thr Asp
Leu Val Ser	Gly Ser Ser Arg Gln Thr	Val Leu Ile Ser Ala	Asp Leu
Arg Lys Glu	Gln Ser Pro Phe Ile Thr	Lys Thr Ala Arg Ala	Ile Glu
Ser Met Ala	Cys Ala Trp Ala Thr	Pro Gly Ser Ser Tyr	His Lys Asp
Pro Glu Ile	Leu Ser Ala Cys Ile	Glu Gly Leu Arg Asp	Phe Cys Arg
Leu Arg Tyr	Asn Pro Ser Gln Asp	Glu Tyr Gly Asn Trp	Trp Asp Trp
Glu Asp Gly	Ala Ser Arg Ala Val	Ala Asp Val Met Cys	Ile Leu His
Asp Val Leu	Pro Pro Glu Val Met	Ser Ala Ala Ala Ala	Gly Ile Asp
His Phe Ile	Pro Asp Pro Trp Phe	Gln Gln Pro Ala Ser	Val Lys Pro
Thr Ala Asn	Pro Val Gln Pro Val	Val Ser Thr Gly Ala	Asn Arg Met
Asp Leu Thr	Arg Ala Val Met Cys	Arg Ser Ile Ala Thr	Gly Asp Glu
Lys Arg Leu	Arg His Ala Val Asp	Gly Leu Pro Asp Ala	Trp Arg Val
Thr Thr Glu	Gly Asp Gly Phe Arg	Ala Asp Gly Gly Phe	Ile Gln His
Ser His Ile	Pro Tyr Thr Gly Gly	Tyr Gly Asp Val Leu	Phe Ser Gly
Leu Ala Met	Leu Phe Pro Leu Val	Ser Gly Met Arg Phe	Asp Ile Val
Glu Ser Ala	Arg Lys Ala Phe His	Asp Gln Val Glu Arg	Gly Phe Ile
Pro Val Met	Tyr Asn Gly Gln Ile	Leu Asp Asp Val Arg	Gly Arg Ser
Ile Ser Arg	Ile Asn Glu Ser Ala	Ala Met His Gly Ile	Ser Ile Ala
Arg Ala Met	Leu Met Met Ala Asp	Ala Leu Pro Thr His	Arg Ala Glu
Gln Trp Arg	Gly Ile Val His Gly	Trp Met Ala Arg Asn	Thr Phe Asp
His Leu Ser	Glu Pro Ser Thr Leu	Val Asp Ile Ser Leu	Phe Asp Ala
Ala Ala Lys	Ala Arg Pro Val Pro	Glu Ser Ser Thr Pro	Ser Tyr Phe
Ala Ser Met	Asp Arg Leu Val His	Arg Thr Ala Asp Trp	Leu Ile Thr
Val Ser Asn	Cys Ser Asp Arg Ile	Ala Trp Tyr Glu Tyr	Gly Asn Gly
Glu Asn Glu	Trp Ala Ser Arg Thr	Ser Gln Gly Met Arg	Tyr Leu Leu
Leu Pro Gly	Asp Met Gly Gln Tyr	Glu Asp Gly Tyr Trp	Ala Thr Val
Asp Tyr Ser	Ala Pro Thr Gly Thr	Thr Val Asp Ser Thr	Pro Leu Lys
Arg Ala Val	Gly Ala Ser Trp Ala	Ala Lys Thr Pro Thr	Asn Glu Trp
Ser Gly Gly	Leu Ala Ser Gly Ser	Trp Ser Ala Ala Ala	Ser His Ile
Thr Ser Gln	Asp Ser Ala Leu Lys	Ala Arg Arg Leu Trp	Val Gly Leu
Lys Asp Ala	Met Val Glu Leu Thr	Thr Thr Asp Val Thr	Thr Asp Ala
Arg Ala Ile	Thr Val Glu His Arg	Lys Val Ala Ser Ser	Ser Ser Thr
Lys Leu Leu	Val Asp Gly Asn Arg	Val Ser Ser Ala Thr	Ser Phe Gln
Asn Pro Arg	Trp Ala His Leu Asp	Gly Val Gly Gly Tyr	Val Phe Ala

3066SEQ PC1.TXT

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

<210> 70

<211> 1072

<212> PRT

<213> Streptococcus agalactiae

<220>

<223> hyaluronan lyase

<400> 70

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

Leu 305	Tyr	Pro	Asn	Ala	Thr 310	Gly	Ser	Thr	Thr 315	Val	Lys	Ile	Ser	Asp	Lys 320
Ser	Gly	Lys	Ile 325	Ile	Lys	Glu	Val	Pro	Leu 330	Ser	Val	Thr	Ala	Ser 335	Thr
Glu	Asp	Lys	Phe 340	Thr	Lys	Leu	Leu	Asp 345	Lys	Trp	Asn	Asp	Val 350	Thr	Ile
Gly	Asn 355	His	Val	Tyr	Asp	Thr	Asn 360	Asp	Ser	Asn	Met	Gln 365	Lys	Ile	Asn
Gln 370	Lys	Leu	Asp	Glu	Thr	Asn 375	Ala	Lys	Asn	Ile	Lys 380	Thr	Ile	Lys	Leu
Asp 385	Ser	Asn	His	Thr	Phe 390	Leu	Trp	Lys	Asp	Leu 395	Asp	Asn	Leu	Asn	Asn 400
Ser	Ala	Gln	Leu	Thr 405	Ala	Thr	Tyr	Arg	Arg 410	Leu	Glu	Asp	Leu	Ala 415	Lys
Gln	Ile	Thr	Asn 420	Pro	His	Ser	Thr	Ile 425	Tyr	Lys	Asn	Glu	Lys 430	Ala	Ile
Arg	Thr	Val 435	Lys	Glu	Ser	Leu	Ala 440	Trp	Leu	His	Gln	Asn 445	Phe	Tyr	Asn
Val	Asn 450	Lys	Asp	Ile	Glu	Gly 455	Ser	Ala	Asn	Trp	Trp 460	Asp	Phe	Glu	Ile
Gly 465	Val	Pro	Arg	Ser	Ile 470	Thr	Ala	Thr	Leu	Ala 475	Leu	Met	Asn	Asn	Tyr 480
Phe	Thr	Asp	Ala 485	Glu	Ile	Lys	Thr	Tyr	Thr 490	Asp	Pro	Ile	Glu	His 495	Phe
Val	Pro	Asp	Ala 500	Gly	Tyr	Phe	Arg	Lys 505	Thr	Leu	Asp	Asn	Pro 510	Phe	Lys
Ala	Leu	Gly 515	Gly	Asn	Leu	Val	Asp 520	Met	Gly	Arg	Val	Lys 525	Ile	Ile	Glu
Gly 530	Leu	Leu	Arg	Lys	Asp	Asn 535	Thr	Ile	Ile	Glu	Lys 540	Thr	Ser	His	Ser
Leu 545	Lys	Asn	Leu	Phe 550	Thr	Ala	Thr	Lys	Ala 555	Glu	Gly	Phe	Tyr	Ala 560	
Asp	Gly	Ser	Tyr	Ile 565	Asp	His	Thr	Asn	Val 570	Ala	Tyr	Thr	Gly	Ala 575	Tyr
Gly	Asn	Val 580	Leu	Ile	Asp	Gly	Leu	Thr 585	Gln	Leu	Leu	Pro	Ile 590	Ile	Gln
Glu	Thr	Asp 595	Tyr	Lys	Ile	Ser	Asn 600	Gln	Glu	Leu	Asp	Met 605	Val	Tyr	Lys
Trp	Ile 610	Asn	Gln	Ser	Phe 615	Leu	Pro	Leu	Ile	Val	Lys 620	Gly	Glu	Leu	Met
Asp 625	Met	Ser	Arg	Gly	Arg 630	Ser	Ile	Ser	Arg	Glu 635	Ala	Ala	Ser	Ser	His 640
Ala	Ala	Ala	Val 645	Glu	Val	Leu	Arg	Gly	Phe 650	Leu	Arg	Leu	Ala	Asn 655	Met
Ser	Asn	Glu	Glu 660	Arg	Asn	Leu	Asp	Leu 665	Lys	Ser	Thr	Ile	Lys 670	Thr	Ile
Ile	Thr	Ser 675	Asn	Lys	Phe	Tyr	Asn 680	Val	Phe	Asn	Asn	Leu 685	Lys	Ser	Tyr
Ser	Asp 690	Ile	Ala	Asn	Met	Asn 695	Lys	Met	Leu	Asn	Asp 700	Ser	Thr	Val	Ala
Thr 705	Lys	Pro	Leu	Lys	Ser 710	Asn	Leu	Ser	Thr	Phe 715	Asn	Ser	Met	Asp	Arg 720
Leu	Ala	Tyr	Tyr	Asn 725	Ala	Glu	Lys	Asp	Phe 730	Gly	Phe	Ala	Leu	Ser 735	Leu
His	Ser	Lys	Arg 740	Thr	Leu	Asn	Tyr	Glu 745	Gly	Met	Asn	Asp	Glu 750	Asn	Thr
Arg	Gly	Trp 755	Tyr	Thr	Gly	Asp	Gly 760	Met	Phe	Tyr	Leu	Tyr 765	Asn	Ser	Asp
Gln	Ser 770	His	Tyr	Ser	Asn 775	His	Phe	Trp	Pro	Thr	Val 780	Asn	Pro	Tyr	Lys
Met 785	Ala	Gly	Thr	Thr	Glu 790	Lys	Asp	Ala	Lys	Arg 795	Glu	Asp	Thr	Thr	Lys 800
Glu	Phe	Met	Ser	Lys 805	His	Ser	Lys	Asp	Ala 810	Lys	Glu	Lys	Thr	Gly 815	Gln
Val	Thr	Gly	Thr 820	Ser	Asp	Phe	Val	Gly 825	Ser	Val	Lys	Leu	Asn 830	Asp	His
Phe	Ala	Leu	Ala 835	Ala	Met	Asp	Phe 840	Thr	Asn	Trp	Asp	Arg 845	Thr	Leu	Thr
Ala	Gln 850	Lys	Gly	Trp	Val	Ile 855	Leu	Asn	Asp	Lys	Ile 860	Val	Phe	Leu	Gly
Ser 865	Asn	Ile	Lys	Asn	Thr 870	Asn	Gly	Ile	Gly	Asn 875	Val	Ser	Thr	Thr	Ile 880
Asp	Gln	Arg	Lys	Asp 885	Asp	Ser	Lys	Thr	Pro 890	Tyr	Thr	Thr	Tyr	Val 895	Asn
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Tyr Ile Phe Phe Lys Asn Ser Thr Ile Asp Ile Glu Arg Lys Glu Gln
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Thr Gly Thr Trp Asn Ser Ile Asn Arg Thr Ser Lys Asn Thr Ser Ile
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Val Ser Asn Pro Phe Ile Thr Ile Ser Gln Lys His Asp Asn Lys Gly
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Asp Ser Tyr Gly Tyr Met Met Val Pro Asn Ile Asp Arg Thr Ser Phe
          985          990          995
Asp Lys Leu Ala Asn Ser Lys Glu Val Glu Leu Leu Glu Asn Ser Ser
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Lys Gln Gln Val Ile Tyr Asp Lys Asn Ser Gln Thr Trp Ala Val Ile
          1015          1020          1025
Lys His Asp Asn Gln Glu Ser Leu Ile Asn Asn Gln Phe Lys Met Asn
          1030          1035          1040
Lys Ala Gly Leu Tyr Leu Val Gln Lys Val Gly Asn Asp Tyr Gln Asn
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<220>
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Leu Thr Lys Thr Thr Ser Thr Ser Gln Ile Arg Asp Thr Gln Thr Asn
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Asn Ile Glu Val Leu Gln Thr Glu Ser Thr Thr Val Lys Glu Thr Ser
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Thr Thr Thr Thr Gln Gln Asp Leu Ser Asn Pro Thr Ala Ser Thr Ala
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Thr Ala Thr Ala Thr His Ser Thr Met Lys Gln Val Val Asp Asn Gln
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Thr Gln Asn Lys Glu Leu Val Lys Asn Gly Asp Phe Asn Gln Thr Asn
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Pro Val Ser Gly Ser Trp Ser His Thr Ser Ala Arg Glu Trp Ser Ala
          115          120          125
Trp Ile Asp Lys Glu Asn Thr Ala Asp Lys Ser Pro Ile Ile Gln Arg
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Thr Glu Gln Gly Gln Val Ser Leu Ser Ser Asp Lys Gly Phe Arg Gly
          145          150          155          160
Ala Val Thr Gln Lys Val Asn Ile Asp Pro Thr Lys Lys Tyr Glu Val
          165          170          175
Lys Phe Asp Ile Glu Thr Ser Asn Lys Ala Gly Gln Ala Phe Leu Arg
          180          185          190
Ile Met Glu Lys Lys Asp Asn Asn Thr Arg Leu Trp Leu Ser Glu Met
          195          200          205
Thr Ser Gly Thr Thr Asn Lys His Thr Leu Thr Lys Ile Tyr Asn Pro
          210          215          220
Lys Leu Asn Val Ser Glu Val Thr Leu Glu Leu Tyr Tyr Glu Lys Gly
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Thr Gly Ser Ala Thr Phe Asp Asn Ile Ser Met Lys Ala Lys Gly Pro
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Lys Asp Ser Glu His Pro Gln Pro Val Thr Thr Gln Ile Glu Glu Ser
          260          265          270
Val Asn Thr Ala Leu Asn Lys Asn Tyr Val Phe Asn Lys Ala Asp Tyr
          275          280          285
Gln Tyr Thr Leu Thr Asn Pro Ser Leu Gly Lys Ile Val Gly Gly Ile
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Leu Tyr Pro Asn Ala Thr Gly Ser Thr Thr Val Lys Ile Ser Asp Lys
          305          310          315          320
Ser Gly Lys Ile Ile Lys Glu Val Pro Leu Ser Val Thr Ala Ser Thr
          325          330          335
Glu Asp Lys Phe Thr Lys Leu Leu Asp Lys Trp Asn Asp Val Thr Ile

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 Val Ser Asn Pro Phe Ile Thr Ile Ser Gln Lys His Asp Asn Lys Gly
 965 970 975
 Asp Ser Tyr Gly Tyr Met Met Val Pro Asn Ile Asp Arg Thr Ser Phe
 980 985 990
 Asp Lys Leu Ala Asn Ser Lys Glu Val Glu Leu Leu Glu Asn Ser Ser
 995 1000 1005
 Lys Gln Gln Val Ile Tyr Asp Lys Asn Ser Gln Thr Trp Ala Val Ile
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 Lys His Asp Asn Gln Glu Ser Leu Ile Asn Asn Gln Phe Lys Met Asn
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 Lys Ala Gly Leu Tyr Leu Val Gln Lys Val Gly Asn Asp Tyr Gln Asn
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<211> 1081

<212> PRT

<213> Streptococcus agalactiae serotype Ia

<220>

<223> hyaluronan lyase

<400> 72

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 35 40 45
 Asn Val Glu Val Pro Gln Thr Glu Ser Thr Thr Val Lys Gly Thr Ser
 50 55 60
 Thr Thr Thr Thr Gln Gln Asp Leu Ser Asn Ser Thr Ala Ser Thr Ala
 65 70 75 80
 Thr Ala Thr Ala Thr His Ser Thr Met Lys Gln Val Val Asp Asn Gln
 85 90 95
 Thr Gln Asn Lys Glu Leu Val Lys Asn Gly Asp Phe Lys Glu Lys Ile
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 Ile Asp Lys Lys Ile Asp Lys Lys Ser Gln Trp Thr Asn Leu Tyr Gly
 115 120 125
 Ala Lys Asp Trp Asn Thr Tyr Ile Asp Gln Thr Lys Ser Val Asn Lys
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 Ser Pro Ile Ile Gln Arg Thr Glu Gln Gly Gln Val Ser Leu Ser Ser
 145 150 155 160

Asp Lys Gly Phe Arg Gly Ala Val Thr Gln Lys Val Asn Ile Asp Pro
 165 170 175
 Thr Lys Lys Tyr Glu Val Lys Phe Asp Ile Glu Thr Ser Asn Lys Val
 180 185 190
 Gly Gln Ala Phe Leu Arg Ile Met Lys Lys Lys Asp Lys Asn Thr Arg
 195 200 205
 Leu Trp Leu Ser Glu Met Thr Ser Gly Thr Thr Asn Lys His Thr Leu
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 Thr Lys Ile Tyr Asn Pro Lys Leu Asn Val Ser Glu Val Thr Leu Glu
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 Leu Tyr Tyr Glu Lys Gly Thr Gly Ser Val Thr Phe Asp Asn Ile Ser
 245 250 255
 Met Lys Ala Lys Gly Pro Lys Asp Ser Glu His Pro Gln Pro Val Thr
 260 265 270
 Thr Gln Ile Glu Glu Ser Val Asn Thr Ala Leu Asn Lys Asn Tyr Val
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 Phe Asn Lys Ala Asp Tyr Gln Tyr Thr Leu Thr Asn Pro Ser Leu Gly
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 Lys Ile Val Gly Gly Ile Leu Tyr Pro Ser Ala Thr Gly Ser Thr Thr
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 Val Lys Ile Ser Asp Lys Ser Gly Lys Ile Ile Lys Glu Val Pro Leu
 325 330 335
 Ser Val Thr Ala Ser Thr Glu Asp Asn Phe Thr Lys Leu Leu Asp Lys
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 Trp Asn Asp Val Thr Ile Gly Asn His Val Tyr Asp Thr Asn Asp Ser
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 Asn Met Gln Lys Leu Asn Gln Lys Leu Asp Glu Thr Asn Ala Lys Asn
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Ile 385	Lys	Asp	Ile	Lys	Leu 390	Asp	Ser	Asn	Arg	Thr	Phe	Leu	Trp	Glu	Asp 400
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Lys	Asn	Glu 435	Lys	Ala	Ile	Arg	Thr 440	Val	Lys	Glu	Ser	Leu 445	Ala	Trp	Leu
His	Gln 450	Asn	Phe	Tyr	Asn 455	Val	Asn	Lys	Asp	Ile	Glu 460	Gly	Ser	Ala	Asn
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Ala	Leu	Met	Asn 485	Asn	Tyr	Phe	Thr	Asp	Ala 490	Glu	Ile	Lys	Thr	Tyr 495	Thr
Asp	Pro	Ile	Glu 500	His	Phe	Val	Pro	Asp 505	Ala	Gly	Tyr	Phe	Arg 510	Lys	Thr
Leu	Val 515	Asn	Pro	Phe	Lys	Ala	Leu 520	Gly	Gly	Asn	Leu	Val 525	Asp	Met	Gly
Arg	Val 530	Lys	Ile	Ile	Glu	Gly 535	Leu	Leu	Arg	Lys	Asp 540	Asn	Thr	Ile	Ile
Lys 545	Lys	Thr	Ser	His	Ser 550	Leu	Lys	Asn	Leu	Phe 555	Thr	Thr	Ala	Thr	Lys 560
Ala	Glu	Gly	Phe	Tyr 565	Ala	Asp	Gly	Ser	Tyr 570	Ile	Asp	His	Thr	Asn 575	Val
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Leu	Asp 610	Met	Val	Tyr	Lys	Trp 615	Ile	Asn	Gln	Ser	Phe 620	Leu	Pro	Leu	Ile
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Glu	Ala	Ala	Ser	Ser 645	His	Ala	Ala	Ala	Val 650	Glu	Val	Leu	Arg	Gly 655	Phe
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Ser	Thr	Ile 675	Lys	Thr	Ile	Ile	Thr 680	Ser	Asn	Lys	Phe	Tyr 685	Asn	Val	Phe
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Asn 705	Asp	Ser	Thr	Val 710	Ala	Thr	Lys	Pro	Leu	Lys 715	Ser	Asn	Leu	Ser	Thr 720
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Gly	Phe	Ala	Leu 740	Ser	Leu	His	Ser	Lys 745	Arg	Thr	Leu	Asn	Tyr 750	Glu	Gly
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Gln	Ala	Ser 915	Ser	Gln	Gln	Phe	Thr 920	Asp	Thr	Lys	Ser	Val 925	Phe	Leu	Glu
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415

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Ala	Ser	Ser	Gln 820	Gln	Phe	Thr	Asp	Thr 825	Lys	Ser	Val	Phe	Leu 830	Glu	Ser
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Lys	Val	Gly	Asn	Asp 965	Tyr	Gln	Asn	Val	Tyr 970	Tyr	Gln	Pro	Gln	Thr 975	Met
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<400> 74

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Glu	Asp	Lys	Asp	Met	Met	Lys	Lys	Ser	Ile	Asp	Ser	Phe	Asn	Lys	Val
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Phe	Thr	Tyr	Val	Gln	Asp	Ser	Ala	Thr	Gly	Lys	Glu	Arg	Asn	Gly	Phe
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Tyr	Lys	Asp	Gly	Ser	Tyr	Ile	Asp	His	Gln	Asp	Val	Pro	Tyr	Thr	Gly
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Ala	Tyr	Gly	Val	Val	Leu	Glu	Gly	Ile	Ser	Gln	Met	Met	Pro	Met	
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Ile	Lys	Glu	Thr	Pro	Phe	Asn	Asp	Lys	Thr	Gln	Asn	Asp	Thr	Thr	Leu
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Lys	Ser	Trp	Ile	Asp	Asp	Gly	Phe	Met	Pro	Leu	Ile	Tyr	Lys	Gly	Glu
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Met	Met	Asp	Leu	Ser	Arg	Gly	Arg	Ala	Ile	Ser	Arg	Glu	Asn	Glu	Thr
		355					360					365			
Ser	His	Ser	Ala	Ser	Ala	Thr	Val	Met	Lys	Ser	Leu	Leu	Arg	Leu	Ser
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Asp	Ala	Met	Asp	Asp	Ser	Thr	Lys	Ala	Lys	Tyr	Lys	Lys	Ile	Val	Lys
385					390					395					400
Ser	Ser	Val	Glu	Ser	Asp	Ser	Ser	Tyr	Lys	Gln	Asn	Asp	Tyr	Leu	Asn
				405					410					415	
Ser	Tyr	Ser	Asp	Ile	Asp	Lys	Met	Lys	Ser	Leu	Met	Thr	Asp	Asn	Ser
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Ile	Ser	Lys	Asn	Gly	Leu	Thr	Gln	Gln	Leu	Lys	Ile	Tyr	Asn	Asp	Met
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Asp	Arg	Val	Thr	Tyr	His	Asn	Lys	Asp	Leu	Asp	Phe	Ala	Phe	Gly	Leu
	450					455					460				
Ser	Met	Thr	Ser	Lys	Asn	Val	Ala	Arg	Tyr	Glu	Ser	Ile	Asn	Gly	Glu
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Ser	Asp	Val	Lys	His	Tyr	His	Asp	Asn	Phe	Trp	Val	Thr	Ala	Asp	Met
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Thr	Leu	Thr	Ala	Lys	Lys	Ser	Tyr	Phe	Ile	Leu	Asn	Asp	Lys	Ile
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Phe	Leu	Gly	Thr	Gly	Ile	Lys	Ser	Thr	Asp	Ser	Ser	Lys	Asn	Pro
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Thr	Thr	Ile	Glu	Asn	Arg	Lys	Ala	Asn	Gly	Tyr	Thr	Leu	Tyr	Thr
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Asp	Lys	Gln	Thr	Thr	Asn	Ser	Asp	Asn	Gln	Glu	Asn	Asn	Ser	Val
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Leu	Glu	Ser	Thr	Asp	Thr	Lys	Lys	Asn	Ile	Gly	Tyr	His	Phe	Leu
625					630					635				640
Lys	Pro	Lys	Ile	Thr	Val	Lys	Lys	Glu	Ser	His	Thr	Gly	Lys	Trp
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Glu	Ile	Asn	Lys	Ser	Gln	Lys	Asp	Thr	Gln	Lys	Thr	Asp	Glu	Tyr
			660					665					670	Tyr
Glu	Val	Thr	Gln	Lys	His	Ser	Asn	Ser	Asp	Asn	Lys	Tyr	Gly	Tyr
		675					680					685		Val
Leu	Tyr	Pro	Gly	Leu	Ser	Lys	Asp	Val	Phe	Lys	Thr	Lys	Lys	Asp
	690					695					700			Glu
Val	Thr	Val	Val	Lys	Gln	Glu	Asp	Asp	Phe	His	Val	Val	Lys	Asp
705					710					715				Asn
Glu	Ser	Val	Trp	Ala	Gly	Val	Asn	Tyr	Ser	Asn	Ser	Thr	Gln	Thr
				725					730					735
Asp	Ile	Asn	Asn	Thr	Lys	Val	Glu	Val	Lys	Ala	Lys	Gly	Met	Phe
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Leu	Lys	Lys	Lys	Asp	Asp	Asn	Thr	Tyr	Glu	Cys	Ser	Phe	Tyr	Asn
		755					760					765		Pro
Glu	Ser	Thr	Asn	Ser	Ala	Ser	Asp	Ile	Glu	Ser	Lys	Ile	Ser	Met
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Gly	Tyr	Ser	Ile	Thr	Asn	Lys	Asn	Thr	Ser	Thr	Ser	Asn	Glu	Ser
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<220>
 <223> hyaluronan lyase

<400> 75

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Tyr	Glu	Lys	Leu	Arg	Asn	Ile	Trp	Leu	Asp	Val	Asn	Tyr	Gly	Tyr	Asp
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Lys	Tyr	Asp	Glu	Asn	Asn	Pro	Asp	Met	Lys	Lys	Lys	Phe	Glu	Ala	Thr
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Glu	Asn	Glu	Ala	Glu	Lys	Leu	Leu	Lys	Glu	Met	Lys	Thr	Glu	Ser	Asp
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Arg	Lys	Tyr	Leu	Trp	Glu	Ser	Ser	Lys	Asp	Leu	Asp	Thr	Lys	Ser	Ala
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Asp	Met	Thr	Arg	Thr	Tyr	Arg	Asn	Ile	Glu	Lys	Ile	Ser	Glu	Ala	Met
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Lys	His	Lys	Asn	Thr	Lys	Leu	Lys	Thr	Asp	Glu	Asn	Lys	Thr	Lys	Val
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Lys	Asp	Ala	Leu	Glu	Trp	Leu	His	Lys	Asn	Ala	Tyr	Gly	Lys	Glu	Pro
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Asp	Lys	Lys	Val	Ala	Asp	Leu	Thr	Ser	Asn	Phe	Lys	Asn	Lys	Thr	Ser
				165					170					175	
Arg	Asn	Thr	Asn	Leu	Asn	Trp	Trp	Asp	Tyr	Glu	Ile	Gly	Thr	Pro	Arg
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Glu	Lys	Lys	Lys	Tyr	Thr	Ala	Pro	Ile	Lys	Thr	Phe	Ala	Pro	Asp	Ser
	210					215					220				

Asp Lys Ile Leu Ser Ser Val Gly Lys Ser Glu Pro Ala Lys Gly Gly
 225 230 235 240
 Asn Leu Val Asp Ile Ser Lys Val Lys Leu Leu Glu Ser Ile Ile Glu
 245 250 255
 Glu Asp Lys Asp Met Met Lys Lys Ser Ile Asp Ser Phe Asn Thr Val
 260 265 270
 Phe Thr Tyr Ala Gln Asn Ser Ala Thr Gly Lys Glu Arg Asn Gly Phe
 275 280 285
 Tyr Lys Asp Gly Ser Tyr Ile Asp His Gln Asp Val Pro Tyr Thr Gly
 290 295 300
 Ala Tyr Gly Val Val Leu Leu Glu Gly Ile Ser Gln Met Met Pro Met
 305 310 315
 Ile Lys Glu Thr Pro Phe Asn Asp Ser Asn Gln Asn Asp Thr Thr Leu
 325 330 335
 Lys Ser Trp Ile Asp Asp Gly Phe Met Pro Leu Ile Tyr Lys Gly Glu
 340 345 350
 Met Met Asp Leu Ser Arg Gly Arg Ala Ile Ser Arg Glu Asn Glu Thr
 355 360 365
 Ser His Ser Ala Ser Ala Thr Val Met Lys Ser Leu Leu Arg Leu Ser
 370 375 380
 Asp Thr Met Asp Lys Ser Thr Lys Ala Lys Tyr Lys Lys Ile Val Lys
 385 390 395 400
 Thr Ser Val Glu Ser Asp Ser Ser Tyr Lys Gln Thr Asp Tyr Leu Ser
 405 410 415
 Ser Tyr Ser Asp
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<210> 76

<211> 806

<212> PRT

<213> Staphylococcus aureus (strain MRSA252)

<220>

<223> hyaluronan lyase

<400> 76

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

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Gly 305	Ala	Tyr	Gly	Val 310	Val 310	Leu	Leu	Glu	Gly 315	Ile 315	Ser	Gln	Met	Met	Pro 320
Met	Ile	Lys	Glu	Ser 325	Pro	Phe	Lys	Thr	Thr 330	Gln	Asp	Asn	Ala	Thr 335	Leu
Ser	Asn	Trp	Ile 340	Asp	Glu	Gly	Phe	Met 345	Pro	Leu	Ile	Tyr	Lys 350	Gly	Glu
Met	Met	Asp 355	Leu	Ser	Arg	Gly	Arg 360	Ala	Ile	Ser	Arg	Glu 365	Asn	Glu	Thr
Ser	His 370	Thr	Ala	Ser	Ala	Thr 375	Val	Met	Lys	Ser	Leu	Leu	Arg	Leu	Asn
Asp 385	Thr	Met	Asp	Asp	Ser 390	Thr	Lys	Thr	Arg	Tyr 395	Lys	Gln	Ile	Val	Lys 400
Thr	Ser	Val	Asn	Ser 405	Asp	Ser	Ser	Tyr	Asn 410	Gln	Asn	Asn	Tyr	Leu 415	Asn
Ser	Tyr	Ser	Asp 420	Ile	Ala	Lys	Met	Lys 425	Lys	Leu	Met	Asn	Asp 430	Ser	Thr
Ile	Ser	Lys 435	Asn	Asp	Leu	Thr	Gln 440	Gln	Leu	Lys	Ile	Tyr 445	Asn	Asp	Met
Asp 450	Arg	Val	Thr	Tyr	His	Asn 455	Lys	Asp	Leu	Asp	Phe 460	Ala	Phe	Gly	Leu
Ser 465	Met	Thr	Ser	Lys	Asn 470	Ile	Ala	Arg	Tyr	Glu 475	Asn	Ile	Asn	Gly	Glu 480
Asn	Leu	Lys	Gly	Trp 485	His	Thr	Gly	Ala	Gly 490	Met	Ser	Tyr	Leu	Tyr 495	Asn
Ser	Asp	Val	Lys 500	His	Tyr	Arg	Asp	Asn 505	Phe	Trp	Ala	Thr	Ala 510	Asp	Met
Thr	Cys	Leu	Pro	Gly	Thr	Thr	Thr 520	Leu	Asn	Asp	Met	Pro 525	Ser	Thr	Asn
Thr 530	Lys	Asn	Asp	Lys	Ser	Phe 535	Val	Gly	Gly	Thr	Lys 540	Leu	Asn	Asn	Lys
Tyr 545	Ala	Ser	Ile	Gly	Met 550	Asp	Phe	Glu	Asn	Gln 555	Asp	Lys	Thr	Leu	Thr 560
Ala	Lys	Lys	Ser	Tyr 565	Phe	Ile	Leu	Asn	Asp 570	Lys	Ile	Val	Phe	Leu 575	Gly
Thr	Gly	Ile	Lys 580	Ser	Thr	Asp	Ser	Ser 585	Lys	Asn	Pro	Val	Thr 590	Ser	Val
Glu	Asn	Arg 595	Lys	Ala	Asn	Gly	Tyr 600	Lys	Leu	Phe	Lys	Asp 605	Asp	Ile	Glu
Ile 610	Thr	Thr	Ser	Asp	Val	Asn 615	Ala	Gln	Glu	Thr	His 620	Ser	Val	Phe	Leu
Glu 625	Ser	Asn	Asp	Thr	Lys 630	Lys	Asn	Ile	Gly	Tyr 635	His	Phe	Leu	Asp	Lys 640
Pro	Lys	Ile	Thr	Val 645	Lys	Lys	Glu	Ser	His 650	Thr	Gly	Lys	Trp	Ser 655	Glu
Ile	Asn	Lys	Ser 660	Gln	Lys	Lys	Asp	Asp 665	Lys	Lys	Asp	Glu	Tyr 670	Tyr	Glu
Val	Thr	Gln	Thr 675	His	Asn	Thr	Ser 680	Asp	Ser	Lys	Tyr	Ala	Tyr 685	Val	Leu
Tyr 690	Pro	Gly	Leu	Ser	Lys	Ser 695	Asp	Phe	Lys	Ser	Lys 700	Asn	Asn	Val	
Ser 705	Ile	Val	Lys	Gln	Asp 710	Glu	Asp	Phe	His	Val 715	Ile	Lys	Asp	Asn	Asp 720
Gly	Val	Phe	Ala	Gly 725	Val	Asn	Tyr	Ser	Asp 730	Asn	Thr	Lys	Ser	Phe	Asp 735
Ile	Asn	Gly	Ile 740	Thr	Val	Glu	Leu	Lys 745	Glu	Lys	Gly	Met	Phe 750	Val	Ile
Lys	Lys	Lys 755	Asp	Asp	Lys	Ala	Tyr 760	Lys	Cys	Ser	Phe	Tyr 765	Asn	Pro	Glu
Thr 770	Thr	Asn	Thr	Ala	Ser	Asn 775	Ile	Glu	Ser	Lys	Ile 780	Phe	Ile	Lys	Gly
Tyr 785	Thr	Ile	Thr	Asn	Lys 790	Ser	Val	Ile	Asn	Ser 795	Asn	Asp	Ala	Gly	Val 800
Asn	Phe	Glu	Leu	Thr 805	Lys										

<210> 77

<211> 815

<212> PRT

<213> Staphylococcus aureus (strain MSSA476)

<220>

<223> hyaluronan lyase

<400> 77

Met	Thr	Tyr	Arg	Ile	Lys	Lys	Trp	Gln	Lys	Leu	Ser	Thr	Ile	Thr	Leu
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Leu	Met	Ala	Gly	Val	Ile	Thr	Leu	Asn	Gly	Gly	Glu	Phe	Arg	Ser	Ile
		20						25					30		
Asp	Lys	Tyr	Gln	Ile	Ala	Val	Ala	Asp	Thr	Asn	Val	Gln	Thr	Pro	Asp
		35					40					45			
Tyr	Glu	Lys	Leu	Arg	Asn	Thr	Trp	Leu	Asp	Val	Asn	Tyr	Gly	Tyr	Asp
	50					55					60				
Lys	Tyr	Asp	Glu	Lys	Asn	Asp	Ala	Met	Lys	Lys	Lys	Phe	Glu	Ala	Thr
65					70					75				80	
Glu	Asn	Glu	Ala	Lys	Lys	Leu	Leu	Ser	Glu	Met	Lys	Thr	Glu	Ser	Asp
				85					90					95	
Arg	Lys	Tyr	Leu	Trp	Glu	Asn	Ser	Lys	Asp	Leu	Asp	Thr	Lys	Ser	Ala
			100					105					110		
Asp	Met	Thr	Arg	Thr	Tyr	Arg	Asn	Ile	Glu	Lys	Ile	Ala	Glu	Ala	Met
		115					120					125			
Lys	His	Lys	Asp	Thr	Lys	Leu	Lys	Ile	Asp	Glu	Asn	Lys	Lys	Lys	Val
	130					135					140				
Lys	Asp	Ala	Leu	Glu	Trp	Leu	His	Lys	Asn	Ala	Tyr	Gly	Lys	Glu	Pro
145					150					155					160
Asp	Lys	Lys	Val	Ala	Asp	Leu	Thr	Ser	Asn	Phe	Lys	Asn	Lys	Thr	Ser
				165					170					175	
Arg	Asn	Thr	Asn	Leu	Asn	Trp	Trp	Asp	Tyr	Glu	Ile	Gly	Thr	Pro	Arg
			180					185					190		
Ala	Leu	Thr	Asn	Thr	Leu	Ile	Leu	Asn	Asp	Gln	Phe	Ser	Asn	Asp	
		195					200				205				
Glu	Lys	Lys	Lys	Tyr	Thr	Ala	Pro	Ile	Lys	Thr	Phe	Ala	Pro	Glu	Ser
	210					215					220				
Asp	Lys	Ile	Leu	Ser	Ser	Val	Gly	Gln	Pro	Glu	Gln	Ala	Lys	Gly	Gly
225					230					235					240
Asn	Leu	Val	Asp	Ile	Ala	Lys	Val	Lys	Leu	Leu	Glu	Ser	Ile	Ile	Glu
				245					250					255	
Glu	Asp	Lys	Asp	Ile	Thr	Lys	Asn	Ser	Ile	Asp	Ala	Phe	Asn	Lys	Val
			260					265					270		
Phe	Thr	Tyr	Val	Gln	Ser	Asn	Ala	Thr	Gly	Lys	Glu	Arg	Asn	Gly	Phe
		275					280					285			
Tyr	Lys	Asp	Gly	Ser	Tyr	Ile	Asp	His	Gln	Asp	Val	Pro	Tyr	Thr	Gly
	290					295					300				
Ala	Tyr	Gly	Val	Val	Leu	Leu	Glu	Gly	Ile	Ser	Gln	Met	Met	Pro	Met
305					310					315					320
Ile	Lys	Glu	Thr	Pro	Phe	Asn	Asp	Lys	Thr	Gln	Asn	Asp	Thr	Thr	Leu
				325										335	
Lys	Ser	Trp	Ile	Asp	Asp	Gly	Phe	Met	Pro	Leu	Ile	Tyr	Lys	Gly	Glu
			340					345					350		
Met	Met	Asp	Leu	Ser	Arg	Gly	Arg	Ala	Ile	Ser	Arg	Glu	Asn	Glu	Thr
		355					360					365			
Ser	His	Thr	Ala	Ser	Ala	Thr	Val	Met	Lys	Ser	Leu	Leu	Arg	Leu	Ser
	370					375					380				
Asp	Ala	Met	Asp	Asp	Ser	Thr	Lys	Ala	Lys	Tyr	Lys	Gln	Ile	Val	Lys
385					390					395					400
Thr	Ser	Val	Lys	Ser	Asp	Ser	Ser	Tyr	Gly	Gln	Asn	Asp	Thr	Leu	Ser
				405					410					415	
Ser	Tyr	Ser	Asp	Ile	Ser	Lys	Met	Lys	Ser	Leu	Met	Glu	Asp	Ser	Thr
			420					425					430		
Ile	Ser	Thr	Asn	Gly	Leu	Thr	Gln	Gln	Leu	Lys	Ile	Tyr	Asn	Asp	Met
			435				440					445			
Asp	Arg	Val	Thr	Tyr	His	Asn	Lys	Asp	Leu	Asp	Phe	Ala	Phe	Gly	Leu
	450					455					460				
Ser	Met	Thr	Ser	Lys	Asn	Val	Ala	Arg	Tyr	Glu	Ser	Ile	Asn	Gly	Glu
465					470					475					480
Asn	Leu	Lys	Gly	Trp	His	Thr	Gly	Ala	Gly	Met	Ser	Tyr	Leu	Tyr	Asn
				485					490					495	
Ser	Asp	Val	Lys	His	Tyr	Arg	Asp	Asn	Phe	Trp	Ala	Thr	Ala	Asp	Met
			500					505					510		
Lys	Arg	Leu	Ala	Gly	Thr	Thr	Thr	Leu	Glu	Asn	Glu	Glu	Pro	Lys	Gly
			515				520					525			
Thr	Asp	Val	Lys	Lys	Ser	Ser	Lys	Thr	Phe	Val	Gly	Gly	Thr	Lys	Phe
	530					535					540				
Asp	Asp	Gln	His	Ala	Ser	Ile	Gly	Met	Asp	Phe	Glu	Asn	Gln	Asp	Lys
545					550					555					560
Thr	Leu	Thr	Ala	Lys	Lys	Ser	Tyr	Phe	Ile	Leu	Asn	Asp	Lys	Ile	Val
				565					570					575	
Phe	Leu	Gly	Thr	Gly	Ile	Lys	Ser	Thr	Asp	Ser	Ser	Lys	Asn	Pro	Val
			580					585					590		
Thr	Thr	Ile	Glu	Asn	Arg	Lys	Ala	Asn	Gly	Tyr	Thr	Leu	Tyr	Thr	Asp

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

<210> 78

<211> 807

<212> PRT

<213> Staphylococcus aureus (strain NCTC 8325)

<220>

<223> hyaluronan lyase

<400> 78

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

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

<210> 79
 <211> 809
 <212> PRT
 <213> Staphylococcus aureus (strain bovine RF122)

<220>
 <223> hyaluronan lyase

<400> 79

Met	Thr	Tyr	Arg	Met	Lys	Lys	Trp	Gln	Lys	Leu	Ser	Thr	Ile	Thr	Leu
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Leu	Met	Ala	Gly	Gly	Ile	Thr	Phe	Asn	Asp	Ser	Glu	Phe	Arg	Ser	Val
		20						25					30		
Asp	Lys	His	Gln	Ile	Ala	Val	Ala	Asp	Thr	Asn	Val	Gln	Thr	Pro	Asn
		35					40					45			
Tyr	Glu	Lys	Leu	Lys	Asn	Thr	Trp	Leu	Asp	Val	Asn	Tyr	Gly	Tyr	Asp
	50				55						60				
Lys	Tyr	Asp	Glu	Ser	Asn	Pro	Asp	Met	Lys	Lys	Lys	Phe	Glu	Ala	Thr
65					70				75					80	
Glu	Lys	Glu	Ala	Arg	Lys	Leu	Leu	Ser	Glu	Met	Lys	Thr	Glu	Ser	Asp
				85					90					95	
Arg	Lys	Tyr	Leu	Trp	Glu	Asn	Ser	Lys	Asp	Leu	Asp	Thr	Lys	Ser	Ala
			100					105					110		
Asp	Met	Thr	Arg	Thr	Tyr	Arg	Asn	Ile	Glu	Lys	Ile	Ala	Glu	Ala	Met
		115					120					125			
Lys	His	Pro	Lys	Thr	Thr	Leu	Lys	Asn	Asp	Glu	Asn	Lys	Lys	Lys	Val
	130					135					140				
Lys	Asp	Ala	Leu	Glu	Trp	Leu	His	Lys	Asn	Ala	Tyr	Gly	Lys	Glu	Pro
145					150					155					160
Gly	Lys	Lys	Val	Ala	Asp	Leu	Lys	Thr	Asn	Phe	Ser	Lys	Ser	Ala	Pro
				165					170					175	
Gln	Lys	Asn	Thr	Asn	Leu	Asn	Trp	Trp	Asp	Tyr	Glu	Ile	Gly	Thr	Pro
			180					185					190		
Arg	Ala	Leu	Thr	Asn	Thr	Leu	Ile	Leu	Leu	Lys	Glu	Asp	Phe	Thr	Asp
		195				200						205			
Glu	Glu	Lys	Lys	Lys	Tyr	Thr	Ala	Pro	Ile	Lys	Thr	Phe	Ala	Pro	Lys
	210					215					220				
Ser	Asp	Glu	Ile	Leu	Ser	Val	Gly	Lys	Ala	Glu	Pro	Ala	Lys	Gly	
225					230				235					240	
Gly	Asn	Leu	Val	Asp	Ile	Ser	Lys	Val	Lys	Leu	Leu	Glu	Ser	Ile	Ile
				245					250					255	
Glu	Glu	Asp	Lys	Asp	Met	Met	Lys	Asn	Ser	Ile	Asp	Ser	Phe	Asn	Lys
			260					265					270		
Val	Phe	Thr	Tyr	Val	Gln	Asp	Ser	Ala	Thr	Asp	Lys	Glu	Arg	Asn	Gly
		275					280					285			
Phe	Tyr	Lys	Asp	Gly	Ser	Tyr	Ile	Asp	His	Lys	Asp	Val	Pro	Tyr	Thr
	290					295					300				
Gly	Ala	Tyr	Gly	Val	Val	Leu	Leu	Glu	Gly	Ile	Ser	Gln	Met	Met	Pro
305					310					315					320
Met	Ile	Lys	Glu	Thr	Pro	Phe	Asn	Asp	Lys	Thr	Gln	Asn	Asn	Thr	Thr
				325					330					335	
Leu	Thr	Ser	Trp	Ile	Asp	Asp	Gly	Phe	Met	Pro	Leu	Ile	Tyr	Lys	Gly
			340					345					350		
Glu	Met	Met	Asp	Leu	Ser	Arg	Gly	Arg	Ala	Ile	Ser	Arg	Glu	Asn	Glu
		355					360					365			
Thr	Ser	His	Ser	Ala	Ser	Ala	Thr	Val	Met	Lys	Ser	Leu	Leu	Arg	Leu
	370					375					380				
Ser	Asp	Ala	Met	Asp	Glu	Ser	Thr	Lys	Ala	Lys	Tyr	Lys	Gln	Ile	Val
385					390					395					400
Lys	Asn	Ser	Val	Lys	Ser	Asp	Ser	Ser	Tyr	Gly	Gln	Asn	Asp	Thr	Leu
				405					410					415	
Ser	Ser	Tyr	Ser	Asp	Ile	Asp	Lys	Met	Lys	Ser	Leu	Met	Thr	Asp	Ser
			420					425					430		
Thr	Ile	Ser	Thr	Asn	Gly	Leu	Thr	Gln	Gln	Leu	Lys	Ile	Tyr	Asn	Ala
			435				440					445			
Met	Asp	Arg	Val	Thr	Tyr	His	Asn	Lys	Asp	Leu	Asp	Phe	Ala	Phe	Gly
	450					455				460					
Leu	Ser	Met	Thr	Ser	Lys	Asn	Val	Ala	Arg	Tyr	Glu	Asn	Ile	Asn	Gly
465					470					475					480
Glu	Asn	Leu	Lys	Gly	Trp	His	Thr	Gly	Ala	Gly	Met	Ser	Tyr	Leu	Tyr
				485					490					495	
Asn	Ser	Asp	Val	Arg	His	Tyr	Arg	Asp	Asn	Phe	Trp	Ala	Thr	Ala	Asp
			500					505					510		
Met	Lys	Arg	Leu	Ala	Asp	Thr	Thr	Thr	Leu	Glu	Asn	Glu	Glu	Pro	Lys
		515					520					525			
Gly	Thr	Asp	Val	Lys	Lys	Ser	Ser	Lys	Thr	Phe	Val	Gly	Gly	Thr	Lys
	530					535					540				
Phe	Asp	Asp	Gln	His	Ala	Ser	Ile	Gly	Met	Asp	Phe	Glu	Asn	Gln	Asp
545					550					555					560
Lys	Thr	Leu	Thr	Ala	Lys	Lys	Ser	Tyr	Phe	Ile	Leu	Asn	Asp	Lys	Ile
				565					570					575	
Val	Phe	Leu	Gly	Thr	Gly	Ile	Lys	Thr	Thr	Asp	Ser	Ser	Lys	Asn	Pro
			580					585					590		

3066SEQ PC1.TXT

Val Thr Thr Ile Glu Asn Arg Lys Ala His Gly Tyr Thr Leu Tyr Thr
 595 600 605
 Asp Asp Lys Gln Thr Thr Asn Ser Asn Asn Gln Glu Thr Asn Ser Val
 610 615 620
 Phe Leu Glu Ser Thr Asn Ser Thr Gln Asn Asn Ile Gly Tyr His Phe
 625 630 635 640
 Leu Asn Lys Ser Lys Ile Thr Val Lys Lys Glu Ser His Thr Gly Lys
 645 650 655
 Trp Ser Asp Ile Asn Lys Ser Gln Lys Asp Thr Gln Lys Thr Asp Glu
 660 665 670
 Tyr Tyr Glu Val Thr Gln Lys His Ser Asn Thr Asp Asp Lys Tyr Ala
 675 680 685
 Tyr Val Leu Tyr Pro Gly Ile Thr Lys Asp Asn Phe Lys Ser Lys Ala
 690 695 700
 Ser Gln Val Thr Val Val Lys Gln Asp Asp Asp Phe His Val Val Lys
 705 710 715 720
 Asp Asn Glu Ser Val Trp Ala Gly Val Asn Tyr Ser Asp Ser Thr Gln
 725 730 735
 Thr Phe Asp Ile Asn Gly Thr Lys Val Glu Val Lys Ala Lys Gly Met
 740 745 750
 Phe Ile Leu Lys Lys Lys Asp Asp Asn Thr Tyr Glu Cys Ser Phe Tyr
 755 760 765
 Asn Pro Glu Ser Thr Asn Ser Ala Ser Asp Ile Glu Ser Lys Ile Ser
 770 775 780
 Met Thr Gly Tyr Ser Ile Thr Asn Lys Asn Thr Ser Asn Thr Asn Glu
 785 790 795 800
 Ser Gly Val Arg Phe Glu Leu Thr Lys
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<210> 80

<211> 807

<212> PRT

<213> Staphylococcus aureus (strain bovine RF122)

<220>

<223> hyaluronan lyase

<400> 80

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

Gly	Phe	Tyr	Lys	Asp	Gly	Ser	Tyr	Ile	Asp	His	Gln	Asp	Val	Pro	Tyr
290						295					300				
Thr	Gly	Ala	Tyr	Gly	Val	Val	Leu	Leu	Glu	Gly	Ile	Ser	Gln	Met	Met
305					310					315					320
Pro	Met	Ile	Lys	Glu	Ser	Pro	Phe	Lys	His	Thr	Gln	Asp	Lys	Ala	Thr
				325					330					335	
Leu	Ser	Asn	Trp	Ile	Asp	Glu	Gly	Phe	Met	Pro	Leu	Ile	Tyr	Lys	Gly
			340					345					350		
Glu	Met	Met	Asp	Leu	Ser	Arg	Gly	Arg	Ala	Ile	Ser	Arg	Glu	Asn	Glu
		355					360					365			
Thr	Ser	His	Thr	Ala	Ser	Ala	Thr	Val	Met	Lys	Ser	Leu	Leu	Arg	Leu
	370					375					380				
Ser	Asp	Thr	Met	Asp	Glu	Ser	Thr	Lys	Thr	Lys	Tyr	Lys	Gln	Ile	Val
385					390					395					400
Lys	Thr	Ser	Val	Lys	Ser	Asp	Ser	Ser	Tyr	Asp	Ser	Asn	Asp	Thr	Leu
				405					410					415	
Asn	Ser	Tyr	Ser	Asp	Ile	Asp	Lys	Met	Lys	Lys	Leu	Met	Asn	Asp	Ser
			420					425					430		
Thr	Ile	Ser	Lys	Asn	Asp	Leu	Thr	Gln	Gln	Leu	Lys	Ile	Tyr	Asn	Asp
		435					440					445			
Met	Asp	Arg	Val	Thr	Tyr	His	Asn	Lys	Glu	Leu	Asp	Phe	Ala	Phe	Gly
	450					455					460				
Leu	Ser	Met	Thr	Ser	Lys	Asn	Ile	Ala	Arg	Tyr	Glu	Asn	Ile	Asn	Gly
465					470					475					480
Glu	Asn	Leu	Lys	Gly	Trp	His	Thr	Gly	Ala	Gly	Met	Ser	Tyr	Leu	Tyr
				485					490					495	
Asn	Ser	Asp	Val	Lys	His	Tyr	Arg	Asp	Asn	Phe	Trp	Ala	Thr	Ala	Asp
			500					505					510		
Met	Thr	Arg	Leu	Pro	Gly	Thr	Thr	Thr	Leu	Asn	Asp	Met	Pro	Ser	Thr
		515					520					525			
Asn	Thr	Lys	Asn	Asp	Lys	Ser	Phe	Val	Gly	Gly	Thr	Lys	Leu	Asn	Asn
	530					535					540				
Lys	Tyr	Ala	Ser	Ile	Gly	Met	Asp	Phe	Glu	Asn	Gln	Asp	Lys	Thr	Leu
545					550					555					560
Thr	Ala	Lys	Lys	Ser	Tyr	Phe	Ile	Leu	Asn	Asp	Lys	Ile	Val	Phe	Ile
				565					570					575	
Gly	Thr	Gly	Ile	Lys	Ser	Thr	Asp	Ser	Ser	Lys	Asn	Pro	Val	Thr	Ser
			580					585					590		
Val	Glu	Asn	Arg	Lys	Ala	Asn	Gly	Tyr	Lys	Leu	Phe	Lys	Gly	Asp	Ile
		595					600					605			
Glu	Ile	Thr	Thr	Ser	Asp	Val	Asn	Ala	Gln	Glu	Thr	His	Ser	Val	Phe
	610					615					620				
Leu	Glu	Ser	Asn	Asp	Thr	Lys	Lys	Asn	Ile	Gly	Tyr	His	Phe	Leu	Asp
625					630					635					640
Lys	Pro	Lys	Ile	Thr	Val	Lys	Lys	Glu	Ser	His	Thr	Gly	Lys	Trp	Ser
				645					650					655	
Glu	Ile	Asn	Lys	Ser	Gln	Lys	Thr	Asp	Lys	Lys	Asp	Glu	Tyr	Tyr	
			660					665				670			
Glu	Val	Thr	Gln	Thr	His	Asn	Thr	Ser	Asp	Ser	Lys	Tyr	Ala	Tyr	Val
		675					680					685			
Leu	Tyr	Pro	Gly	Leu	Ser	Lys	Ser	Asp	Phe	Lys	Ser	Lys	Asn	Asn	Asn
	690					695					700				
Val	Ser	Ile	Val	Lys	Gln	Asp	Glu	Asp	Phe	His	Val	Ile	Lys	Asp	Asn
705					710					715					720
Asp	Gly	Val	Phe	Ala	Gly	Val	Asn	Tyr	Ser	Asp	Ser	Thr	Lys	Ser	Phe
				725					730					735	
Asp	Ile	Asn	Gly	Thr	Ile	Val	Glu	Leu	Lys	Glu	Lys	Gly	Met	Phe	Val
			740					745					750		
Ile	Lys	Lys	Lys	Asp	Asp	Asn	Thr	Tyr	Glu	Cys	Ser	Phe	Tyr	Asn	Pro
		755					760					765			
Thr	Ser	Thr	Asn	Ser	Thr	Ser	Asn	Lys	Glu	Ser	Lys	Ile	Ser	Val	Thr
	770					775					780				
Gly	Tyr	Thr	Ile	Thr	Asn	Gln	Ser	Val	Ser	Asn	Phe	Lys	Glu	Ser	Asp
785					790					795					800
Ile	His	Phe	Glu	Leu	Thr	Lys									
				805											

<210> 81

<211> 807

<212> PRT

<213> Staphylococcus aureus (strain USA300)

<220>

<223> hyaluronan lyase

<400> 81

Met	Thr	Tyr	Arg	Ile	Lys	Lys	Trp	Gln	Lys	Leu	Ser	Thr	Ile	Thr	Leu
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Leu	Met	Ala	Gly	Val	Ile	Thr	Leu	Asn	Gly	Gly	Glu	Phe	Arg	Ser	Val
		20						25					30		
Asp	Lys	His	Gln	Ile	Ala	Val	Ala	Asp	Thr	Asn	Val	Gln	Thr	Pro	Asp
		35					40					45			
Tyr	Glu	Lys	Leu	Arg	Asn	Thr	Trp	Leu	Asp	Val	Asn	Tyr	Gly	Tyr	Asp
	50					55					60				
Lys	Tyr	Asp	Glu	Asn	Asn	Pro	Asp	Met	Lys	Lys	Lys	Phe	Asp	Ala	Thr
65					70					75					80
Glu	Lys	Glu	Ala	Thr	Asn	Leu	Leu	Lys	Glu	Met	Lys	Thr	Glu	Ser	Gly
				85					90					95	
Arg	Lys	Tyr	Leu	Trp	Ser	Gly	Ala	Glu	Thr	Leu	Glu	Thr	Asn	Ser	Ser
			100					105					110		
His	Met	Thr	Arg	Thr	Tyr	Arg	Asn	Ile	Glu	Lys	Ile	Ala	Glu	Ala	Met
		115					120					125			
Arg	Asn	Pro	Lys	Thr	Thr	Leu	Asn	Thr	Asp	Glu	Asn	Lys	Lys	Lys	Val
	130					135					140				
Lys	Asp	Ala	Leu	Glu	Trp	Leu	His	Lys	Asn	Ala	Tyr	Gly	Lys	Glu	Pro
145					150					155					160
Asp	Lys	Lys	Val	Lys	Glu	Leu	Ser	Glu	Asn	Phe	Thr	Lys	Thr	Thr	Gly
				165					170					175	
Lys	Asn	Thr	Asn	Leu	Asn	Trp	Trp	Asp	Tyr	Glu	Ile	Gly	Thr	Pro	Lys
			180					185					190		
Ser	Leu	Thr	Asn	Thr	Leu	Ile	Leu	Leu	Asn	Asp	Gln	Phe	Ser	Asn	Glu
		195					200					205			
Glu	Lys	Lys	Lys	Phe	Thr	Ala	Pro	Ile	Lys	Thr	Phe	Ala	Pro	Asp	Ser
	210					215					220				
Asp	Lys	Ile	Leu	Ser	Ser	Val	Gly	Lys	Ala	Glu	Leu	Ala	Lys	Gly	Gly
225					230					235					240
Asn	Leu	Val	Asp	Ile	Ser	Lys	Val	Lys	Leu	Leu	Glu	Cys	Ile	Ile	Glu
				245					250					255	
Glu	Asp	Lys	Asp	Met	Met	Lys	Lys	Ser	Ile	Asp	Ser	Phe	Asn	Lys	Val
			260					265					270		
Phe	Thr	Tyr	Val	Gln	Asp	Ser	Ala	Thr	Gly	Lys	Glu	Arg	Asn	Gly	Phe
		275					280					285			
Tyr	Lys	Asp	Gly	Ser	Tyr	Ile	Asp	His	Gln	Asp	Val	Pro	Tyr	Thr	Gly
	290					295					300				
Ala	Tyr	Gly	Val	Val	Leu	Glu	Gly	Ile	Ser	Gln	Met	Met	Pro	Met	
305					310					315					320
Ile	Lys	Glu	Thr	Pro	Phe	Asn	Asp	Lys	Thr	Gln	Asn	Asp	Thr	Thr	Leu
				325					330					335	
Lys	Ser	Trp	Ile	Asp	Asp	Gly	Phe	Met	Pro	Leu	Ile	Tyr	Lys	Gly	Glu
			340					345					350		
Met	Met	Asp	Leu	Ser	Arg	Gly	Arg	Ala	Ile	Ser	Arg	Glu	Asn	Glu	Thr
		355					360					365			
Ser	His	Ser	Ala	Ser	Ala	Thr	Val	Met	Lys	Ser	Leu	Leu	Arg	Leu	Ser
	370					375					380				
Asp	Ala	Met	Asp	Asp	Ser	Thr	Lys	Ala	Lys	Tyr	Lys	Lys	Ile	Val	Lys
385					390					395					400
Ser	Ser	Val	Glu	Ser	Asp	Ser	Ser	Tyr	Lys	Gln	Asn	Asp	Tyr	Leu	Asn
				405					410					415	
Ser	Tyr	Ser	Asp	Ile	Asp	Lys	Met	Lys	Ser	Leu	Met	Thr	Asp	Asn	Ser
			420					425					430		
Ile	Ser	Lys	Asn	Gly	Leu	Thr	Gln	Gln	Leu	Lys	Ile	Tyr	Asn	Asp	Met
		435					440					445			
Asp	Arg	Val	Thr	Tyr	His	Asn	Lys	Asp	Leu	Asp	Phe	Ala	Phe	Gly	Leu
	450					455					460				
Ser	Met	Thr	Ser	Lys	Asn	Val	Ala	Arg	Tyr	Glu	Ser	Ile	Asn	Gly	Glu
465					470					475					480
Asn	Leu	Lys	Gly	Trp	His	Thr	Gly	Ala	Gly	Met	Ser	Tyr	Leu	Tyr	Asn
				485					490					495	
Ser	Asp	Val	Lys	His	Tyr	His	Asp	Asn	Phe	Trp	Val	Thr	Ala	Asp	Met
			500					505					510		
Lys	Arg	Leu	Ser	Gly	Thr	Thr	Thr	Leu	Asp	Asn	Glu	Ile	Leu	Lys	Asp
		515					520					525			
Thr	Asp	Asp	Lys	Lys	Ser	Ser	Lys	Thr	Phe	Val	Gly	Gly	Thr	Lys	Val
	530					535					540				
Asp	Asp	Gln	His	Ala	Ser	Ile	Gly	Met	Asp	Phe	Glu	Asn	Gln	Asp	Lys
545					550					555					560
Thr	Leu	Thr	Ala	Lys	Lys	Ser	Tyr	Phe	Ile	Leu	Asn	Asp	Lys	Ile	Val
				565					570					575	
Phe	Leu	Gly	Thr	Gly	Ile	Lys	Ser	Thr	Asp	Ser	Ser	Lys	Asn	Pro	Val

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Thr	Thr	Ile	580	Glu	Asn	Arg	Lys	Ala	585	Asn	Gly	Tyr	Thr	Leu	590	Tyr	Thr	Asp
Asp	Lys	Gln	595	Thr	Thr	Asn	Ser	600	Asp	Asn	Gln	Glu	Asn	605	Asn	Ser	Val	Phe
Leu	610	Glu	Ser	Thr	Asp	Thr	615	Lys	Lys	Asn	Ile	Gly	Tyr	620	His	Phe	Leu	Asn
625	Lys	Pro	Lys	Ile	Thr	Val	630	Lys	Lys	Glu	Ser	His	Thr	635	Gly	Lys	Trp	Lys
Glu	Ile	Asn	Lys	Ser	Gln	Lys	645	Asp	Thr	Gln	Lys	Thr	Asp	650	Glu	Tyr	Tyr	
Glu	Val	Thr	Gln	Lys	His	Ser	660	Asn	Ser	Asp	Asn	Lys	Tyr	665	Gly	Tyr	Val	
Leu	Tyr	Pro	Gly	Leu	Ser	Lys	675	Asp	Val	Phe	Lys	Thr	Lys	680	Lys	Lys	Asp	Glu
Val	Thr	Val	Val	Lys	Gln	Glu	690	Asp	Asp	Phe	His	Val	Val	700	Lys	Lys	Asp	Asn
705	Glu	Ser	Val	Trp	Ala	Gly	710	Val	Asn	Tyr	Ser	Asn	Ser	715	Thr	Gln	Thr	Phe
Asp	Ile	Asn	Asn	Thr	Lys	Val	725	Glu	Val	Lys	Ala	Lys	Gly	730	Met	Phe	Ile	
Leu	Lys	Lys	Lys	Asp	Asp	Asn	740	Thr	Tyr	Glu	Cys	Ser	Phe	745	Tyr	Asn	Pro	
Glu	Ser	Thr	Asn	Ser	Ala	Ser	755	Asp	Ile	Glu	Ser	Lys	Ile	760	Ser	Met	Thr	
Gly	Tyr	Ser	Ile	Thr	Asn	Lys	770	Asn	Thr	Ser	Thr	Ser	Asn	775	Glu	Ser	Gly	
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							805											

<210> 82

<211> 1066

<212> PRT

<213> Streptococcus pneumoniae

<220>

<223> hyaluronan lyase

<400> 82

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	Thr	Thr	Thr	Asn	Thr	Ile	Gln	Gln	Ser	Gln	Lys	Glu	Val	Gln	Tyr	Gln		
	Gln	Arg	Asp	Thr	Lys	Asn	Leu	Val	Glu	Asn	Gly	Asp	Phe	Gly	Gln	Thr		
	50						55					60						
Glu	Asp	Gly	Ser	Ser	Pro	Trp	Thr	Gly	Ser	Lys	Ala	Gln	Gly	Trp	Ser			
65	Ala	Trp	Val	Asp	Gln	Lys	Asn	Ser	Ala	Asp	Ala	Ser	Thr	Arg	Val	Ile		
	Glu	Ala	Lys	Asp	Gly	Ala	Ile	Thr	Ile	Ser	Ser	His	Glu	Lys	Leu	Arg		
	Ala	Ala	Leu	His	Arg	Met	Val	Pro	Ile	Glu	Ala	Lys	Lys	Lys	Tyr	Lys		
	Leu	Arg	Phe	Lys	Ile	Lys	Thr	Asp	Asn	Lys	Ile	Gly	Ile	Ala	Lys	Val		
	130	Ile	Ile	Glu	Glu	Ser	Gly	Lys	Asp	Lys	Arg	Leu	Trp	Asn	Ser	Ala		
145	Thr	Thr	Ser	Gly	Thr	Lys	Asp	Trp	Gln	Thr	Ile	Glu	Ala	Asp	Tyr	Ser		
	Pro	Thr	Leu	Asp	Val	Asp	Lys	Ile	Lys	Leu	Glu	Leu	Phe	Tyr	Glu	Thr		
	Gly	Thr	Gly	Thr	Val	Ser	Phe	Lys	Asp	Ile	Glu	Leu	Val	Glu	Val	Ala		
	195																	
Asp	Gln	Leu	Ser	Glu	Asp	Ser	Gln	Thr	Asp	Lys	Gln	Leu	Glu	Glu	Lys			
	Ile	Asp	Leu	Pro	Ile	Gly	Lys	Lys	His	Val	Phe	Ser	Leu	Ala	Asp	Tyr		
225	Thr	Tyr	Lys	Val	Glu	Asn	Pro	Asp	Val	Ala	Ser	Val	Lys	Asn	Gly	Ile		
	Leu	Glu	Pro	Leu	Lys	Glu	Gly	Thr	Thr	Asn	Val	Ile	Val	Ser	Lys	Asp		
	Gly	Lys	Glu	Val	Lys	Lys	Ile	Pro	Leu	Lys	Ile	Leu	Ala	Ser	Val	Lys		

Asp	Ala	Tyr	Thr	Asp	Arg	Leu	Asp	Asp	Trp	Asn	Gly	Ile	Ile	Ala	Gly
275	290					280	295				285				
Asn	Gln	Tyr	Tyr	Asp	Ser	Lys	Asn	Glu	Gln	Met	Ala	Lys	Leu	Asn	Gln
305					310					315					320
Glu	Leu	Glu	Gly	Lys	Val	Ala	Asp	Ser	Leu	Ser	Ser	Ile	Ser	Ser	Gln
				325					330					335	
Ala	Asp	Arg	Thr	Tyr	Leu	Trp	Glu	Lys	Phe	Ser	Asn	Tyr	Lys	Thr	Ser
			340					345					350		
Ala	Asn	Leu	Thr	Ala	Thr	Tyr	Arg	Lys	Leu	Glu	Glu	Met	Ala	Lys	Gln
	355						360					365			
Val	Thr	Asn	Pro	Ser	Ser	Arg	Tyr	Tyr	Gln	Asp	Glu	Thr	Val	Val	Arg
	370					375					380				
Thr	Val	Arg	Asp	Ser	Met	Glu	Trp	Met	His	Lys	His	Val	Tyr	Asn	Ser
385					390					395					400
Glu	Lys	Ser	Ile	Val	Gly	Asn	Trp	Trp	Asp	Tyr	Glu	Ile	Gly	Thr	Pro
				405					410					415	
Arg	Ala	Ile	Asn	Asn	Thr	Leu	Ser	Leu	Met	Lys	Glu	Tyr	Phe	Ser	Asp
			420					425					430		
Glu	Glu	Ile	Lys	Lys	Tyr	Thr	Asp	Val	Ile	Glu	Lys	Phe	Val	Pro	Asp
	435						440					445			
Pro	Glu	His	Phe	Arg	Lys	Thr	Thr	Asp	Asn	Pro	Phe	Lys	Ala	Leu	Gly
	450					455					460				
Gly	Asn	Leu	Val	Asp	Met	Gly	Arg	Val	Lys	Val	Ile	Ala	Gly	Leu	Leu
465					470					475					480
Arg	Lys	Asp	Asp	Gln	Glu	Ile	Ser	Ser	Thr	Ile	Arg	Ser	Ile	Glu	Gln
				485					490					495	
Val	Phe	Lys	Leu	Val	Asp	Gln	Gly	Glu	Gly	Phe	Tyr	Gln	Asp	Gly	Ser
			500					505					510		
Tyr	Ile	Asp	His	Thr	Asn	Val	Ala	Tyr	Thr	Gly	Ala	Tyr	Gly	Asn	Val
	515					520						525			
Leu	Ile	Asp	Gly	Leu	Ser	Gln	Leu	Leu	Pro	Val	Ile	Gln	Lys	Thr	Lys
	530					535					540				
Asn	Pro	Ile	Asp	Lys	Asp	Lys	Met	Gln	Thr	Met	Tyr	His	Trp	Ile	Asp
545					550					555					560
Lys	Ser	Phe	Ala	Pro	Leu	Leu	Val	Asn	Gly	Glu	Leu	Met	Asp	Met	Ser
				565					570					575	
Arg	Gly	Arg	Ser	Ile	Ser	Arg	Ala	Asn	Ser	Glu	Gly	His	Val	Ala	Ala
			580					585					590		
Val	Glu	Val	Leu	Arg	Gly	Ile	His	Arg	Ile	Ala	Asp	Met	Ser	Glu	Gly
	595					600						605			
Glu	Thr	Lys	Gln	Cys	Leu	Gln	Ser	Leu	Val	Lys	Thr	Ile	Val	Gln	Ser
	610					615					620				
Asp	Ser	Tyr	Tyr	Asp	Val	Phe	Lys	Asn	Leu	Lys	Thr	Tyr	Lys	Asp	Ile
625					630					635					640
Ser	Leu	Met	Gln	Ser	Leu	Leu	Ser	Asp	Ala	Gly	Val	Ala	Ser	Val	Pro
				645					650					655	
Arg	Pro	Ser	Tyr	Leu	Ser	Ala	Phe	Asn	Lys	Met	Asp	Lys	Thr	Ala	Met
			660					665					670		
Tyr	Asn	Ala	Glu	Lys	Gly	Phe	Gly	Phe	Gly	Leu	Ser	Leu	Phe	Ser	Ser
	675						680					685			
Arg	Thr	Leu	Asn	Tyr	Glu	His	Met	Asn	Lys	Glu	Asn	Lys	Arg	Gly	Trp
	690					695						700			
Tyr	Thr	Ser	Asp	Gly	Met	Phe	Tyr	Leu	Tyr	Asn	Gly	Asp	Leu	Ser	His
705					710					715					720
Tyr	Ser	Asp	Gly	Tyr	Trp	Pro	Thr	Val	Asn	Pro	Tyr	Lys	Met	Pro	Gly
				725					730					735	
Thr	Thr	Glu	Thr	Asp	Ala	Lys	Arg	Ala	Asp	Ser	Asp	Thr	Gly	Lys	Val
			740					745					750		
Leu	Pro	Ser	Ala	Phe	Val	Gly	Thr	Ser	Lys	Leu	Asp	Asp	Ala	Asn	Ala
	755					760						765			
Thr	Ala	Thr	Met	Asp	Phe	Thr	Asn	Trp	Asn	Gln	Thr	Leu	Thr	Ala	His
	770					775					780				
Lys	Ser	Trp	Phe	Met	Leu	Lys	Asp	Lys	Ile	Ala	Phe	Leu	Gly	Ser	Asn
785					790					795					800
Ile	Gln	Asn	Thr	Ser	Thr	Asp	Thr	Ala	Ala	Thr	Thr	Ile	Asp	Gln	Arg
				805					810					815	
Lys	Leu	Glu	Ser	Gly	Asn	Pro	Tyr	Lys	Val	Tyr	Val	Asn	Asp	Lys	Glu
			820					825					830		
Ala	Ser	Leu	Thr	Glu	Gln	Glu	Lys	Asp	Tyr	Pro	Glu	Thr	Gln	Ser	Val
	835						840					845			
Phe	Leu	Glu	Ser	Phe	Asp	Ser	Lys	Lys	Asn	Ile	Gly	Tyr	Phe	Phe	Phe
	850					855					860				
Lys	Lys	Ser	Ser	Ile	Ser	Met	Ser	Lys	Ala	Leu	Gln	Lys	Gly	Ala	Trp
865					870					875					880

Lys Asp Ile Asn Glu Gly Gln Ser Asp Lys Glu Val Glu Asn Glu Phe
 885 890 895
 Leu Thr Ile Ser Gln Ala His Lys Gln Asn Arg Asp Ser Tyr Gly Tyr
 900 905 910
 Met Leu Ile Pro Asn Val Asp Arg Ala Thr Phe Asn Gln Met Ile Lys
 915 920 925
 Glu Leu Glu Ser Ser Leu Ile Glu Asn Asn Glu Thr Leu Gln Ser Val
 930 935 940
 Tyr Asp Ala Lys Gln Gly Val Trp Gly Ile Val Lys Tyr Asp Asp Ser
 945 950 955 960
 Val Ser Thr Ile Ser Asn Gln Phe Gln Val Leu Lys Arg Gly Val Tyr
 965 970 975
 Thr Ile Arg Lys Glu Gly Asp Glu Tyr Lys Ile Ala Tyr Tyr Asn Pro
 980 985 990
 Glu Thr Gln Glu Ser Ala Pro Asp Gln Glu Val Phe Lys Lys Leu Glu
 995 1000 1005
 Gln Ala Ala Gln Pro Gln Val Gln Asn Ser Lys Glu Lys Glu Lys Ser
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 Glu Glu Glu Lys Asn His Ser Asp Gln Lys Asn Leu Pro Gln Thr Gly
 1025 1030 1035 1040
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 Phe Tyr Leu Phe Arg Arg Gly Lys Asn Asn
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<210> 83

<211> 1078

<212> PRT

<213> Streptococcus pneumoniae (strain ATCC BAA-255/R6)

<220>

<223> hyaluronan lyase

<400> 83

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 35 40 45
 Ile Gln Gln Ser Gln Lys Glu Val Gln Tyr Gln Gln Arg Asp Thr Lys
 50 55 60
 Asn Leu Val Glu Asn Gly Asp Phe Gly Gln Thr Glu Asp Gly Ser Ser
 65 70 75 80
 Pro Trp Thr Gly Ser Lys Ala Gln Gly Trp Ser Ala Trp Val Asp Gln
 85 90 95

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

Asp	Ser	Lys	Asn	Glu	Gln	Met	Ala	Lys	Leu	Asn	Gln	Glu	Leu	Glu	Gly
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Lys	Val	Ala	Asp	Ser	Leu	Ser	Ser	Ile	Ser	Ser	Gln	Ala	Asp	Arg	Ile
			340					345					350		
Tyr	Leu	Trp	Glu	Lys	Phe	Ser	Asn	Tyr	Lys	Thr	Ser	Ala	Asn	Leu	Thr
		355					360					365			
Ala	Thr	Tyr	Arg	Lys	Leu	Glu	Glu	Met	Ala	Lys	Gln	Val	Thr	Asn	Pro
	370					375					380				
Ser	Ser	Arg	Tyr	Tyr	Gln	Asp	Glu	Thr	Val	Val	Arg	Thr	Val	Arg	Asp
385					390					395					400
Ser	Met	Glu	Trp	Met	His	Lys	His	Val	Tyr	Asn	Ser	Glu	Lys	Ser	Ile
				405					410					415	
Val	Gly	Asn	Trp	Trp	Asp	Tyr	Glu	Ile	Gly	Thr	Pro	Arg	Ala	Ile	Asn
		420						425					430		
Asn	Thr	Leu	Ser	Leu	Met	Lys	Glu	Tyr	Phe	Ser	Asp	Glu	Glu	Ile	Lys
		435					440					445			
Lys	Tyr	Thr	Asp	Val	Ile	Glu	Lys	Phe	Val	Pro	Asp	Pro	Glu	His	Phe
	450					455					460				
Arg	Lys	Thr	Thr	Asp	Asn	Pro	Phe	Lys	Ala	Leu	Gly	Gly	Asn	Leu	Val
465					470					475					480
Asp	Met	Gly	Arg	Val	Lys	Val	Ile	Ala	Gly	Leu	Leu	Arg	Lys	Asp	Asp
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Gln	Glu	Ile	Ser	Ser	Thr	Ile	Arg	Ser	Ile	Glu	Gln	Val	Phe	Lys	Leu
			500					505					510		
Val	Asp	Gln	Gly	Glu	Gly	Phe	Tyr	Gln	Asp	Gly	Ser	Tyr	Ile	Asp	His
	515						520					525			
Thr	Asn	Val	Ala	Tyr	Thr	Gly	Ala	Tyr	Gly	Asn	Val	Leu	Ile	Asp	Gly
	530					535					540				
Leu	Ser	Gln	Leu	Leu	Pro	Val	Ile	Gln	Lys	Thr	Lys	Asn	Pro	Ile	Asp
545					550					555					560
Lys	Asp	Lys	Met	Gln	Thr	Met	Tyr	His	Trp	Ile	Asp	Lys	Ser	Phe	Ala
			565						570					575	
Pro	Leu	Leu	Val	Asn	Gly	Glu	Leu	Met	Asp	Met	Ser	Arg	Gly	Arg	Ser
			580					585					590		
Ile	Ser	Arg	Ala	Asn	Ser	Glu	Gly	His	Val	Ala	Ala	Val	Glu	Val	Leu
		595					600					605			
Arg	Gly	Ile	His	Arg	Ile	Ala	Asp	Met	Ser	Glu	Gly	Glu	Thr	Lys	Gln
	610					615					620				
Arg	Leu	Gln	Ser	Leu	Val	Lys	Thr	Ile	Val	Gln	Ser	Asp	Ser	Tyr	Tyr
625					630					635					640
Asp	Val	Phe	Lys	Asn	Leu	Lys	Thr	Tyr	Lys	Asp	Ile	Ser	Leu	Met	Gln
				645					650					655	
Ser	Leu	Leu	Ser	Asp	Ala	Gly	Val	Ala	Ser	Val	Pro	Arg	Thr	Ser	Tyr
			660					665					670		
Leu	Ser	Ala	Phe	Asn	Lys	Met	Asp	Lys	Thr	Ala	Met	Tyr	Asn	Ala	Glu
		675					680					685			
Lys	Gly	Phe	Gly	Phe	Gly	Leu	Ser	Leu	Phe	Ser	Ser	Arg	Thr	Leu	Asn
	690					695					700				
Tyr	Glu	His	Met	Asn	Lys	Glu	Asn	Lys	Arg	Gly	Trp	Tyr	Thr	Ser	Asp
705					710					715					720
Gly	Met	Phe	Tyr	Leu	Tyr	Asn	Gly	Asp	Leu	Ser	His	Tyr	Ser	Asp	Gly
				725					730					735	
Tyr	Trp	Pro	Thr	Val	Asn	Pro	Tyr	Lys	Met	Pro	Gly	Thr	Thr	Glu	Thr
			740					745					750		
Asp	Ala	Lys	Arg	Ala	Asp	Ser	Asp	Thr	Gly	Lys	Val	Leu	Pro	Ser	Ala
		755					760					765			
Phe	Val	Gly	Thr	Ser	Lys	Leu	Asp	Asp	Ala	Asn	Ala	Thr	Ala	Thr	Met
	770					775					780				
Asp	Phe	Thr	Asn	Trp	Asn	Gln	Thr	Leu	Thr	Ala	His	Lys	Ser	Trp	Phe
785					790					795					800
Met	Leu	Lys	Asp	Lys	Ile	Ala	Phe	Leu	Gly	Ser	Asn	Ile	Gln	Asn	Thr
				805					810					815	
Ser	Thr	Asp	Thr	Ala	Ala	Thr	Thr	Ile	Asp	Gln	Arg	Lys	Leu	Glu	Ser
			820					825					830		
Ser	Asn	Pro	Tyr	Lys	Val	Tyr	Val	Asn	Asp	Lys	Glu	Ala	Ser	Leu	Thr
		835					840					845			
Glu	Gln	Glu	Lys	Asp	Tyr	Pro	Glu	Thr	Gln	Ser	Val	Phe	Leu	Glu	Ser
	850					855					860				
Ser	Asp	Ser	Lys	Lys	Asn	Ile	Gly	Tyr	Phe	Phe	Phe	Lys	Lys	Ser	Ser
865					870					875					880
Ile	Ser	Met	Ser	Lys	Ala	Leu	Gln	Lys	Gly	Ala	Trp	Lys	Asp	Ile	Asn
				885					890					895	
Glu	Gly	Gln	Ser	Asp	Lys	Glu	Val	Glu	Asn	Glu	Phe	Leu	Thr	Ile	Ser
			900					905					910		
Gln	Ala	His	Lys	Gln	Asn	Gly	Asp	Ser	Tyr	Gly	Tyr	Met	Leu	Ile	Pro

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 Asn Val Asp Arg Ala Thr Phe Asn Gln Met Ile Lys Glu Leu Glu Ser
 930 935 940
 Ser Leu Ile Glu Asn Asn Glu Thr Leu Gln Ser Val Tyr Asp Ala Lys
 945 950 955 960
 Gln Gly Val Trp Gly Ile Val Lys Tyr Asp Asp Ser Val Ser Thr Ile
 965 970 975
 Ser Asn Gln Phe Gln Val Leu Lys Arg Gly Val Tyr Thr Ile Arg Lys
 980 985 990
 Glu Gly Asp Glu Tyr Lys Ile Ala Tyr Tyr Asn Pro Glu Thr Gln Glu
 995 1000 1005
 Ser Ala Pro Asp Gln Glu Val Phe Lys Lys Leu Glu Gln Ala Ala Gln
 1010 1015 1020
 Pro Gln Val Gln Asn Ser Lys Glu Lys Glu Lys Ser Glu Glu Glu Lys
 1025 1030 1035 1040
 Asn His Ser Asp Gln Lys Asn Leu Pro Gln Thr Gly Glu Gly Gln Ser
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 Ile Leu Ala Ser Leu Gly Phe Leu Leu Gly Ala Phe Tyr Leu Phe
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 Arg Arg Gly Lys Asn Asn
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<210> 84

<211> 1067

<212> PRT

<213> Streptococcus pneumoniae serotype 2 (strain D39/N)

<220>

<223> hyaluronan lyase

<400> 84

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 Thr Thr Thr Asn Thr Ile Gln Gln Ser Gln Lys Glu Val Gln Tyr Gln
 35 40 45
 Gln Arg Asp Thr Lys Asn Leu Val Glu Asn Gly Asp Phe Gly Gln Thr
 50 55 60
 Glu Asp Gly Ser Ser Pro Trp Thr Gly Ser Lys Ala Gln Gly Trp Ser
 65 70 75 80
 Ala Trp Val Asp Gln Lys Asn Ser Ser Ala Asp Ala Ser Thr Arg Val
 85 90 95
 Ile Glu Ala Lys Asp Gly Ala Ile Thr Ile Ser Ser Pro Glu Lys Leu
 100 105 110
 Arg Ala Ala Val His Arg Met Val Pro Ile Glu Ala Lys Lys Tyr
 115 120 125
 Lys Leu Arg Phe Lys Ile Lys Thr Asp Asn Lys Val Gly Ile Ala Lys
 130 135 140
 Val Arg Ile Ile Glu Glu Ser Gly Lys Asp Lys Arg Leu Trp Asn Ser
 145 150 155 160
 Ala Thr Thr Ser Gly Thr Lys Asp Trp Gln Thr Ile Glu Ala Asp Tyr
 165 170 175
 Ser Pro Thr Leu Asp Val Asp Lys Ile Lys Leu Glu Leu Phe Tyr Glu
 180 185 190
 Thr Gly Thr Gly Thr Val Ser Phe Lys Asp Ile Glu Leu Val Glu Val
 195 200 205
 Ala Asp Gln Pro Ser Glu Asp Ser Gln Thr Asp Lys Gln Leu Glu Glu
 210 215 220
 Lys Ile Asp Leu Pro Ile Gly Lys Lys His Val Phe Ser Leu Ala Asp
 225 230 235 240
 Tyr Thr Tyr Lys Val Glu Asn Pro Asp Val Ala Ser Val Lys Asn Gly
 245 250 255
 Ile Leu Glu Pro Leu Lys Glu Gly Thr Asn Val Ile Val Ser Lys
 260 265 270
 Asp Gly Lys Glu Val Lys Lys Ile Pro Leu Lys Ile Leu Ala Ser Val
 275 280 285
 Lys Asp Thr Tyr Thr Asp Arg Leu Asp Asp Trp Asn Gly Ile Ile Ala
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 Gly Asn Gln Tyr Tyr Asp Ser Lys Asn Glu Gln Met Ala Lys Leu Asn
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 Gln Glu Leu Glu Gly Lys Val Ala Asp Ser Leu Ser Ser Ile Ser Ser
 325 330 335
 Gln Ala Asp Arg Ile Tyr Leu Trp Glu Lys Phe Ser Asn Tyr Lys Thr

3066SEQ PC1.TXT

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	370					375					380				
Arg	Thr	Val	Arg	Asp	Ser	Met	Glu	Trp	Met	His	Lys	His	Val	Tyr	Asn
385					390					395				400	
Ser	Glu	Lys	Ser	Ile	Val	Gly	Asn	Trp	Trp	Asp	Tyr	Glu	Ile	Gly	Thr
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Pro	Arg	Ala	Ile	Asn	Asn	Thr	Leu	Ser	Leu	Met	Lys	Glu	Tyr	Phe	Ser
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Asp	Glu	Glu	Ile	Lys	Lys	Tyr	Thr	Asp	Val	Ile	Glu	Lys	Phe	Val	Pro
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Asp	Pro	Glu	His	Phe	Arg	Lys	Thr	Thr	Asp	Asn	Pro	Phe	Lys	Ala	Leu
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Gly	Gly	Asn	Leu	Val	Asp	Met	Gly	Arg	Val	Lys	Val	Ile	Ala	Gly	Leu
465					470					475					480
Leu	Arg	Lys	Asp	Asp	Gln	Glu	Ile	Ser	Ser	Thr	Ile	Arg	Ser	Ile	Glu
				485					490					495	
Gln	Val	Phe	Lys	Leu	Val	Asp	Gln	Gly	Glu	Gly	Phe	Tyr	Gln	Asp	Gly
			500					505					510		
Ser	Tyr	Ile	Asp	His	Thr	Asn	Val	Ala	Tyr	Thr	Gly	Ala	Tyr	Gly	Asn
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Val	Leu	Ile	Asp	Gly	Leu	Ser	Gln	Leu	Leu	Pro	Val	Ile	Gln	Lys	Thr
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Lys	Asn	Pro	Ile	Asp	Lys	Asp	Lys	Met	Gln	Thr	Met	Tyr	His	Trp	Ile
545					550					555					560
Asp	Lys	Ser	Phe	Ala	Pro	Leu	Leu	Val	Asn	Gly	Glu	Leu	Met	Asp	Met
				565					570					575	
Ser	Arg	Gly	Arg	Ser	Ile	Ser	Arg	Ala	Asn	Ser	Glu	Gly	His	Val	Ala
			580					585					590		
Ala	Val	Glu	Val	Leu	Arg	Gly	Ile	His	Arg	Ile	Ala	Asp	Met	Ser	Glu
		595					600					605			
Gly	Glu	Thr	Lys	Gln	Arg	Leu	Gln	Ser	Leu	Val	Lys	Thr	Ile	Val	Gln
	610					615					620				
Ser	Asp	Ser	Tyr	Tyr	Asp	Val	Phe	Lys	Asn	Leu	Lys	Thr	Tyr	Lys	Asp
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Ile	Ser	Leu	Met	Gln	Ser	Leu	Leu	Ser	Asp	Ala	Gly	Val	Ala	Ser	Val
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Pro	Arg	Thr	Ser	Tyr	Leu	Ser	Ala	Phe	Asn	Lys	Met	Asp	Lys	Thr	Ala
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Met	Tyr	Asn	Ala	Glu	Lys	Gly	Phe	Gly	Phe	Gly	Leu	Ser	Leu	Phe	Ser
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Trp	Tyr	Thr	Ser	Asp	Gly	Met	Phe	Tyr	Leu	Tyr	Asn	Gly	Asp	Leu	Ser
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His	Tyr	Ser	Asp	Gly	Tyr	Trp	Pro	Thr	Val	Asn	Pro	Tyr	Lys	Met	Pro
				725					730					735	
Gly	Thr	Thr	Glu	Thr	Asp	Ala	Lys	Arg	Ala	Asp	Ser	Asp	Thr	Gly	Lys
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Val	Leu	Pro	Ser	Ala	Phe	Val	Gly	Thr	Ser	Lys	Leu	Asp	Asp	Ala	Asn
		755					760					765			
Ala	Thr	Ala	Thr	Met	Asp	Phe	Thr	Asn	Trp	Asn	Gln	Thr	Leu	Thr	Ala
	770					775					780				
His	Lys	Ser	Trp	Phe	Met	Leu	Lys	Asp	Lys	Ile	Ala	Phe	Leu	Gly	Ser
785					790					795					800
Asn	Ile	Gln	Asn	Thr	Ser	Thr	Asp	Thr	Ala	Ala	Thr	Thr	Ile	Asp	Gln
			805						810					815	
Arg	Lys	Leu	Glu	Ser	Ser	Asn	Pro	Tyr	Lys	Val	Tyr	Val	Asn	Asp	Lys
			820					825					830		
Glu	Ala	Ser	Leu	Thr	Glu	Gln	Glu	Lys	Asp	Tyr	Pro	Glu	Thr	Gln	Ser
		835					840					845			
Val	Phe	Leu	Glu	Ser	Ser	Asp	Ser	Lys	Lys	Asn	Ile	Gly	Tyr	Phe	Phe
	850					855					860				
Phe	Lys	Lys	Ser	Ser	Ile	Ser	Met	Ser	Lys	Ala	Leu	Gln	Lys	Gly	Ala
865					870					875					880
Trp	Lys	Asp	Ile	Asn	Glu	Gly	Gln	Ser	Asp	Lys	Glu	Val	Glu	Asn	Glu
				885					890					895	
Phe	Leu	Thr	Ile	Ser	Gln	Ala	His	Lys	Gln	Asn	Gly	Asp	Ser	Tyr	Gly
			900					905					910		
Tyr	Met	Leu	Ile	Pro	Asn	Val	Asp	Arg	Ala	Thr	Phe	Asn	Gln	Met	Ile
		915					920					925			
Lys	Glu	Leu	Glu	Ser	Ser	Leu	Ile	Glu	Asn	Asn	Glu	Thr	Leu	Gln	Ser
	930					935					940				

Val Tyr Asp Ala Lys Gln Gly Val Trp Gly Ile Val Lys Tyr Asp Asp
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 965 970 975
 Tyr Thr Ile Arg Lys Glu Gly Asp Glu Tyr Lys Ile Ala Tyr Tyr Asn
 980 985 990
 Pro Glu Thr Gln Glu Ser Ala Pro Asp Gln Glu Val Phe Lys Lys Leu
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 1010 1015 1020
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<210> 85

<211> 805

<212> PRT

<213> Streptococcus pyogenes serotype M1

<220>

<223> hyaluronan lyase

<400> 85

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 Ala Asp Thr Leu Thr Ser Asn Ser Glu Pro Asn Asn Thr Tyr Phe Gln
 35 40 45
 Thr Gln Thr Leu Thr Thr Thr Asp Ser Glu Lys Lys Val Val Gln Pro
 50 55 60
 Gln Gln Lys Asp Tyr Tyr Thr Glu Leu Leu Asp Gln Trp Asn Ser Ile
 65 70 75 80
 Ile Ala Gly Asn Asp Ala Tyr Asp Lys Thr Asn Pro Asp Met Val Thr
 85 90 95
 Phe His Asn Lys Ala Glu Lys Asp Ala Gln Asn Ile Ile Lys Ser Tyr
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 Gln Gly Pro Asp His Glu Asn Arg Thr Tyr Leu Trp Glu His Ala Lys
 115 120 125
 Asp Tyr Ser Ala Ser Ala Asn Ile Thr Lys Thr Tyr Arg Asn Ile Glu
 130 135 140
 Lys Ile Ala Lys Gln Ile Thr Asn Pro Glu Ser Cys Tyr Tyr Gln Asp
 145 150 155 160

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

Arg	Phe	Asn	Ala	Gln	Ser	His	Val	Ala	Gly	Ile	Glu	Ala	Leu	Arg	Ala
385					390					395					400
Ile	Leu	Arg	Ile	Ala	Asp	Met	Ser	Glu	Glu	Pro	His	Arg	Leu	Ala	Leu
			405						410					415	
Lys	Thr	Arg	Ile	Lys	Thr	Leu	Val	Thr	Gln	Gly	Asn	Ala	Phe	Tyr	Asn
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Val	Tyr	Asp	Asn	Leu	Lys	Thr	Tyr	His	Asp	Ile	Lys	Leu	Met	Lys	Glu
		435					440					445			
Leu	Leu	Ser	Asp	Thr	Ser	Val	Pro	Val	Gln	Lys	Leu	Asp	Ser	Tyr	Val
	450					455					460				
Ala	Ser	Phe	Asn	Ser	Met	Asp	Lys	Leu	Ala	Leu	Tyr	Asn	Asn	Lys	His
465					470					475					480
Asp	Phe	Ala	Phe	Gly	Leu	Ser	Met	Phe	Ser	Asn	Arg	Thr	Gln	Asn	Tyr
			485						490					495	
Glu	Ala	Met	Asn	Asn	Glu	Asn	Leu	His	Gly	Trp	Phe	Thr	Ser	Asp	Gly
			500					505					510		
Met	Phe	Tyr	Leu	Tyr	Asn	Asn	Asp	Leu	Gly	His	Tyr	Ser	Glu	Asn	Tyr
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Trp	Ala	Thr	Val	Asn	Pro	Tyr	Arg	Leu	Pro	Gly	Thr	Thr	Glu	Thr	Glu
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Gln	Lys	Pro	Leu	Glu	Gly	Thr	Pro	Glu	Asn	Ile	Lys	Thr	Asn	Tyr	Gln
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Gln	Val	Gly	Met	Thr	Gly	Leu	Ser	Asp	Asp	Ala	Phe	Val	Ala	Ser	Lys
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Lys	Leu	Asn	Asn	Thr	Ser	Ala	Leu	Ala	Ala	Met	Thr	Phe	Thr	Asn	Trp
		580						585					590		
Asn	Lys	Ser	Leu	Thr	Leu	Asn	Lys	Gly	Trp	Phe	Ile	Leu	Gly	Asn	Lys
		595					600					605			
Ile	Ile	Phe	Val	Gly	Ser	Asn	Ile	Lys	Asn	Gln	Ser	Ser	His	Lys	Ala
	610					615					620				
Tyr	Thr	Thr	Ile	Glu	Gln	Arg	Lys	Glu	Asn	Gln	Lys	Tyr	Pro	Tyr	Cys
625					630					635					640
Ser	Tyr	Val	Asn	Asn	Gln	Pro	Val	Asp	Leu	Asn	Asn	Gln	Leu	Val	Asp
			645						650				655		
Phe	Thr	Asn	Thr	Lys	Ser	Ile	Phe	Leu	Glu	Ser	Asp	Asp	Pro	Ala	Gln
		660						665					670		
Asn	Ile	Gly	Tyr	Tyr	Phe	Phe	Lys	Pro	Thr	Thr	Leu	Ser	Ile	Ser	Lys
		675					680					685			
Ala	Leu	Gln	Thr	Gly	Lys	Trp	Gln	Asn	Ile	Lys	Ala	Asp	Asp	Lys	Ser
	690					695					700				
Pro	Glu	Ala	Ile	Lys	Glu	Val	Ser	Asn	Thr	Phe	Ile	Thr	Ile	Met	Gln
705					710					715					720
Asn	His	Thr	Gln	Asp	Gly	Asp	Arg	Tyr	Ala	Tyr	Met	Met	Leu	Pro	Asn
			725						730					735	
Met	Thr	Arg	Gln	Glu	Phe	Glu	Thr	Tyr	Ile	Ser	Lys	Leu	Asp	Ile	Asp
		740						745					750		
Leu	Leu	Glu	Asn	Asn	Asp	Lys	Leu	Ala	Ala	Val	Tyr	Asp	His	Asp	Ser
		755					760					765			
Gln	Gln	Met	His	Val	Ile	His	Tyr	Gly	Lys	Lys	Ala	Thr	Met	Phe	Ser
	770					775					780				
Asn	His	Asn	Leu	Ser	His	Gln	Gly	Phe	Tyr	Ser	Phe	Pro	His	Pro	Val
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<210> 86

<211> 780

<212> PRT

<213> Streptococcus pyogenes serotype M2 (strain MGAS10)

<220>

<223> hyaluronan lyase

<400> 86

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

Glu	Lys	Lys	Val	Val	Gln	Pro	Gln	Gln	Lys	Asp	Tyr	Tyr	Thr	Glu	Leu
85				85					90					95	
Leu	Asp	Gln	Trp	Asn	Ser	Ile	Ile	Ala	Gly	Asn	Asp	Ala	Tyr	Asp	Lys
100								105						110	
Thr	Asn	Pro	Asp	Met	Val	Thr	Phe	His	Asn	Lys	Ala	Glu	Lys	Asp	Ala
115							120					125			
Gln	Asn	Ile	Ile	Lys	Ser	Tyr	Gln	Gly	Pro	Asp	His	Glu	Asn	Arg	Thr
130						135					140				
Tyr	Leu	Trp	Glu	His	Ala	Lys	Asp	Tyr	Ser	Ala	Ser	Thr	Asn	Ile	Thr
145				150					155					160	
Lys	Thr	Tyr	Arg	Asn	Ile	Glu	Lys	Ile	Ala	Lys	Gln	Ile	Thr	Asn	Pro
165									170					175	
Glu	Ser	Cys	Tyr	Tyr	Gln	Asp	Ser	Lys	Ala	Ile	Ala	Ile	Val	Lys	Asp
180								185					190		
Gly	Met	Ala	Phe	Met	Tyr	Glu	His	Ala	Tyr	Asn	Leu	Asn	Arg	Glu	Asn
195							200					205			
His	Gln	Thr	Thr	Gly	Lys	Glu	Asn	Lys	Glu	Asn	Trp	Trp	Val	Tyr	Glu
210						215					220				
Ile	Gly	Thr	Pro	Arg	Ala	Ile	Asn	Asn	Thr	Leu	Ser	Leu	Met	Tyr	Pro
225					230					235					240
Tyr	Phe	Thr	Gln	Glu	Glu	Ile	Leu	Lys	Tyr	Thr	Ala	Pro	Ile	Glu	Lys
245									250					255	
Phe	Val	Pro	Asp	Pro	Thr	Arg	Phe	Arg	Val	Arg	Ala	Ala	Asn	Phe	Ser
260								265					270		
Pro	Phe	Glu	Ala	Asn	Ser	Gly	Asn	Lys	Ile	Asp	Met	Gly	Arg	Val	Lys
275							280					285			
Leu	Ile	Ser	Gly	Ile	Leu	Arg	Lys	Asp	Asp	Leu	Glu	Ile	Ser	Asp	Thr
290						295					300				
Ile	Lys	Ala	Ile	Glu	Lys	Val	Phe	Thr	Leu	Val	Asp	Glu	Gly	Asn	Gly
305					310					315					320
Phe	Tyr	Gln	Asp	Gly	Ser	Leu	Ile	Asp	His	Val	Val	Thr	Asn	Thr	Gln
325									330					335	
Ser	Pro	Leu	Tyr	Lys	Lys	Gly	Ile	Ala	Tyr	Thr	Gly	Ala	Tyr	Gly	Asn
340								345					350		
Val	Leu	Ile	Asp	Gly	Leu	Ser	Gln	Leu	Ile	Pro	Ile	Ile	Gln	Lys	Thr
355							360					365			
Lys	Ser	Pro	Ile	Glu	Ala	Asp	Lys	Met	Ala	Thr	Ile	Tyr	His	Trp	Ile
370						375					380				
Asn	His	Ser	Phe	Phe	Pro	Ile	Ile	Val	Arg	Gly	Glu	Met	Met	Asp	Met
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Thr	Arg	Gly	Arg	Ser	Ile	Ser	Arg	Phe	Asn	Ala	Gln	Ser	His	Val	Ala
405									410					415	
Gly	Ile	Glu	Ala	Leu	Arg	Ala	Ile	Leu	Arg	Ile	Ala	Asp	Met	Ser	Glu
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Glu	Pro	His	Arg	Leu	Glu	Leu	Lys	Thr	Arg	Ile	Lys	Thr	Leu	Val	Thr
435							440					445			
Gln	Gly	Asn	Ala	Phe	Tyr	Asn	Val	Tyr	Asp	Asn	Leu	Lys	Thr	Tyr	His
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Asp	Ile	Lys	Leu	Met	Lys	Glu	Leu	Leu	Ser	Asp	Thr	Ser	Val	Pro	Val
465					470					475					480
Gln	Lys	Leu	Asp	Ser	Tyr	Val	Ala	Ser	Phe	Asn	Ser	Met	Asp	Lys	Leu
485									490					495	
Ala	Leu	Tyr	Asn	Asn	Lys	His	Asp	Phe	Ala	Phe	Gly	Leu	Ser	Met	Phe
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Ser	Asn	Arg	Thr	Gln	Asn	Tyr	Glu	Ala	Met	Asn	Asn	Glu	Asn	Leu	His
515							520					525			
Gly	Trp	Phe	Thr	Ser	Asp	Gly	Met	Phe	Tyr	Leu	Tyr	Asn	Asn	Asp	Leu
530						535					540				
Gly	His	Tyr	Ser	Glu	Asn	Tyr	Trp	Ala	Thr	Val	Asn	Pro	Tyr	Arg	Leu
545					550					555					560
Pro	Gly	Thr	Thr	Glu	Thr	Glu	Gln	Lys	Pro	Leu	Glu	Gly	Thr	Pro	Glu
565									570					575	
Asn	Ile	Lys	Thr	Asn	Tyr	Gln	Gln	Val	Gly	Met	Thr	Ser	Leu	Ser	Asp
580								585					590		
Asp	Ala	Phe	Val	Ala	Ser	Lys	Lys	Leu	Asn	Asn	Thr	Ser	Ala	Leu	Ala
595							600					605			
Ala	Met	Thr	Phe	Thr	Asn	Trp	Asn	Lys	Ser	Leu	Thr	Leu	Asn	Lys	Gly
610						615					620				
Trp	Phe	Ile	Leu	Gly	Asn	Lys	Ile	Ile	Phe	Val	Gly	Ser	Asn	Ile	Lys
625					630					635					640
Asn	Gln	Ser	Ser	His	Lys	Ala	Tyr	Thr	Thr	Ile	Glu	Gln	Arg	Lys	Glu
645									650					655	
Asn	Gln	Lys	His	Pro	Tyr	Cys	Ser	Tyr	Val	Asn	Asn	Gln	Pro	Val	Asp
660								665					670		
Leu	Asn	Asn	Gln	Leu	Val	Asp	Phe	Thr	Asn	Thr	Lys	Ser	Ile	Phe	Leu

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675	Glu	Ser	Asp	Asp	Pro	Ala	Gln	Asn	Ile	Gly	Tyr	Tyr	Phe	Phe	Lys	Pro
690	Arg	Thr	Leu	Ser	Ile	Ser	Lys	Ala	Leu	Gln	Thr	Gly	Lys	Trp	Gln	Asn
705	Ile	Lys	Ala	Asp	Asp	Lys	Ser	Pro	Glu	Ala	Ile	Lys	Glu	Val	Ser	Asn
	Thr	Phe	Ile	Thr	Ile	Met	Gln	Asn	His	Thr	Gln	Glu	Gly	Asp	Arg	Tyr
	Ala	Tyr	Met	Met	Leu	Pro	Asn	Met	Thr	Arg	Gln	Glu	Phe	Glu	Thr	Tyr
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<210> 87

<211> 868

<212> PRT

<213> Streptococcus pyogenes serotype M4 (strain MGAS10)

<220>

<223> hyaluronan lyase

<400> 87

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

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				405					410					415	
Lys	Thr	Arg	Ile	Lys	Thr	Leu	Val	Thr	Gln	Gly	Asn	Val	Phe	Tyr	Asn
			420					425					430		
Val	Tyr	Asp	Asn	Leu	Lys	Thr	Tyr	His	Asp	Ile	Lys	Leu	Met	Lys	Glu
		435					440					445			
Leu	Leu	Ser	Asp	Thr	Ser	Val	Pro	Val	Gln	Lys	Leu	Asp	Ser	Tyr	Val
	450					455					460				
Ala	Ser	Phe	Asn	Ser	Met	Asp	Lys	Leu	Ala	Leu	Tyr	Asn	Asn	Lys	His
465					470				475					480	
Asp	Phe	Ala	Phe	Gly	Leu	Ser	Met	Phe	Ser	Asn	Arg	Thr	Gln	Asn	Tyr
				485					490					495	
Glu	Ala	Met	Asn	Asn	Glu	Asn	Leu	His	Gly	Trp	Phe	Thr	Ser	Asp	Gly
			500					505					510		
Met	Phe	Tyr	Leu	Tyr	Asn	Asn	Asp	Leu	Gly	His	Tyr	Ser	Glu	Asn	Tyr
		515					520					525			
Trp	Ala	Thr	Val	Asn	Pro	Tyr	Arg	Leu	Pro	Gly	Thr	Thr	Glu	Thr	Glu
	530					535					540				
Gln	Lys	Pro	Leu	Glu	Gly	Thr	Pro	Glu	Asn	Ile	Lys	Thr	Asn	Tyr	Gln
545					550					555					560
Gln	Val	Gly	Met	Thr	Ser	Leu	Ser	Asp	Asp	Ala	Phe	Val	Ala	Ser	Lys
				565					570					575	
Lys	Leu	Asn	Asn	Thr	Ser	Ala	Leu	Ala	Ala	Met	Thr	Phe	Thr	Asn	Trp
			580					585					590		
Asn	Lys	Ser	Leu	Thr	Leu	Asn	Lys	Gly	Trp	Phe	Ile	Leu	Gly	Asn	Lys
		595					600					605			
Ile	Ile	Phe	Val	Gly	Ser	Asn	Ile	Lys	Asn	Gln	Ser	Ser	His	Lys	Ala
	610					615					620				
Tyr	Thr	Thr	Ile	Glu	Gln	Arg	Lys	Glu	Asn	Gln	Lys	His	Pro	Tyr	Cys
625					630					635					640
Ser	Tyr	Val	Asn	Asn	Gln	Pro	Val	Asp	Leu	Asn	Asn	Gln	Leu	Val	Asp
				645					650					655	
Phe	Thr	Asn	Thr	Lys	Ser	Ile	Phe	Leu	Glu	Ser	Asp	Asp	Pro	Ala	Gln
			660					665					670		
Asn	Ile	Gly	Tyr	Tyr	Phe	Phe	Lys	Pro	Thr	Thr	Leu	Ser	Ile	Ser	Lys
		675					680					685			
Ala	Leu	Gln	Thr	Gly	Lys	Trp	Gln	Asn	Ile	Lys	Ala	Asp	Asp	Lys	Ser
	690					695					700				
Pro	Glu	Ala	Ile	Lys	Glu	Val	Ser	Asn	Thr	Phe	Ile	Thr	Ile	Met	Gln
705					710					715				720	
Asn	His	Thr	Gln	Asp	Gly	Asp	Arg	Tyr	Ala	Tyr	Met	Met	Leu	Pro	Asn
				725					730					735	
Met	Thr	Arg	Gln	Glu	Phe	Glu	Thr	Tyr	Ile	Ser	Lys	Leu	Asp	Ile	Asp
			740					745					750		
Leu	Leu	Glu	Asn	Asn	Asp	Lys	Leu	Ala	Ala	Val	Tyr	Asp	His	Asp	Ser
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<210> 88
<211> 828
<212> PRT
<213> Streptococcus pyogenes serotype M6
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<220>
<223> hyaluronan lyase

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Leu Tyr Trp Arg Asn Leu Ser Val Asn Thr Tyr Phe Cys Thr His His
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Lys Gln Leu Leu Leu Tyr Ser Asn Leu Phe Leu Ser Phe Ala Met Met

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Asn	Gln	Ser	Ser	His	Lys	Ala	Tyr	Thr	Thr	Ile	Glu	Gln	Arg	Lys	Glu
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Asn	Gln	Lys	His	Pro	Tyr	Cys	Ser	Tyr	Val	Asn	Asn	Gln	Pro	Val	Asp
			660					665					670		
Leu	Asn	Asn	Gln	Leu	Val	Asp	Phe	Thr	Asn	Thr	Lys	Ser	Ile	Phe	Leu
		675					680					685			
Glu	Ser	Asp	Asp	Pro	Ala	Gln	Asn	Ile	Gly	Tyr	Tyr	Phe	Phe	Lys	Pro
	690					695					700				
Thr	Thr	Leu	Ser	Ile	Ser	Lys	Ala	Leu	Gln	Thr	Gly	Lys	Trp	Gln	Asn
	705				710					715					720
Ile	Lys	Ala	Asp	Asp	Lys	Ser	Pro	Glu	Ala	Ile	Lys	Glu	Val	Ser	Asn
			725						730					735	
Thr	Phe	Ile	Thr	Ile	Met	Gln	Asn	His	Thr	Gln	Asp	Gly	Asp	Arg	Tyr
			740					745					750		
Ala	Tyr	Met	Met	Leu	Pro	Asn	Met	Thr	Arg	Gln	Glu	Phe	Glu	Thr	Tyr
		755					760					765			
Ile	Ser	Lys	Leu	Asp	Ile	Asp	Leu	Leu	Glu	Asn	Asn	Asp	Lys	Leu	Ala
	770					775					780				
Ala	Val	Tyr	Asp	His	Asp	Ser	Gln	Gln	Met	His	Val	Ile	His	Tyr	Glu
	785				790					795					800
Lys	Lys	Ala	Thr	Met	Phe	Ser	Asn	His	Asn	Leu	Ser	His	Gln	Gly	Phe
				805					810					815	
Tyr	Ser	Phe	Pro	His	Pro	Val	Lys	Gln	Asn	Gln	Gln				
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<211> 556

<212> PRT

<213> Streptococcus pyogenes serotype M12 (strain MGAS2)

<220>

<223> hyaluronan lyase

<400> 89

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			20					25				30			
Lys	Gln	Leu	Leu	Tyr	Ser	Asn	Leu	Phe	Leu	Ser	Phe	Ala	Met	Met	
		35				40					45				
Gly	Gln	Gly	Thr	Ala	Ile	Tyr	Ala	Asp	Thr	Leu	Thr	Ser	Asn	Ser	Glu
	50					55				60					
Pro	Asn	Asn	Thr	Tyr	Phe	Gln	Thr	Gln	Thr	Leu	Thr	Thr	Thr	Asp	Ser
	65				70					75				80	
Glu	Lys	Lys	Val	Val	Gln	Pro	Gln	Gln	Lys	Asp	Tyr	Tyr	Thr	Glu	Leu
				85					90					95	
Leu	Asp	Gln	Trp	Asn	Ser	Ile	Ile	Ala	Gly	Asn	Asp	Ala	Tyr	Asp	Lys
			100					105					110		
Thr	Asn	Pro	Asp	Met	Val	Thr	Phe	His	Asn	Lys	Ala	Glu	Lys	Asp	Ala
			115				120					125			
Gln	Asn	Ile	Ile	Lys	Ser	Tyr	Gln	Gly	Pro	Asp	His	Glu	Asn	Arg	Thr
	130					135					140				
Tyr	Leu	Gly	Asn	Met	Gln	Arg	Ile	Ile	Pro	Leu	Leu	Ile	Ser	Arg	
	145				150					155				160	
Lys	Leu	Thr	Ala	Ile	Leu	Lys	Lys	Ile	Ser	Lys	Met	Lys	Ser	Leu	Met
				165					170					175	
Glu	Asp	Ser	Thr	Ile	Ser	Thr	Asn	Gly	Leu	Thr	Gln	Gln	Leu	Lys	Ile
			180					185					190		
Tyr	Asn	Asp	Met	Asp	Arg	Val	Thr	Tyr	His	Asn	Lys	Gly	Leu	Asp	Phe
		195					200					205			
Ala	Phe	Gly	Leu	Ser	Met	Thr	Ser	Lys	Asn	Val	Ala	Arg	Tyr	Glu	Ser
	210					215					220				
Ile	Asn	Gly	Glu	Asn	Leu	Lys	Gly	Trp	His	Thr	Gly	Ala	Gly	Met	Ser
	225				230					235					240
Tyr	Leu	Tyr	Asn	Ser	Asp	Val	Lys	His	Tyr	Arg	Asp	Asn	Phe	Trp	Ala
				245					250					255	
Thr	Ala	Asp	Met	Lys	Arg	Leu	Ala	Gly	Thr	Thr	Thr	Leu	Asp	Asn	Glu
			260					265					270		
Glu	Pro	Lys	Ser	Thr	Asp	Val	Lys	Lys	Ser	Ser	Lys	Thr	Phe	Val	Gly
		275					280					285			
Gly	Thr	Lys	Phe	Asp	Asp	Gln	His	Ala	Ser	Ile	Gly	Met	Asp	Phe	Glu
	290					295					300				
Asn	Gln	Asp	Lys	Thr	Leu	Thr	Ala	Lys	Lys	Ser	Tyr	Phe	Ile	Leu	Asn
	305				310					315					320

Asp Lys Ile Val Phe Leu Gly Thr Gly Ile Lys Ser Thr Asp Ser Ser
 325 330 335
 Lys Asn Pro Val Thr Thr Ile Glu Asn Arg Lys Ala Asn Asp Tyr Lys
 340 345 350
 Leu Tyr Lys Asp Asp Thr Gln Thr Thr Asn Ser Asp Asn Gln Glu Thr
 355 360 365
 Asn Ser Leu Phe Leu Glu Ser Thr Asn Ser Thr Gln Asn Asn Ile Gly
 370 375 380
 Tyr His Phe Leu Asn Glu Ser Lys Ile Thr Val Lys Lys Glu Ser His
 385 390 395 400
 Thr Gly Lys Trp Ser Asp Ile Asn Lys Ser Gln Lys Asp Ile Gln Lys
 405 410 415
 Thr Asp Glu Tyr Tyr Glu Val Thr Gln Lys His Ser Asn Thr Asp Ser
 420 425 430
 Lys Tyr Ala Tyr Val Leu Tyr Pro Gly Leu Ser Lys Asp Val Phe Lys
 435 440 445
 Ser Lys Ala Ser Lys Val Thr Val Val Lys Gln Glu Asp Asp Phe His
 450 455 460
 Val Val Lys Asp Asn Glu Ser Val Trp Ala Gly Ile Asn Tyr Ser Asp
 465 470 475 480
 Ser Ala Lys Thr Phe Glu Ile Asn Asn Thr Lys Val Glu Val Lys Ala
 485 490 495
 Lys Gly Met Phe Ile Leu Thr Lys Lys Asp Asp Asn Thr Tyr Glu Cys
 500 505 510
 Ser Phe Tyr Asn Pro Glu Ser Thr Asn Ser Val Ser Asp Ile Glu Ser
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<210> 90

<211> 635

<212> PRT

<213> Streptococcus pyogenes serotype M12 (strain MGAS2)

<220>

<223> hyaluronan lyase

<400> 90

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

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Ile Lys Leu Met Lys Glu Leu Leu Ser Asp Thr Ser Val Pro Val Gln
275 280 285
Lys Leu Asp Ser Tyr Val Ala Ser Phe Asn Ser Met Asp Lys Leu Ala
290 295 300
Leu Tyr Asn Asn Lys His Asp Phe Ala Phe Gly Leu Ser Met Phe Ser
305 310 315
Asn Arg Thr Gln Asn Tyr Glu Ala Met Asn Asn Glu Asn Leu His Gly
325 330 335
Trp Phe Thr Ser Asp Gly Met Phe Tyr Leu Tyr Asn Asn Asp Leu Gly
340 345 350
His Tyr Ser Glu Asn Tyr Trp Ala Thr Val Asn Pro Tyr Arg Leu Pro
355 360 365
Gly Thr Thr Glu Thr Glu Gln Lys Pro Leu Glu Gly Thr Pro Glu Asn
370 375 380
Ile Lys Thr Asn Tyr Gln Gln Val Gly Met Thr Ser Leu Ser Asp Asp
385 390 395
Ala Phe Val Ala Ser Lys Lys Leu Asn Asn Thr Ser Ala Leu Ala Ala
405 410 415
Met Thr Phe Thr Asn Trp Asn Lys Ser Leu Thr Leu Asn Lys Gly Trp
420 425 430
Phe Ile Leu Gly Asn Lys Ile Ile Phe Val Gly Ser Asn Ile Lys Asn
435 440 445
Gln Ser Ser His Lys Ala Tyr Thr Thr Ile Glu Gln Arg Lys Glu Asn
450 455 460
Gln Lys His Pro Tyr Cys Ser Tyr Val Asn Asn Gln Pro Val Asp Leu
465 470 475
Asn Asn Gln Leu Val Asp Phe Thr Asn Thr Lys Ser Ile Phe Leu Glu
485 490 495
Ser Asp Asp Pro Ala Gln Asn Ile Gly Tyr Tyr Phe Phe Lys Pro Thr
500 505 510
Thr Leu Ser Ile Ser Lys Ala Leu Gln Thr Gly Lys Trp Gln Asn Ile
515 520 525
Lys Ala Asp Asp Lys Ser Pro Glu Ala Ile Lys Glu Val Ser Asn Thr
530 535 540
Phe Ile Thr Ile Met Gln Asn His Thr Gln Asp Gly Asp Arg Tyr Ala
545 550 555
Tyr Met Met Leu Pro Asn Met Thr Arg Gln Glu Phe Glu Thr Tyr Ile
565 570 575
Ser Lys Leu Asp Ile Asp Leu Leu Glu Asn Asn Asp Lys Leu Ala Ala
580 585 590
Val Tyr Asp His Asp Ser Gln Gln Met His Val Ile His Tyr Glu Lys
595 600 605
Lys Ala Thr Met Phe Ser Asn His Asn Leu Ser His Gln Gly Phe Tyr
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Ser Phe Pro His Pro Val Lys Gln Asn Gln Gln
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<210> 91

<211> 828

<212> PRT

<213> Streptococcus pyogenes serotype M12 (strain MGAS9)

<220>

<223> hyaluronan lyase

<400> 91

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Lys Gln Leu Leu Leu Tyr Ser Asn Leu Phe Leu Ser Phe Ala Met Met
35 40 45
Gly Gln Gly Thr Ala Ile Tyr Ala Asp Thr Leu Thr Ser Asn Ser Glu
50 55 60
Pro Asn Asn Thr Tyr Phe Gln Thr Gln Thr Leu Thr Thr Thr Asp Ser
65 70 75 80
Glu Lys Lys Val Val Gln Pro Gln Gln Lys Asp Tyr Tyr Thr Glu Leu
85 90 95
Leu Asp Gln Trp Asn Ser Ile Ile Ala Gly Asn Asp Ala Tyr Val Lys
100 105 110
Thr Asn Pro Asp Met Val Thr Phe His Asn Lys Ala Glu Lys Asp Ala
115 120 125
Gln Asn Ile Ile Lys Ser Tyr Gln Gly Pro Asp His Glu Asn Arg Thr
130 135 140

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Tyr 145	Leu	Trp	Glu	His 150	Ala 150	Lys	Asp	Tyr	Ser 155	Ala 155	Ser	Thr	Asn	Ile	Thr 160
Lys	Thr	Tyr	Arg	Asn 165	Ile 165	Glu	Lys	Ile	Ala 170	Lys 170	Gln	Ile	Thr	Asn 175	Pro 175
Glu	Ser	Cys	Tyr 180	Tyr	Gln	Asp	Ser	Lys 185	Ala 185	Ile	Ala	Ile	Val 190	Lys 190	Asp 190
Gly	Met	Ala 195	Phe	Met	Tyr	Glu	His 200	Ala	Tyr	Asn	Leu	Asn 205	Arg	Glu	Asn 205
His	Gln 210	Thr	Thr	Gly	Lys	Glu 215	Asn	Lys	Glu	Asn	Trp 220	Trp	Val	Tyr	Glu 220
Ile 225	Gly	Thr	Pro	Arg	Ala 230	Ile	Asn	Asn	Thr	Leu 235	Ser	Leu	Met	Tyr	Pro 240
Tyr	Phe	Thr	Gln	Glu 245	Glu	Ile	Leu	Lys	Tyr 250	Thr	Ala	Pro	Ile	Glu 255	Lys 255
Phe	Val	Pro	Asp 260	Pro	Thr	Arg	Phe	Arg 265	Val	Arg	Ala	Ala	Asn 270	Phe	Ser 270
Pro	Phe	Glu 275	Ala	Ser	Ser	Gly	Asn 280	Leu	Ile	Asp	Met	Gly 285	Arg	Val	Lys 285
Leu	Ile 290	Ser	Gly	Ile	Leu	Arg 295	Lys	Asp	Asp	Leu	Glu 300	Ile	Ser	Asp	Thr 300
Ile 305	Lys	Ala	Ile	Glu	Lys 310	Val	Phe	Thr	Leu	Val 315	Asp	Glu	Gly	Asn	Gly 320
Phe	Tyr	Gln	Asp	Gly 325	Ser	Leu	Ile	Asp	His 330	Val	Val	Thr	Asn	Ala 335	Gln 335
Ser	Pro	Leu	Tyr 340	Lys	Lys	Gly	Ile	Ala 345	Tyr	Thr	Gly	Ala	Tyr 350	Gly	Asn 350
Val	Leu	Ile 355	Asp	Gly	Leu	Ser	Gln 360	Leu	Ile	Pro	Ile	Ile 365	Gln	Lys	Thr 365
Lys	Ser 370	Pro	Ile	Glu	Ala 375	Lys	Met	Ala	Thr	Ile	Ile 380	Tyr	His	Trp	Ile 380
Asn 385	His	Ser	Phe	Phe	Pro 390	Ile	Ile	Val	Arg	Gly 395	Glu	Met	Met	Asp	Met 400
Thr	Arg	Gly	Arg	Ser 405	Ile	Ser	Arg	Phe	Asn 410	Ala	Gln	Ser	His	Val 415	Ala 415
Gly	Ile	Glu	Ala 420	Leu	Arg	Ala	Ile	Leu	Arg 425	Ile	Ala	Asp	Met 430	Ser	Glu 430
Glu	Pro	His 435	Arg	Leu	Ala	Leu	Lys 440	Thr	Arg	Ile	Lys	Thr 445	Leu	Val	Thr 445
Gln	Gly 450	Asn	Ala	Phe	Tyr	Asn 455	Val	Tyr	Asp	Asn	Leu 460	Lys	Thr	Tyr	His 460
Asp 465	Ile	Lys	Leu	Met	Lys 470	Glu	Leu	Leu	Ser	Asp 475	Thr	Ser	Val	Pro	Val 480
Gln	Lys	Leu	Asp	Ser 485	Tyr	Val	Ala	Ser	Phe 490	Asn	Ser	Met	Asp	Lys 495	Leu 495
Ala	Leu	Tyr	Asn 500	Asn	Lys	His	Asp	Phe 505	Ala	Phe	Gly	Leu	Ser 510	Met	Phe 510
Ser	Asn	Arg 515	Thr	Gln	Asn	Tyr	Glu 520	Ala	Met	Asn	Asn	Glu 525	Asn	Leu	His 525
Gly	Trp 530	Phe	Thr	Ser	Asp	Gly 535	Met	Phe	Tyr	Leu	Tyr 540	Asn	Asn	Asp	Leu 540
Gly 545	His	Tyr	Ser	Glu	Asn 550	Tyr	Trp	Ala	Thr	Val 555	Asn	Pro	Tyr	Arg	Leu 560
Pro	Gly	Thr	Thr	Glu 565	Thr	Glu	Gln	Lys	Pro 570	Leu	Glu	Gly	Thr	Pro 575	Glu 575
Asn	Ile	Lys	Thr 580	Asn	Tyr	Gln	Gln	Val 585	Gly	Met	Thr	Ser	Leu 590	Ser	Asp 590
Asp	Ala	Phe 595	Val	Ala	Ser	Lys	Lys 600	Leu	Asn	Asn	Thr	Ser 605	Ala	Leu	Ala 605
Ala	Met 610	Thr	Phe	Thr	Asn	Trp 615	Asn	Lys	Ser	Leu	Thr 620	Leu	Asn	Lys	Gly 620
Trp	Phe	Ile	Leu	Gly	Asn 630	Lys	Ile	Ile	Phe	Val 635	Gly	Ser	Asn	Ile	Lys 640
Asn	Gln	Ser	Ser	His 645	Lys	Ala	Tyr	Thr	Thr 650	Ile	Glu	Gln	Arg	Lys 655	Glu 655
Asn	Gln	Lys	His 660	Pro	Tyr	Cys	Ser	Tyr 665	Val	Asn	Asn	Gln	Pro 670	Val	Asp 670
Leu	Asn	Asn 675	Gln	Leu	Val	Asp	Phe 680	Thr	Asn	Thr	Lys	Ser 685	Ile	Phe	Leu 685
Glu	Ser 690	Asp	Asp	Pro	Ala	Gln 695	Asn	Ile	Gly	Tyr	Tyr 700	Phe	Phe	Lys	Pro 700
Thr	Thr 705	Leu	Ser	Ile	Ser 710	Lys	Ala	Leu	Gln	Thr 715	Gly	Lys	Trp	Gln	Asn 720
Ile	Lys	Ala	Asp	Asp 725	Lys	Ser	Pro	Glu	Ala 730	Ile	Lys	Glu	Val	Ser	Asn 735
Thr	Phe	Ile	Thr	Ile	Met	Gln	Asn	His	Thr	Gln	Asp	Gly	Asp	Arg	Tyr 735

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Ala	Tyr	Met	Met	Leu	Pro	Asn	Met	Thr	Arg	Gln	Glu	Phe	Glu	Thr	Tyr
		755					760					765			
Ile	Ser	Lys	Leu	Asp	Ile	Asp	Leu	Leu	Glu	Asn	Asn	Asp	Lys	Leu	Ala
	770					775					780				
Ala	Val	Tyr	Asp	His	Asp	Ser	Gln	Gln	Met	His	Val	Ile	His	Tyr	Glu
785					790					795					800
Lys	Lys	Ala	Thr	Met	Phe	Ser	Asn	His	Asn	Leu	Ser	His	Gln	Gly	Phe
				805					810					815	
Tyr	Ser	Phe	Pro	His	Pro	Val	Lys	Gln	Asn	Gln	Gln				
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<210> 92

<211> 828

<212> PRT

<213> Streptococcus pyogenes serotype M28

<220>

<223> hyaluronan lyase

<400> 92

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			20					25					30		
Lys	Gln	Leu	Leu	Leu	Tyr	Ser	Asn	Leu	Phe	Leu	Ser	Phe	Ala	Met	Met
		35					40					45			
Gly	Gln	Gly	Thr	Ala	Ile	Tyr	Ala	Asp	Thr	Leu	Thr	Ser	Asn	Ser	Glu
	50					55					60				
Pro	Asn	Asn	Thr	Tyr	Phe	Gln	Thr	Gln	Met	Leu	Thr	Thr	Thr	Asp	Ser
65					70					75					80
Glu	Lys	Lys	Val	Val	Gln	Pro	Gln	Gln	Lys	Asp	Tyr	Tyr	Thr	Glu	Leu
			85						90					95	
Leu	Asp	Gln	Trp	Asn	Ser	Ile	Ile	Ala	Gly	Asn	Asp	Ala	Tyr	Asp	Lys
			100					105					110		
Thr	Asn	Pro	Asp	Met	Val	Thr	Phe	His	Asn	Lys	Ala	Glu	Lys	Asp	Ala
		115					120					125			
Gln	Asn	Ile	Ile	Lys	Ser	Tyr	Gln	Gly	Pro	Asp	His	Glu	Asn	Arg	Thr
	130					135					140				
Tyr	Leu	Trp	Glu	His	Ala	Lys	Asp	Tyr	Ser	Ala	Ser	Ala	Asn	Ile	Thr
145					150					155					160
Lys	Thr	Tyr	Arg	Asn	Ile	Glu	Lys	Ile	Ala	Lys	Gln	Ile	Thr	Asn	Pro
				165					170					175	
Glu	Ser	Cys	Tyr	Tyr	Gln	Asp	Ser	Lys	Ala	Ile	Ala	Ile	Val	Lys	Asp
			180					185					190		
Gly	Met	Ala	Phe	Met	Tyr	Glu	His	Ala	Tyr	Asn	Leu	Asp	Arg	Glu	Asn
		195					200					205			
His	Gln	Thr	Thr	Gly	Lys	Glu	Asn	Lys	Glu	Asn	Trp	Trp	Val	Tyr	Glu
	210					215					220				
Ile	Gly	Thr	Pro	Arg	Ala	Ile	Asn	Asn	Thr	Leu	Ser	Leu	Met	Tyr	Pro
225					230					235					240
Tyr	Phe	Thr	Gln	Glu	Ile	Leu	Lys	Tyr	Thr	Ala	Pro	Ile	Glu	Lys	
			245						250				255		
Phe	Val	Pro	Asp	Pro	Thr	Arg	Phe	Arg	Val	Arg	Ala	Ala	Asn	Phe	Ser
			260					265					270		
Pro	Phe	Glu	Ala	Asn	Ser	Gly	Asn	Leu	Ile	Asp	Met	Gly	Arg	Val	Lys
		275					280					285			
Leu	Ile	Ser	Gly	Ile	Leu	Arg	Lys	Asp	Asp	Leu	Glu	Ile	Ser	Asp	Thr
	290					295					300				
Ile	Lys	Ala	Ile	Glu	Lys	Val	Phe	Thr	Leu	Val	Asp	Glu	Gly	Asn	Gly
305					310					315					320
Phe	Tyr	Gln	Asp	Gly	Ser	Leu	Ile	Asp	His	Val	Val	Thr	Asn	Ala	Gln
				325					330					335	
Ser	Pro	Leu	Tyr	Lys	Lys	Gly	Ile	Ala	Tyr	Thr	Gly	Ala	Tyr	Gly	Asn
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Val	Leu	Ile	Asp	Gly	Leu	Ser	Gln	Leu	Ile	Pro	Ile	Ile	Gln	Lys	Thr
		355					360					365			
Lys	Ser	Ser	Ile	Glu	Ala	Asp	Lys	Met	Ala	Thr	Ile	Tyr	His	Trp	Ile
	370					375					380				
Asn	His	Ser	Phe	Phe	Pro	Ile	Ile	Val	Arg	Gly	Glu	Met	Met	Asp	Met
385					390					395					400
Thr	Arg	Gly	Arg	Ser	Ile	Ser	Arg	Phe	Asn	Ala	Gln	Ser	His	Val	Ala
				405					410					415	
Gly	Ile	Glu	Ala	Leu	Arg	Ala	Ile	Leu	Arg	Ile	Ala	Asp	Met	Ser	Glu

3066SEQ PC1.TXT

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Gln	Gly	Asn	Ala	Phe	Tyr	Asn	Val	Tyr	Asp	Asn	Leu	Lys	Thr	Tyr	His																			
	450					455					460																							
Asp	Ile	Lys	Leu	Met	Lys	Glu	Leu	Leu	Ser	Asp	Thr	Ser	Val	Pro	Val																			
465					470					475					480																			
Gln	Lys	Leu	Asp	Ser	Tyr	Val	Ala	Ser	Phe	Asn	Ser	Met	Asp	Lys	Leu																			
			485						490					495																				
Ala	Leu	Tyr	Asn	Asn	Lys	His	Asp	Phe	Ala	Phe	Gly	Leu	Ser	Met	Phe																			
		500					505						510																					
Ser	Asn	Arg	Thr	Gln	Asn	Tyr	Glu	Ala	Met	Asn	Asn	Glu	Asn	Leu	His																			
	515					520						525																						
Gly	Trp	Phe	Thr	Ser	Asp	Gly	Met	Phe	Tyr	Leu	Tyr	Asn	Asn	Asp	Leu																			
	530				535						540																							
Gly	His	Tyr	Ser	Glu	Asn	Tyr	Trp	Ala	Thr	Val	Asn	Pro	Tyr	Arg	Leu																			
545					550					555					560																			
Pro	Gly	Thr	Thr	Glu	Thr	Glu	Gln	Lys	Pro	Leu	Glu	Gly	Thr	Pro	Glu																			
			565					570						575																				
Asn	Ile	Lys	Thr	Asn	Tyr	Gln	Gln	Val	Gly	Met	Thr	Ser	Leu	Ser	Asp																			
		580					585						590																					
Asp	Ala	Phe	Val	Ala	Ser	Lys	Lys	Leu	Asn	Asn	Thr	Ser	Ala	Leu	Ala																			
	595					600						605																						
Ala	Met	Thr	Phe	Thr	Asn	Trp	Asn	Lys	Ser	Leu	Thr	Leu	Asn	Lys	Gly																			
	610				615						620																							
Trp	Phe	Ile	Leu	Gly	Asn	Lys	Ile	Ile	Phe	Val	Gly	Ser	Asn	Ile	Lys																			
625					630					635					640																			
Asn	Gln	Ser	Ser	His	Lys	Ala	Tyr	Thr	Thr	Ile	Glu	Gln	Arg	Lys	Glu																			
			645						650					655																				
Asn	Gln	Lys	His	Pro	Tyr	His	Ala	Tyr	Val	Asn	Asn	Gln	Pro	Val	Asp																			
		660					665						670																					
Leu	Asn	Asn	Gln	Leu	Val	Asp	Phe	Thr	Asn	Thr	Lys	Ser	Ile	Phe	Leu																			
	675					680					685																							
Glu	Ser	Asp	Asp	Ser	Ala	Gln	Asn	Ile	Gly	Tyr	Tyr	Phe	Phe	Lys	Pro																			
	690				695					700																								
Thr	Thr	Leu	Ser	Ile	Ser	Lys	Ala	Leu	Gln	Thr	Gly	Lys	Trp	Gln	Asn																			
705					710					715					720																			
Ile	Lys	Ala	Asp	Asp	Lys	Ser	Pro	Glu	Ala	Ile	Lys	Glu	Val	Ser	Asn																			
			725						730					735																				
Thr	Phe	Ile	Thr	Ile	Met	Gln	Asn	His	Thr	Gln	Asp	Gly	Asp	Arg	Tyr																			
		740					745						750																					
Ala	Tyr	Met	Met	Leu	Pro	Asn	Met	Thr	Arg	Gln	Glu	Phe	Glu	Thr	Tyr																			
	755					760						765																						
Ile	Ser	Lys	Leu	Asp	Ile	Asp	Leu	Leu	Glu	Asn	Asn	Asp	Lys	Leu	Ala																			
	770				775						780																							
Ala	Val	Tyr	Asp	His	Asp	Ser	Gln	Gln	Met	His	Val	Ile	His	Tyr	Glu																			
785					790				795					800																				
Lys	Lys	Ala	Thr	Met	Phe	Ser	Asn	His	Asn	Leu	Ser	His	Gln	Gly	Phe																			
			805					810					815																					
Tyr	Ser	Phe	Pro	His	Pro	Val	Lys	Gln	Asn	Gln	Gln																							
		820					825																											

<210> 93
 <211> 522
 <212> PRT
 <213> Streptococcus suis

<220>
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<400> 93
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 Thr Arg Val Ala Ala Asn Gln Leu Pro Ser Thr Glu Thr Ala Ser Pro
 35 40 45
 Gln Ser Ser Gln Leu Val Glu Thr Thr Pro Glu Thr Thr Glu Ala Val
 50 55 60
 Asn Leu Thr Thr Glu Ala Val Met Thr Ser Glu Val Ser Ser Glu Val
 65 70 75 80
 Ser Pro Val Thr Ser Thr Glu Thr Gln Pro Ser Ser Thr Ala Ala Glu
 85 90 95
 Thr Leu Ala Ser Pro Gln Ala Val Gln Ala Thr Lys Glu Glu Glu Lys

3066SEQ PC1.TXT

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

<210> 94
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 <212> PRT
 <213> Streptococcus suis

<220>
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Met	Gly	Phe	Phe	Ile	Ser	Gln	Ser	Lys	Gln	His	Tyr	Gly	Ile	Arg	Lys
1	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75
Tyr	Lys	Val	Gly	Val	Cys	Ser	Ala	Leu	Ile	Ala	Leu	Ser	Ile	Leu	Gly
1	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75
Thr	Arg	Val	Ala	Ala	Asn	Gln	Leu	Pro	Ser	Thr	Glu	Thr	Ala	Ser	Pro
1	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75
Gln	Ser	Ser	Gln	Leu	Val	Glu	Thr	Thr	Pro	Glu	Thr	Thr	Glu	Ala	Val
1	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75
Met	Thr	Ser	Glu	Val	Ser	Ser	Glu	Val	Ser	Pro	Val	Thr	Ser	Thr	Glu
1	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75
Thr	Gln	Pro	Ser	Ser	Thr	Thr	Ala	Glu	Thr	Leu	Ala	Ser	Pro	Gln	Ala

Val	Gln	Ala	Thr	85	Lys	Glu	Glu	Lys	Asn	90	Leu	Val	Ala	Asn	95	Gly	Glu	Phe
Ile	Ser	Thr	100	Thr	Ala	Pro	Ser	Gly	105	Asn	Trp	Lys	Glu	Leu	110	Ala	Ala	Thr
Asn	Trp	115	Thr	Trp	Ile	Pro	120	Ala	Asn	Val	Lys	Lys	125	Glu	Asn	Gly	Gln	
Val	Arg	Ile	Asp	Glu	Gly	Arg	Leu	His	Ile	Ser	Ser	Thr	Ala	Ser	Tyr			
Arg	Val	Ala	Val	150	His	Gln	Thr	Val	Asp	Val	Asp	Pro	Asn	Lys	Arg	Tyr		
Leu	Phe	Ser	Tyr	165	Asp	Val	Glu	Thr	Lys	170	Asp	Leu	Lys	Gly	Ser	Gly	Val	
Arg	Val	Arg	Leu	180	Arg	Ser	Leu	Thr	Ala	Glu	Gly	Lys	Asp	Leu	Ser	Pro		
Gln	Glu	Phe	Ala	Tyr	Thr	Pro	Tyr	Lys	Asn	Gly	Ser	Gln	Ala	Glu	His			
Ile	Glu	Gln	Ile	Leu	Thr	Val	Ser	Pro	Glu	Thr	Arg	Lys	Leu	Lys	Val			
Glu	Leu	Phe	Phe	Glu	Asn	Ser	Val	Gly	Gln	Ala	Trp	Leu	Asp	Asn	Ile			
Ser	Leu	Val	Glu	Tyr	Val	Glu	Lys	Thr	Pro	Glu	Thr	Pro	Glu	Gln	Ser			
Pro	Glu	Leu	Val	Gln	Pro	Glu	Thr	Gly	Gln	Ile	Ser	Leu	Ala	Ser	Asn			
Lys	Val	Tyr	Leu	Pro	Val	Arg	Pro	Asp	Leu	Thr	Tyr	Arg	Ile	Ala	Asp			
Ala	Ala	Val	Ala	Thr	Val	Glu	Lys	Asn	Met	Ile	Arg	Pro	Leu	Ala	Ala			
Gly	Lys	Thr	Gln	Val	Asp	Val	Tyr	Asp	Lys	Asp	Thr	Lys	Leu	Ser	Ser			
Phe	Glu	Leu	Ile	Val	Thr	Glu	His	Gln	Ala	Thr	Val	Phe	Asp	Thr	Leu			
Arg	Asn	Asn	Trp	Glu	Asp	Ile	Ser	Leu	Ala	Asn	Lys	Arg	Tyr	Gln	Ser			
Asn	Asp	Ala	Gln	Met	Lys	Ala	Phe	Leu	Gly	Arg	Leu	Asp	Ala	Gly	Val			
Ala	Ser	Ser	Leu	Glu	Lys	Trp	Val	Glu	Pro	Thr	Glu	Gln	Ser	Lys	Thr			
Ile	Phe	Asn	Asp	Ile	Asp	Phe	Ser	Lys	Ser	His	Leu	Thr	Thr	Val				
Tyr	Arg	Arg	Leu	Glu	Gln	Met	Ala	Gln	Val	Val	Glu	Asn	Pro	Asp	Ser			
Ala	Tyr	Tyr	His	Asp	Arg	Ser	Leu	Ile	Asp	Leu	Val	Arg	Lys	Gly	Met			
Asn	Trp	Leu	Tyr	Ala	Asn	Val	Tyr	Asn	Glu	Asn	Lys	Ser	Ile	Asp	Gly			
Asn	Trp	Trp	Asp	Tyr	Glu	Ile	Gly	Thr	Ser	Arg	Ala	Val	Val	Asn	Thr			
Leu	Ile	Tyr	Met	His	Pro	Tyr	Phe	Ser	Gln	Glu	Glu	Ile	Leu	Thr	Tyr			
Thr	Lys	Pro	Ile	Ser	Lys	Phe	Val	Pro	Asp	Pro	Thr	Thr	Ile	Arg	Lys			
Thr	Leu	Thr	Asn	Pro	Val	Pro	Ala	Val	Gly	Gly	Asn	Gln	Thr	Asp	Leu			
Ser	Lys	Val	Ala	Ile	Leu	Glu	Gly	Ala	Leu	Arg	Glu	Asp	Ala	Asp	Arg			
Val	Arg	Ala	Gly	Ala	Gln	Gly	Leu	Thr	Thr	Ile	Met	Lys	Phe	Val	Asp			
Lys	Gly	Glu	Gly	Phe	Tyr	Arg	Asp	Gly	Ser	Phe	Ile	Asp	His	Thr	Asn			
Val	Ala	Tyr	Thr	Gly	Ala	Tyr	Gly	Asn	Val	Leu	Ile	Glu	Gly	Phe	Ser			
Gln	Leu	Leu	Pro	Val	Ile	Gln	Pro	Thr	Glu	Phe	Ala	Leu	Lys	Glu	Glu			
Gln	Thr	Asn	Ile	Leu	Tyr	Glu	Trp	Ile	Glu	Lys	Ala	Phe	Met	Pro	Ile			
Leu	Val	Arg	Gly	Glu	Leu	Met	Asp	Met	Thr	Arg	Gly	Arg	Ser	Ile	Ser			
Arg	Ala	Thr	Gly	Glu	Ser	His	Val	Gln	Ala	Met	Glu	Ile	Leu	Arg	Ser			
Leu	Val	Arg	Ile	Ala	Glu	Ser	Ala	Gln	Pro	Glu	Gln	Lys	Thr	Lys	Leu			
Leu	Ser	Phe	Val	Lys	Ala	Gln	Leu	Thr	Ser	Asp	Thr	Phe	Tyr	Asp	Ser			

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Tyr	Arg	Ser	Leu	Lys	Ser	Tyr	Lys	Asp	Ile	Asp	Leu	Val	Asn	Lys	Leu
690						695					700				
Leu	Ala	Asp	Asn	Gln	Ile	Pro	Ala	Glu	Val	Asp	Lys	Asp	Tyr	Ile	Ala
705					710					715					720
Ala	Phe	Asn	Asn	Met	Asp	Lys	Phe	Val	Tyr	Arg	Ser	Ala	Gln	Glu	Gly
				725					730						735
Phe	Thr	Phe	Ala	Leu	Ser	Met	Tyr	Ser	Ser	Arg	Thr	Gln	Asn	Tyr	Glu
			740					745					750		
Asp	Met	Asn	Asn	Glu	Asn	Arg	Lys	Gly	Trp	Tyr	Thr	Ala	Asp	Gly	Met
		755					760					765			
Val	Tyr	Leu	Tyr	Asn	Asp	Asp	Leu	Ser	His	Tyr	Ser	Asn	His	Tyr	Trp
		770				775					780				
Ala	Thr	Val	Asp	Pro	Tyr	Arg	Leu	Pro	Gly	Thr	Thr	Thr	Thr	Lys	Asp
785					790					795					800
Lys	Arg	Glu	Asp	Gly	Ser	Gly	Glu	Val	Thr	Leu	Ala	Ser	Asp	Phe	Val
				805					810					815	
Gly	Ala	Ser	Gln	Leu	Gly	Asn	Arg	Leu	Ala	Thr	Ile	Ala	Met	Asp	Phe
			820					825					830		
Asn	Asn	Trp	Asn	Asn	Ser	Leu	Thr	Ala	Arg	Lys	Ala	Trp	Ile	Val	Leu
		835					840					845			
Gly	Asn	Lys	Ile	Val	Phe	Leu	Gly	Thr	Asp	Ile	Gln	His	Gln	Ser	Ala
		850				855					860				
Gln	Gly	Ala	Glu	Thr	Thr	Ile	Glu	Asn	Arg	Lys	Leu	Leu	Thr	Gly	Glu
865					870					875					880
Lys	Tyr	Ser	Tyr	Tyr	Ile	Asn	Gly	Gln	Pro	Val	Asp	Leu	Ser	Lys	Glu
				885					890					895	
Val	Val	Thr	Asp	Lys	Thr	Gln	Ser	Phe	Tyr	Met	Thr	Asn	Gly	Lys	Asp
			900					905					910		
Asn	Gln	Ser	Ile	Gly	Tyr	Val	Phe	Leu	Asn	Gln	Leu	Pro	Thr	His	Ala
			915				920					925			
Lys	Leu	Asp	Gln	Arg	Thr	Gly	Lys	Trp	Ser	Asp	Ile	Asn	Tyr	Asn	Gln
		930				935					940				
Ser	Lys	Glu	Glu	Val	Ser	Asn	Ser	Phe	Val	Ser	Leu	Trp	His	Glu	His
945					950					955					960
Ala	Gln	Thr	Ser	Ser	Asn	Tyr	Ala	Tyr	Val	Leu	Val	Pro	Asn	Gln	Ser
				965					970					975	
Met	Glu	Lys	Val	Asn	Gln	Ala	Ala	Ala	Ser	Val	Lys	Leu	Leu	His	Gln
			980					985					990		
Asp	Arg	Asp	Leu	Gln	Val	Val	Tyr	Asp	Gln	Glu	Gln	Asn	Val	Trp	Gly
		995					1000					1005			
Val	Val	Lys	Tyr	Thr	Asp	Thr	Ala	Tyr	Lys	Leu	Thr	Asp	Asp	Ile	Thr
		1010				1015						1020			
Leu	Thr	Asp	Ala	Gly	Leu	Tyr	Thr	Ile	Gln	Lys	Val	Glu	Gly	Gly	Tyr
1025					1030					1035					1040
Arg	Ile	Ala	Phe	Tyr	Asn	Pro	Ser	Thr	Arg	Thr	Val	Lys	Asn	Gly	Ile
				1045					1050					1055	
Glu	Leu	Thr	Lys	Ala	Gly	Ser	Ser	Leu	Thr	Val	Glu	Met	Glu	Pro	Thr
			1060					1065					1070		
Ala	Ala	Tyr	Pro	Ser	Thr	Val	Trp	Lys	Val	Thr	Met	Pro	Glu	Gly	Ser
		1075					1080					1085			
Asp	Lys	Gln	Thr	Gly	Ser	Val	Glu	Lys	Thr	Glu	Lys	Glu	Glu	Lys	Gln
		1090				1095					1100				
Leu	Lys	Glu	Asn	Gln	Pro	Ser	Ser	Glu	Val	Lys	Gln	Val	Val	His	His
1105					1110					1115					1120
Ala	Ala	Glu	Lys	Thr	Lys	Pro	Ser	Lys	Pro	Arg	Leu	Pro	Gln	Thr	Gly
				1125					1130					1135	
Glu	Glu	Ala	Ser	Leu	Gly	Leu	Gly	Phe	Leu	Gly	Leu	Leu	Thr	Leu	Gly
			1140					1145					1150		
Ala	Val	Val	Asp	Phe	Lys	Cys	Arg	Arg	Ser	His	Ser				
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<220>
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<400> 95
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 Tyr Lys Val Gly Val Cys Ser Ala Leu Ile Ala Leu Ser Ile Leu Gly
 20 25 30

Thr	Arg	Val	Ala	Ala	Asn	Gln	Leu	Pro	Ser	Thr	Glu	Thr	Ala	Ser	Pro
		35					40					45			
Gln	Ser	Ser	Gln	Leu	Val	Glu	Thr	Thr	Pro	Glu	Thr	Thr	Glu	Ala	Val
		50				55					60				
Met	Thr	Ser	Glu	Val	Ser	Ser	Glu	Val	Ser	Pro	Val	Thr	Ser	Thr	Glu
65					70				75					80	
Thr	Gln	Pro	Ser	Ser	Thr	Thr	Ala	Glu	Thr	Leu	Ala	Ser	Pro	Gln	Ala
				85					90					95	
Val	Gln	Ala	Thr	Lys	Glu	Glu	Lys	Asn	Leu	Val	Ala	Asn	Gly	Glu	Phe
			100					105					110		
Ile	Ser	Thr	Thr	Ala	Pro	Ser	Gly	Asn	Trp	Lys	Glu	Leu	Ala	Ala	Thr
		115					120					125			
Asn	Trp	Glu	Thr	Trp	Ile	Pro	Ala	Asn	Val	Lys	Lys	Glu	Asn	Gly	Gln
		130				135					140				
Val	Arg	Ile	Asp	Glu	Gly	Arg	Leu	His	Ile	Ser	Ser	Thr	Ala	Ser	Tyr
145					150					155					160
Arg	Val	Ala	Val	His	Gln	Thr	Val	Asp	Val	Asp	Pro	Asn	Lys	Arg	Tyr
				165					170					175	
Leu	Phe	Ser	Tyr	Asp	Val	Glu	Thr	Lys	Asp	Leu	Lys	Gly	Ser	Gly	Val
			180					185					190		
Arg	Val	Arg	Leu	Arg	Ser	Leu	Thr	Ala	Glu	Gly	Lys	Asp	Leu	Ser	Pro
		195					200					205			
Gln	Glu	Phe	Ala	Tyr	Thr	Pro	Tyr	Lys	Asn	Gly	Ser	Gln	Ala	Glu	His
		210				215					220				
Ile	Glu	Gln	Ile	Leu	Thr	Val	Ser	Pro	Glu	Thr	Arg	Lys	Leu	Lys	Val
225					230					235					240
Glu	Leu	Phe	Phe	Glu	Asn	Ser	Val	Gly	Gln	Ala	Trp	Leu	Asp	Asn	Ile
				245					250					255	
Ser	Leu	Val	Glu	Tyr	Val	Glu	Lys	Thr	Pro	Glu	Thr	Pro	Glu	Gln	Ser
			260					265					270		
Pro	Glu	Leu	Val	Gln	Pro	Glu	Thr	Gly	Gln	Ile	Ser	Leu	Ala	Ser	Asn
		275					280					285			
Lys	Val	Tyr	Leu	Pro	Val	Arg	Pro	Asp	Leu	Thr	Tyr	Arg	Ile	Ala	Asp
		290				295					300				
Ala	Ala	Val	Ala	Thr	Val	Glu	Lys	Asn	Met	Ile	Arg	Pro	Leu	Ala	Ala
305					310					315					320
Gly	Lys	Thr	Gln	Val	Asp	Val	Tyr	Asp	Lys	Asp	Thr	Lys	Leu	Ser	Ser
				325					330					335	
Phe	Glu	Leu	Ile	Val	Thr	Glu	His	Gln	Ala	Thr	Val	Phe	Asp	Thr	Leu
			340					345					350		
Arg	Asn	Asn	Trp	Glu	Asp	Ile	Ser	Leu	Ala	Asn	Lys	Arg	Tyr	Gln	Ser
		355					360						365		
Asn	Asp	Ala	Gln	Met	Lys	Ala	Phe	Leu	Gly	Arg	Leu	Asp	Ala	Gly	Val
		370				375					380				
Ala	Ser	Ser	Leu	Glu	Lys	Trp	Val	Glu	Pro	Thr	Glu	Gln	Ser	Lys	Thr
385					390					395					400
Ile	Phe	Asn	Asp	Ile	Asp	Phe	Ser	Lys	Ser	Ser	His	Leu	Thr	Thr	Val
				405					410					415	
Tyr	Arg	Arg	Leu	Glu	Gln	Met	Ala	Gln	Val	Val	Glu	Asn	Pro	Asp	Ser
			420					425					430		
Ala	Tyr	Tyr	His	Asp	Arg	Ser	Leu	Ile	Asp	Leu	Val	Arg	Lys	Gly	Met
		435					440					445			
Asn	Trp	Leu	Tyr	Thr	Asn	Val	Tyr	Asn	Glu	Asn	Lys	Ser	Ile	Asp	Gly
		450				455					460				
Asn	Trp	Trp	Asp	Tyr	Glu	Ile	Gly	Thr	Pro	Arg	Ala	Val	Val	Asn	Thr
465					470					475					480
Leu	Ile	Tyr	Met	His	Pro	Tyr	Phe	Ser	Gln	Glu	Glu	Ile	Leu	Thr	Tyr
				485					490					495	
Thr	Lys	Pro	Ile	Ser	Lys	Phe	Val	Pro	Asp	Pro	Thr	Thr	Ile	Arg	Lys
			500					505					510		
Thr	Leu	Thr	Asn	Pro	Val	Pro	Ala	Val	Gly	Gly	Asn	Gln	Thr	Asp	Leu
		515					520					525			
Ser	Lys	Val	Ala	Ile	Leu	Glu	Gly	Ala	Leu	Arg	Glu	Asp	Ala	Asp	Arg
		530				535					540				
Val	Arg	Ala	Gly	Ala	Gln	Gly	Leu	Thr	Thr	Ile	Met	Lys	Phe	Val	Asp
545					550					555					560
Lys	Gly	Glu	Gly	Phe	Tyr	Arg	Asp	Gly	Ser	Phe	Ile	Asp	His	Thr	Asn
				565					570					575	
Val	Ala	Tyr	Thr	Gly	Ala	Tyr	Gly	Asn	Val	Leu	Ile	Glu	Gly	Phe	Ser
			580					585					590		
Gln	Leu	Leu	Pro	Val	Ile	Gln	Pro	Thr	Glu	Phe	Ala	Leu	Lys	Glu	Glu
		595					600					605			
Gln	Thr	Asn	Ile	Leu	Tyr	Glu	Trp	Ile	Glu	Lys	Ala	Phe	Met	Pro	Ile
		610				615					620				
Leu	Val	Arg	Gly	Glu	Leu	Met	Asp	Met	Thr	Arg	Gly	Arg	Ser	Ile	Ser

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625	Arg	Ala	Thr	Gly	Glu	Ser	His	Val	Gln	Ala	Met	Glu	Ile	Leu	Arg	Ser	640
	Leu	Val	Arg	Ile	Ala	Glu	Ser	Ala	Gln	Pro	Glu	Gln	Lys	Thr	Lys	Leu	655
	Leu	Ser	Phe	Val	Lys	Ala	Gln	Leu	Thr	Ser	Asp	Thr	Phe	Tyr	Asp	Ser	670
	Tyr	Arg	Ser	Leu	Lys	Ser	Tyr	Lys	Asp	Ile	Asp	Leu	Val	Asn	Lys	Leu	685
	Leu	Ala	Asp	Asn	Gln	Ile	Pro	Ala	Glu	Val	Asp	Lys	Asp	Tyr	Ile	Ala	700
	Ala	Phe	Asn	Asn	Met	Asp	Lys	Phe	Val	Tyr	Arg	Ser	Ala	Gln	Glu	Gly	715
	Phe	Thr	Phe	Ala	Leu	Ser	Met	Tyr	Ser	Ser	Arg	Thr	Gln	Asn	Tyr	Glu	730
	Asp	Met	Asn	Asn	Glu	Asn	Arg	Lys	Gly	Trp	Tyr	Thr	Ala	Asp	Gly	Met	745
	Val	Tyr	Leu	Tyr	Asn	Asp	Asp	Leu	Ser	His	Tyr	Ser	Asn	His	Tyr	Trp	760
	Ala	Thr	Val	Asp	Pro	Tyr	Arg	Leu	Pro	Gly	Thr	Thr	Thr	Thr	Lys	Asp	775
	Lys	Arg	Glu	Asp	Gly	Ser	Gly	Glu	Val	Thr	Leu	Ala	Ser	Asp	Phe	Val	790
	Gly	Ala	Ser	Gln	Leu	Gly	Asn	Arg	Leu	Ala	Thr	Ile	Ala	Met	Asp	Phe	805
	Asn	Asn	Trp	Asn	Asn	Ser	Leu	Thr	Ala	Arg	Lys	Ala	Trp	Ile	Val	Leu	820
	Gly	Asn	Lys	Ile	Val	Phe	Leu	Gly	Thr	Asp	Ile	Gln	His	Gln	Ser	Ala	835
	Gln	Gly	Ala	Glu	Thr	Thr	Ile	Glu	Asn	Arg	Lys	Leu	Leu	Thr	Gly	Glu	850
	Lys	Tyr	Ser	Tyr	Tyr	Ile	Asn	Gly	Gln	Pro	Val	Asp	Leu	Ser	Lys	Glu	865
	Val	Val	Thr	Asp	Lys	Thr	Gln	Ser	Phe	Tyr	Met	Thr	Asn	Gly	Lys	Asp	880
	Asn	Gln	Ser	Ile	Gly	Tyr	Val	Phe	Leu	Asn	Gln	Leu	Pro	Thr	His	Ala	895
	Lys	Leu	Asp	Gln	Arg	Thr	Gly	Lys	Trp	Ser	Asp	Ile	Asn	Tyr	Asn	Gln	910
	Ser	Lys	Glu	Glu	Val	Ser	Asn	Ser	Phe	Val	Ser	Leu	Trp	His	Glu	His	925
	Ala	Gln	Thr	Ser	Ser	Asn	Tyr	Ala	Tyr	Val	Ser	Leu	Val	Pro	Asn	Gln	940
	Met	Glu	Lys	Val	Asn	Gln	Ala	Ala	Ala	Ser	Val	Lys	Leu	Leu	His	Gln	955
	Asp	Arg	Asp	Leu	Gln	Val	Val	Tyr	Asp	Gln	Glu	Gln	Asn	Val	Trp	Gly	970
	Val	Val	Lys	Tyr	Thr	Asp	Thr	Ala	Tyr	Lys	Leu	Thr	Asp	Asp	Ile	Thr	1000
	Leu	Thr	Asp	Ala	Gly	Leu	Tyr	Thr	Ile	Gln	Lys	Val	Glu	Gly	Gly	Tyr	1015
	Arg	Ile	Ala	Phe	Tyr	Asn	Pro	Ser	Thr	Arg	Thr	Val	Lys	Asn	Gly	Ile	1030
	Glu	Leu	Thr	Lys	Ala	Gly	Ser	Ser	Leu	Thr	Val	Glu	Met	Glu	Pro	Thr	1045
	Ala	Ala	Tyr	Pro	Ser	Thr	Val	Trp	Lys	Val	Thr	Met	Pro	Glu	Gly	Ser	1060
	Asp	Lys	Gln	Thr	Gly	Ser	Val	Glu	Lys	Thr	Glu	Lys	Glu	Glu	Lys	Gln	1075
	Leu	Lys	Glu	Asn	Gln	Pro	Ser	Ser	Glu	Val	Lys	Gln	Val	Val	His	His	1090
	Ala	Ala	Glu	Lys	Thr	Lys	Pro	Ser	Lys	Pro	Arg	Leu	Pro	Gln	Thr	Gly	1105
	Glu	Glu	Ala	Ser	Leu	Gly	Leu	Gly	Phe	Leu	Gly	Leu	Leu	Thr	Leu	Gly	1120
	Ala	Val	Val	Asp	Phe	Lys	Cys	Arg	Arg	Ser	His	Ser					1135

<210> 96

<211> 802

<212> PRT

<213> *Vibrio fischeri* (strain ATCC 700601 / ES114)

<220>

<223> hyaluronan lyase

<400> 96

Met	Tyr	Met	Ile	Lys	Lys	His	Arg	Leu	Asn	Thr	Ile	Ala	Leu	Ser	Met
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Leu	Phe	Leu	Phe	Thr	Gly	Asn	Ala	Tyr	Ala	Ala	Lys	Asn	Thr	Gln	Thr
			20					25					30		
Pro	Gln	Tyr	Leu	Pro	Ser	Asp	Phe	Glu	Gln	Val	Arg	Glu	Asn	Trp	Ala
		35					40					45			
Glu	Asn	Tyr	Leu	Gly	Asp	Pro	Ala	Ile	Thr	Phe	Asp	Gln	Thr	Leu	Lys
	50					55					60				
Asn	Met	Val	Thr	Ser	Thr	Asn	Ser	Ser	Ala	Gln	Lys	His	Trp	Asp	Ser
65					70					75				80	
Met	Thr	Pro	Gln	Pro	Asn	Ala	Ser	Gly	Ile	Trp	Asp	Asp	Leu	Pro	Leu
				85					90					95	
Ile	Asp	Lys	Asp	Thr	Thr	Leu	Gly	Pro	Asn	Ile	Arg	Asn	Ser	Tyr	Gln
			100					105					110		
Arg	Leu	Phe	Thr	Met	Ala	Lys	Ala	Tyr	Arg	Leu	Arg	Asp	Gly	Asn	Leu
		115					120					125			
Glu	Asn	Asn	Gln	Leu	Met	Leu	Asn	Asp	Ile	Met	Thr	Ala	Met	Asn	Tyr
	130					135					140				
Ile	Asn	Gln	Asn	Phe	Tyr	Phe	Val	Asn	Gln	Leu	Glu	Tyr	Gly	Asn	Trp
145				150					155					160	
Trp	Gln	Trp	Glu	Leu	Ala	Ile	Pro	Lys	Asp	Ile	His	Asn	Ile	Leu	Val
				165					170					175	
Leu	Leu	Phe	Asp	Asp	Ile	Lys	Asp	Asn	Tyr	Gln	Thr	Ile	Ile	Thr	Asn
			180					185					190		
His	Leu	Asn	Ala	Thr	Arg	Tyr	Phe	Thr	Pro	Asp	Pro	Thr	His	Leu	Gly
		195					200					205			
Val	Ser	Pro	Gly	Ala	Ala	Glu	Ser	Thr	Asn	Pro	Asn	Tyr	Arg	Glu	Ser
	210					215					220				
Thr	Gly	Gly	Asn	Arg	Thr	Asp	Asn	Ala	Gln	Val	Val	Leu	Ile	Arg	Gly
225					230				235					240	
Met	Leu	Glu	Asn	Asn	Ser	Glu	Glu	Ile	Ser	Gln	Ala	Ile	Ala	Ala	Leu
			245						250				255		
Pro	Ala	Val	Ile	Glu	Tyr	Val	Ser	Glu	Gly	Asp	Gly	Tyr	Tyr	Thr	Asp
			260					265					270		
Gly	Ser	Phe	Leu	Gln	His	Ser	Asp	Ile	Ala	Tyr	Asn	Gly	Thr	Tyr	Gly
		275					280					285			
Asn	Val	Leu	Leu	Gly	Gly	Leu	Gly	Ile	Gln	Met	Asn	Ala	Val	Ala	Gly
	290					295					300				
Ser	Pro	Trp	Ser	Met	Asp	Asn	Gln	Thr	Ile	Ser	Asn	Val	Tyr	Asn	Ile
305					310					315				320	
Ile	Asn	Gln	Ser	Tyr	Glu	Pro	Leu	Leu	Tyr	Lys	Gly	Ala	Met	Met	Asp
				325					330					335	
Met	Val	Asn	Gly	Arg	Ser	Ile	Ser	Arg	Ser	Ala	Glu	Gln	Asn	His	Asp
			340					345					350		
Val	Gly	Leu	Asn	Ile	Val	Asn	Ser	Met	Leu	Phe	Tyr	Thr	Asn	Gly	Pro
		355					360					365			
Asp	Ser	Asp	Lys	Asn	Lys	Gln	Leu	Ser	Ser	Leu	Ile	Lys	Thr	Gln	Ile
	370					375					380				
Thr	Asp	Asp	Thr	Tyr	Gln	Asn	Phe	Phe	Asp	Lys	Ile	Tyr	Tyr	Val	Ser
385					390					395				400	
Thr	Tyr	Gln	Ala	Ala	Gln	His	Ile	Val	Asn	Asp	Pro	Thr	Val	Ser	Leu
			405						410					415	
Lys	Asp	Pro	Leu	Ile	Gly	Asn	Phe	Ser	Tyr	Pro	Ser	Met	Asp	Arg	Ile
			420					425					430		
Val	His	Arg	Arg	Thr	Asp	Trp	Ala	Phe	Ala	Leu	Ala	Met	His	Ser	Tyr
		435					440					445			
Arg	Ile	Gly	Asn	Tyr	Glu	Cys	Met	Asn	Gly	Glu	Asn	Leu	Lys	Gly	Trp
	450					455					460				
Phe	Thr	Gly	Asp	Gly	Met	Ile	Tyr	Leu	Tyr	Asn	Asp	Gln	Leu	Asp	His
465					470					475				480	
Tyr	Thr	Gly	Tyr	Trp	Pro	Thr	Val	Asn	Ala	Ser	Arg	Met	Pro	Gly	Thr
				485					490					495	
Thr	Val	Asp	Ser	Gln	Ile	Met	Ala	Asp	Cys	Ser	Gly	Glu	Arg	Val	Gly
			500					505					510		
Gly	Asn	Val	Asn	Thr	Asn	Met	Gln	Trp	Val	Gly	Ser	Thr	Ser	Leu	Asn
		515					520					525			
Asn	Tyr	Gly	Ile	Ala	Gly	Met	Gln	Phe	Tyr	Asn	Trp	Ser	Asp	Thr	Leu
	530					535					540				
Ser	Ala	Tyr	Lys	Ser	Trp	Met	Phe	Asp	Asn	Glu	Val	Val	Met	Leu	
545					550				555					560	
Gly	Ser	Asn	Ile	Lys	Asp	Gln	Ser	Asn	Ala	Asn	Asn	Ile	Thr	Thr	Ile
				565					570					575	

3066SEQ PC1.TXT

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

<210> 97

<211> 517

<212> PRT

<213> Synechococcus sp. (strain RCC307)

<220>

<223> hyaluronan lyase

<400> 97

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

3066SEQ PC1.TXT

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

<210> 98
 <211> 1021
 <212> PRT
 <213> Proteus vulgaris

<220>
 <223> chondroitin-sulfate-ABC endolyase

<400> 98

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

Ile	Asp	Leu	Ile	Arg	Gln	Arg	Leu	Ile	Asn	Glu	Phe	Val	Gly	Gly	Glu
			260					265					270		
Lys	Glu	Thr	Asn	Leu	Ala	Leu	Glu	Glu	Asn	Ile	Ser	Lys	Leu	Lys	Ser
		275					280					285			
Asp	Phe	Asp	Ala	Leu	Asn	Ile	His	Thr	Leu	Ala	Asn	Gly	Gly	Thr	Gln
	290					295					300				
Gly	Arg	His	Leu	Ile	Thr	Asp	Lys	Gln	Ile	Ile	Ile	Tyr	Gln	Pro	Glu
305					310					315					320
Asn	Leu	Asn	Ser	Gln	Asp	Lys	Gln	Leu	Phe	Asp	Asn	Tyr	Val	Ile	Leu
				325					330					335	
Gly	Asn	Tyr	Thr	Thr	Leu	Met	Phe	Asn	Ile	Ser	Arg	Ala	Tyr	Val	Leu
			340					345					350		
Glu	Lys	Asp	Pro	Thr	Gln	Lys	Ala	Gln	Leu	Lys	Gln	Met	Tyr	Leu	Leu
		355					360					365			
Met	Thr	Lys	His	Leu	Leu	Asp	Gln	Gly	Phe	Val	Lys	Gly	Ser	Ala	Leu
	370					375					380				
Val	Thr	Thr	His	His	Trp	Gly	Tyr	Ser	Ser	Arg	Trp	Trp	Tyr	Ile	Ser
385					390					395					400
Thr	Leu	Leu	Met	Ser	Asp	Ala	Leu	Lys	Glu	Ala	Asn	Leu	Gln	Thr	Gln
				405					410					415	
Val	Tyr	Asp	Ser	Leu	Leu	Trp	Tyr	Ser	Arg	Glu	Phe	Lys	Ser	Ser	Phe
			420					425					430		
Asp	Met	Lys	Val	Ser	Ala	Asp	Ser	Ser	Asp	Leu	Asp	Tyr	Phe	Asn	Thr
		435					440					445			
Leu	Ser	Arg	Gln	His	Leu	Ala	Leu	Leu	Leu	Glu	Pro	Asp	Asp	Gln	
	450					455				460					
Lys	Arg	Ile	Asn	Leu	Val	Asn	Thr	Phe	Ser	His	Tyr	Ile	Thr	Gly	Ala
465					470					475					480
Leu	Thr	Gln	Val	Pro	Pro	Gly	Gly	Lys	Asp	Gly	Leu	Arg	Pro	Asp	Gly
				485					490					495	
Thr	Ala	Trp	Arg	His	Glu	Gly	Asn	Tyr	Pro	Gly	Tyr	Ser	Phe	Pro	Ala
			500					505					510		
Phe	Lys	Asn	Ala	Ser	Gln	Leu	Ile	Tyr	Leu	Leu	Arg	Asp	Thr	Pro	Phe
		515					520					525			
Ser	Val	Gly	Glu	Ser	Gly	Trp	Asn	Asn	Leu	Lys	Lys	Ala	Met	Val	Ser
	530					535					540				
Ala	Trp	Ile	Tyr	Ser	Asn	Pro	Glu	Val	Gly	Leu	Pro	Leu	Ala	Gly	Arg
545					550					555					560
His	Pro	Phe	Asn	Ser	Pro	Ser	Leu	Lys	Ser	Val	Ala	Gln	Gly	Tyr	Tyr
			565						570					575	
Trp	Leu	Ala	Met	Ser	Ala	Lys	Ser	Ser	Pro	Asp	Lys	Thr	Leu	Ala	Ser
			580					585					590		
Ile	Tyr	Leu	Ala	Ile	Ser	Asp	Lys	Thr	Gln	Asn	Glu	Ser	Thr	Ala	Ile
		595					600					605			
Phe	Gly	Glu	Thr	Ile	Thr	Pro	Ala	Ser	Leu	Pro	Gln	Gly	Phe	Tyr	Ala
	610					615					620				
Phe	Asn	Gly	Gly	Ala	Phe	Gly	Ile	His	Arg	Trp	Gln	Asp	Lys	Met	Val
625					630					635					640
Thr	Leu	Lys	Ala	Tyr	Asn	Thr	Asn	Val	Trp	Ser	Ser	Glu	Ile	Tyr	Asn
				645					650					655	
Lys	Asp	Asn	Arg	Tyr	Gly	Arg	Tyr	Gln	Ser	His	Gly	Val	Ala	Gln	Ile
			660					665					670		
Val	Ser	Asn	Gly	Ser	Gln	Leu	Ser	Gln	Gly	Tyr	Gln	Gln	Glu	Gly	Trp
		675					680					685			
Asp	Trp	Asn	Arg	Met	Glu	Gly	Ala	Thr	Thr	Ile	His	Leu	Pro	Leu	Lys
	690					695					700				
Asp	Leu	Asp	Ser	Pro	Lys	Pro	His	Thr	Leu	Met	Gln	Arg	Gly	Glu	Arg
705					710					715					720
Gly	Phe	Ser	Gly	Thr	Ser	Ser	Leu	Glu	Gly	Gln	Tyr	Gly	Met	Met	Ala
			725						730				735		
Phe	Asn	Leu	Ile	Tyr	Pro	Ala	Asn	Leu	Glu	Arg	Phe	Asp	Pro	Asn	Phe
		740						745					750		
Thr	Ala	Lys	Lys	Ser	Val	Leu	Ala	Ala	Asp	Asn	His	Leu	Ile	Phe	Ile
		755					760					765			
Gly	Ser	Asn	Ile	Asn	Ser	Ser	Asp	Lys	Asn	Lys	Asn	Val	Glu	Thr	Thr
	770					775					780				
Leu	Phe	Gln	His	Ala	Ile	Thr	Pro	Thr	Leu	Asn	Thr	Leu	Trp	Ile	Asn
785					790					795					800
Gly	Gln	Lys	Ile	Glu	Asn	Met	Pro	Tyr	Gln	Thr	Thr	Leu	Gln	Gln	Gly
			805						810					815	
Asp	Trp	Leu	Ile	Asp	Ser	Asn	Gly	Asn	Gly	Tyr	Leu	Ile	Thr	Gln	Ala
		820						825					830		
Glu	Lys	Val	Asn	Val	Ser	Arg	Gln	His	Gln	Val	Ser	Ala	Glu	Asn	Lys
	835						840					845			
Asn	Arg	Gln	Pro	Thr	Glu	Gly	Asn	Phe	Ser	Ser	Ala	Trp	Ile	Asp	His

3066SEQ PC1.TXT

850	855	860	
Ser Thr Arg Pro Lys Asp Ala Ser Tyr Glu Tyr Met Val Phe Leu Asp			
865	870	875	880
Ala Thr Pro Glu Lys Met Gly Glu Met Ala Gln Lys Phe Arg Glu Asn			
	885	890	895
Asn Gly Leu Tyr Gln Val Leu Arg Lys Asp Lys Asp Val His Ile Ile			
	900	905	910
Leu Asp Lys Leu Ser Asn Val Thr Gly Tyr Ala Phe Tyr Gln Pro Ala			
	915	920	925
Ser Ile Glu Asp Lys Trp Ile Lys Lys Val Asn Lys Pro Ala Ile Val			
930	935	940	
Met Thr His Arg Gln Lys Asp Thr Leu Ile Val Ser Ala Val Thr Pro			
945	950	955	960
Asp Leu Asn Met Thr Arg Gln Lys Ala Ala Thr Pro Val Thr Ile Asn			
	965	970	975
Val Thr Ile Asn Gly Lys Trp Gln Ser Ala Asp Lys Asn Ser Glu Val			
	980	985	990
Lys Tyr Gln Val Ser Gly Asp Asn Thr Glu Leu Thr Phe Thr Ser Tyr			
	995	1000	1005
Phe Gly Ile Pro Gln Glu Ile Lys Leu Ser Pro Leu Pro			
1010	1015	1020	

<210> 99
 <211> 684
 <212> PRT
 <213> Pedobacter heparinus

<220>
 <223> chondroitin AC lyase

<400> 99

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	20
Val Ala Glu Lys Asn Leu Asn Thr Leu Gln Pro Asp Gly Ser Trp Lys	
	35
Asp Val Pro Tyr Lys Asp Asp Ala Met Thr Asn Trp Leu Pro Asn Asn	
	50
His Leu Leu Gln Leu Glu Thr Ile Ile Gln Ala Tyr Ile Glu Lys Asp	
65	70
Ser His Tyr Tyr Gly Asp Asp Lys Val Phe Asp Gln Ile Ser Lys Ala	
	85
Phe Lys Tyr Trp Tyr Asp Ser Asp Pro Lys Ser Arg Asn Trp Trp His	
	100
Asn Glu Ile Ala Thr Pro Gln Ala Leu Gly Glu Met Leu Ile Leu Met	
	115
Arg Tyr Gly Lys Lys Pro Leu Asp Glu Ala Leu Val His Lys Leu Thr	
	130
Glu Arg Met Lys Arg Gly Glu Pro Glu Lys Lys Thr Gly Ala Asn Lys	
145	150
Thr Asp Ile Ala Leu His Tyr Phe Tyr Arg Ala Leu Leu Thr Ser Asp	
	165
Glu Ala Leu Leu Ser Phe Ala Val Lys Glu Leu Phe Tyr Pro Val Gln	
	180
Phe Val His Tyr Glu Glu Gly Leu Gln Tyr Asp Tyr Ser Tyr Leu Gln	
	195
His Gly Pro Gln Leu Gln Ile Ser Ser Tyr Gly Ala Val Phe Ile Thr	
	210
Gly Val Leu Lys Leu Ala Asn Tyr Val Arg Asp Thr Pro Tyr Ala Leu	
225	230
Ser Thr Glu Lys Leu Ala Ile Phe Ser Lys Tyr Tyr Arg Asp Ser Tyr	
	245
Leu Lys Ala Ile Arg Gly Ser Tyr Met Asp Phe Asn Val Glu Gly Arg	
	260
Gly Val Ser Arg Pro Asp Ile Leu Asn Lys Lys Ala Glu Lys Lys Arg	
	275
Leu Leu Val Ala Lys Met Ile Asp Leu Lys His Thr Glu Glu Trp Ala	
	290
Asp Ala Ile Ala Arg Thr Asp Ser Thr Val Ala Ala Gly Tyr Lys Ile	
305	310
Glu Pro Tyr His His Gln Phe Trp Asn Gly Asp Tyr Val Gln His Leu	
	325
Arg Pro Ala Tyr Ser Phe Asn Val Arg Met Val Ser Lys Arg Thr Arg	
	330

3066SEQ PC1.TXT

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Arg Ser Glu Ser Gly Asn Lys Glu Asn Leu Leu Gly Arg Tyr Leu Ser
      355      360      365
Asp Gly Ala Thr Asn Ile Gln Leu Arg Gly Pro Glu Tyr Tyr Asn Ile
      370      375      380
Met Pro Val Trp Glu Trp Asp Lys Ile Pro Gly Ile Thr Ser Arg Asp
      385      390      395      400
Tyr Leu Thr Asp Arg Pro Leu Thr Lys Leu Trp Gly Glu Gln Gly Ser
      405      410      415
Asn Asp Phe Ala Gly Gly Val Ser Asp Gly Val Tyr Gly Ala Ser Ala
      420      425      430
Tyr Ala Leu Asp Tyr Asp Ser Leu Gln Ala Lys Lys Ala Trp Phe Phe
      435      440      445
Phe Asp Lys Glu Ile Val Cys Leu Gly Ala Gly Ile Asn Ser Asn Ala
      450      455      460
Pro Glu Asn Ile Thr Thr Thr Leu Asn Gln Ser Trp Leu Asn Gly Pro
      465      470      475      480
Val Ile Ser Thr Ala Gly Lys Thr Gly Arg Gly Lys Ile Thr Thr Phe
      485      490      495
Lys Ala Gln Gly Gln Phe Trp Leu Leu His Asp Ala Ile Gly Tyr Tyr
      500      505      510
Phe Pro Glu Gly Ala Asn Leu Ser Leu Ser Thr Gln Ser Gln Lys Gly
      515      520      525
Asn Trp Phe His Ile Asn Asn Ser His Ser Lys Asp Glu Val Ser Gly
      530      535      540
Asp Val Phe Lys Leu Trp Ile Asn His Gly Ala Arg Pro Glu Asn Ala
      545      550      555      560
Gln Tyr Ala Tyr Ile Val Leu Pro Gly Ile Asn Lys Pro Glu Glu Ile
      565      570      575
Lys Lys Tyr Asn Gly Thr Ala Pro Lys Val Leu Ala Asn Thr Asn Gln
      580      585      590
Leu Gln Ala Val Tyr His Gln Gln Leu Asp Met Val Gln Ala Ile Phe
      595      600      605
Tyr Thr Ala Gly Lys Leu Ser Val Ala Gly Ile Glu Ile Glu Thr Asp
      610      615      620
Lys Pro Cys Ala Val Leu Ile Lys His Ile Asn Gly Lys Gln Val Ile
      625      630      635      640
Trp Ala Ala Asp Pro Leu Gln Lys Glu Lys Thr Ala Val Leu Ser Ile
      645      650      655      660
Arg Asp Leu Lys Thr Gly Lys Thr Asn Arg Val Lys Ile Asp Phe Pro
      660      665      670
Gln Gln Glu Phe Ala Gly Ala Thr Val Glu Leu Lys
      675      680

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<210> 100

<211> 844

<212> PRT

<213> Victivallis vadensis ATCC BAA-548

<220>

<223> chondroitin AC lyase

<400> 100

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

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Pro	Glu	Gln	Ile	165	Arg	Glu	Cys	Glu	Leu	170	Ile	Glu	Ser	Arg	Leu	175	Arg	Ala
Gly	Leu	Pro	180	Ala	Gln	Gln	Pro	Pro	185	Pro	Gly	Pro	Glu	Val	190	Lys	Lys	Leu
Leu	Glu	195	Arg	Gln	Asn	Gly	Asp	200	Gly	Thr	Phe	Arg	Asp	205	Leu	Asp	Tyr	Ser
Ala	Arg	Asn	Arg	Ser	Ile	230	Trp	Pro	Ala	Ala	Arg	His	Leu	Lys	Arg	Thr	240	Thr
Phe	Glu	Leu	Ala	Leu	245	Ala	Arg	Ala	Thr	Pro	Gly	His	Ala	Leu	Tyr	Arg	255	Arg
Asp	Pro	Ala	Ala	Gly	Glu	Ala	Val	Arg	265	Lys	Ala	Val	Ala	Trp	Trp	Ala	270	Ala
Glu	Arg	Gln	Pro	Gln	Asn	Gly	Asn	Trp	Trp	Trp	Trp	Asn	Asp	Met	Tyr	Val	285	Val
Pro	Lys	Ile	Leu	Gly	Asn	Ile	Leu	Leu	Leu	Ser	Pro	Glu	Leu	Phe	Pro	290	Pro	Pro
Asp	Gly	Pro	Arg	Arg	Arg	Ala	Ala	Leu	Asn	Val	Cys	Arg	Gln	Ala	Cys	305	Cys	Cys
Phe	Leu	Ser	Arg	Tyr	Thr	Gly	Asn	Asn	Arg	Val	Phe	Ile	Ala	Ala	Asn	320	Ala	Asn
Ile	Phe	Cys	Arg	Ala	Leu	Leu	Glu	Arg	Asn	Leu	Pro	Val	Met	Asp	Arg	335	Asp	Arg
Ala	Ala	Ala	Val	Leu	Ser	Glu	Glu	Ile	Arg	Phe	Ala	Pro	Ala	Glu	His	350	Glu	His
Lys	Thr	Ala	Trp	Ser	Phe	Gly	Gly	Ile	Arg	Ala	Asp	Gly	Cys	Tyr	His	365	Tyr	His
Gln	His	Gly	Pro	Gln	Ile	Gln	Phe	Gly	Asn	Tyr	Gly	Gly	Glu	Phe	Leu	380	Phe	Leu
Ala	Asn	Ile	Ala	Tyr	Trp	Ser	Asn	Ile	Leu	Lys	Glu	Thr	His	Trp	Glu	400	Trp	Glu
Leu	Ser	Pro	Glu	Gln	Trp	Arg	Ile	Met	Arg	His	Leu	Thr	Phe	Asn	Gly	415	Asn	Gly
Phe	Gln	Trp	Val	Leu	Trp	Arg	Gly	Arg	Met	Asp	Leu	Leu	Ala	Cys	Gly	430	Ala	Cys
Arg	Gln	Leu	Gly	Arg	Asn	Ala	Ala	Glu	Thr	Lys	Gly	Glu	Arg	Thr	Leu	445	Arg	Thr
Asn	Ala	Phe	Ala	Gln	Leu	Arg	Asn	Ala	Asp	Pro	Gly	Asp	Arg	Ala	Pro	460	Asp	Arg
Tyr	Asp	Ala	Ala	Leu	Arg	Arg	Asn	Arg	Asp	Gly	Glu	Asn	Thr	Leu	Thr	475	Glu	Thr
Gly	Asn	Arg	His	Phe	Trp	Asn	Ser	Asp	Tyr	Met	Val	His	Arg	Arg	Pro	490	Arg	Pro
Thr	Trp	Tyr	Ala	Ser	Val	Arg	Met	Asn	Ser	Val	Arg	Val	Arg	Pro	Ile	505	Arg	Ile
Glu	Asp	Asp	Thr	Asn	Trp	Asp	Asn	Ala	Leu	Gly	Arg	Tyr	Phe	Ser	Asp	520	Phe	Asp
Gly	Ala	Cys	Leu	Val	Met	Arg	Ser	Gly	Arg	Glu	Tyr	Glu	Asn	Ile	Thr	535	Asn	Ile
Ala	Cys	Trp	Asp	Trp	Thr	Arg	Leu	Pro	Gly	Thr	Thr	Leu	Pro	Lys	Thr	550	Pro	Lys
Pro	Val	Tyr	Thr	Gly	Glu	Asp	Ala	Arg	Arg	Phe	Gly	Leu	Lys	Ile	Gly	565	Arg	Gly
Gly	Gly	Asp	Leu	Pro	Arg	Trp	Thr	His	Ser	Arg	Asn	Trp	Arg	Gln	Leu	580	Trp	Arg
Gly	Glu	Thr	Gly	Phe	Val	Gly	Gly	Val	Thr	Asp	Gly	Glu	Arg	Gly	Ala	595	Glu	Arg
Ala	Val	Tyr	Thr	Gln	Asp	Leu	Asp	Gly	Val	Arg	Ala	Arg	Lys	Ala	Trp	610	Ala	Trp
Phe	Phe	Asp	Arg	Asp	Ala	Ile	Tyr	Cys	Leu	Gly	Ser	Gly	Ile	Thr	Ser	625	Gly	Ser
Thr	Ser	Pro	Tyr	Glu	Val	Ala	Thr	Thr	Val	Asn	Ser	Cys	Leu	Arg	Asn	635	Thr	Arg
Gly	Glu	Ile	Gln	Gln	Gly	Asp	Gly	Trp	Phe	Arg	His	Asp	Gly	Ile	Gly	650	Arg	Gly
Tyr	Arg	Gly	Glu	Asn	Leu	Lys	Leu	Thr	Ala	Gly	Pro	Arg	Thr	Gly	Asp	665	Thr	Asp
Trp	Arg	Tyr	Val	Glu	Gly	Gly	Leu	Thr	Arg	Pro	Val	Pro	Glu	Thr	Lys	680	Pro	Lys
Glu	Leu	Phe	Thr	Leu	Thr	Val	Glu	His	Gly	Val	Lys	Pro	Arg	Asn	Ala	695	Arg	Ala
Ser	Tyr	Ile	Tyr	Thr	Ile	Leu	Pro	Gly	Ala	Thr	Pro	Glu	Glu	Thr	Ala	710	Thr	Ala
Gly	Thr	Pro	Pro	Gly	Arg	Val	Leu	Arg	Asn	Thr	Pro	Glu	Cys	Gln	Ala	725	Thr	Ala
																740		
																755		
																760		
																765		

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Val Glu Phe Ala Asp Gly Val Arg Ala Ala Ile Phe Tyr Glu Pro Gly
 770 775 780
 Arg Leu Asp Asp Phe Glu Thr Asp Thr Pro Gly Val Phe Leu Ile Gly
 785 790 795 800
 Lys Gly Thr Val His Ala Ala Asp Pro Thr Gly Arg His Ser Ser Phe
 805 810 815
 Thr Leu Lys Leu Asn Gly Val Ser Arg Lys Val Pro Leu Pro Ala Gly
 820 825 830
 Glu Phe Ala Gly Gln Ser Val Lys Ile Val Leu Lys
 835 840

<210> 101
 <211> 510
 <212> PRT
 <213> Pan troglodytes

<220>
 <223> Chimpanzee PH20

<400> 101
 Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1 5 10 15
 Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
 20 25 30
 Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
 35 40 45
 Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
 50 55 60
 Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
 65 70 75 80
 Ile Asn Val Thr Gly Gln Asp Val Thr Ile Phe Tyr Val Asp Arg Leu
 85 90 95
 Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
 100 105 110
 Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
 115 120 125
 Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
 130 135 140
 Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
 145 150 155 160
 Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
 165 170 175
 Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
 180 185 190
 Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
 195 200 205
 Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
 210 215 220
 Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
 225 230 235 240
 Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
 245 250 255
 Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
 260 265 270
 Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Gln Glu Ala Ile Arg Val
 275 280 285
 Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Val Tyr Thr
 290 295 300
 Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
 305 310 315 320
 Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
 325 330 335
 Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
 340 345 350
 Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
 355 360 365
 Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
 370 375 380
 Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
 385 390 395 400
 Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
 405 410 415
 Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
 420 425 430

Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
 435 440 445
 Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
 450 455 460
 Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Ser Gln Ile
 465 470 475
 Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Asp
 485 490 495
 Leu Cys Asp Leu Tyr Leu Val Pro Thr Ser Tyr Leu Ile Leu
 500 505 510

<210> 102

<211> 512

<212> PRT

<213> Macaca mulatta

<220>

<223> Rhesus Monkey PH20

<400> 102

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

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

Phe Tyr Cys Ser Cys Tyr Thr Asn Leu Ser Cys Lys Glu Lys Ala Asp
 435 440 445
 Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
 450 455 460
 Ile Asp Ala Ser Leu Lys Pro Pro Val Glu Thr Glu Gly Ser Pro Pro
 465 470 475 480
 Ile Phe Tyr Asn Thr Ser Ser Ser Thr Val Ser Thr Thr Met Phe Ile
 485 490 495
 Trp Arg Leu Glu Val Trp Asp Gln Gly Ile Ser Arg Ile Gly Phe Phe
 500 505 510

<210> 103

<211> 505

<212> PRT

<213> Homo sapiens

<220>

<223> HuPH20 precursor 1-505

<400> 103

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

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

Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
 435 440 445
 Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
 450 455 460
 Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
 465 470 475 480
 Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
 485 490 495
 Ser Ile Leu Phe Leu Ile Ile Ser Ser
 500 505

<210> 104
 <211> 503
 <212> PRT
 <213> Homo sapiens

<220>
 <223> HuPH20 precursor 1-503

<400> 104
 Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1 5 10 15
 Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
 20 25 30
 Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
 35 40 45
 Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
 50 55 60
 Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
 65 70 75 80
 Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
 85 90 95
 Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
 100 105 110
 Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
 115 120 125
 Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
 130 135 140
 Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
 145 150 155 160
 Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
 165 170 175
 Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
 180 185 190
 Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
 195 200 205

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

Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
 435 440 445
 Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
 450 455 460
 Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
 465 470 475 480
 Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
 485 490 495
 Ser Ile Leu Phe Leu Ile Ile
 500

<210> 105
 <211> 501
 <212> PRT
 <213> Homo sapiens

<220>
 <223> HuPH20 precursor 1-501

<400> 105
 Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1 5 10 15
 Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
 20 25 30
 Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
 35 40 45
 Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
 50 55 60
 Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
 65 70 75 80
 Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
 85 90 95
 Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
 100 105 110
 Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
 115 120 125
 Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
 130 135 140
 Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
 145 150 155 160
 Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
 165 170 175
 Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
 180 185 190
 Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
 195 200 205

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

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Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
 435 440 445
 Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
 450 455 460
 Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
 465 470 475 480
 Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
 485 490 495
 Ser Ile Leu Phe Leu
 500

<210> 106

<211> 499

<212> PRT

<213> Homo sapiens

<220>

<223> HuPH20 precursor 1-499

<400> 106

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

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

3066SEQ PC1.TXT

Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
 435 440 445
 Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
 450 455 460
 Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
 465 470 475
 Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
 485 490 495
 Ser Ile Leu

<210> 107

<211> 497

<212> PRT

<213> Homo sapiens

<220>

<223> HuPH20 precursor 1-497

<400> 107

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

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

Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
 435 440 445
 Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
 450 455 460
 Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
 465 470 475 480
 Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
 485 490 495
 Ser

<210> 108

<211> 495

<212> PRT

<213> Homo sapiens

<220>

<223> HuPH20 precursor 1-495

<400> 108

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

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

3066SEQ PC1.TXT

Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
 435 440 445
 Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
 450 455 460
 Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
 465 470 475
 Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile
 485 490 495

<210> 109

<211> 493

<212> PRT

<213> Homo sapiens

<220>

<223> HuPH20 precursor 1-493

<400> 109

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

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

3066SEQ PC1.TXT

Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
 450 455 460
 Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
 465 470 475 480
 Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met
 485 490

<210> 110
 <211> 491
 <212> PRT
 <213> Homo sapiens

<220>
 <223> HuPH20 precursor 1-489

<400> 110
 Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1 5 10 15
 Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
 20 25 30
 Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
 35 40 45
 Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
 50 55 60
 Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
 65 70 75 80
 Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
 85 90 95
 Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
 100 105 110
 Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
 115 120 125
 Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
 130 135 140
 Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
 145 150 155 160
 Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
 165 170 175
 Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
 180 185 190
 Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
 195 200 205
 Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
 210 215 220
 Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
 225 230 235 240

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

Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
 465 470 475 480
 Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala
 485 490

<210> 111
 <211> 489
 <212> PRT
 <213> Homo sapiens

<220>
 <223> HuPH20 precursor 1-489

<400> 111
 Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1 5 10 15
 Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
 20 25 30
 Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
 35 40 45
 Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
 50 55 60
 Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
 65 70 75 80
 Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
 85 90 95
 Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
 100 105 110
 Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
 115 120 125
 Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
 130 135 140
 Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
 145 150 155 160
 Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
 165 170 175
 Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
 180 185 190
 Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
 195 200 205
 Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
 210 215 220
 Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
 225 230 235 240
 Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
 245 250 255

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

Phe Tyr Asn Ala Ser Pro Ser Thr Leu
485

<210> 112

<211> 490

<212> PRT

<213> Homo sapiens

<220>

<223> HuPH20 precursor 1-490

<400> 112

Met	Gly	Val	Leu	Lys	Phe	Lys	His	Ile	Phe	Phe	Arg	Ser	Phe	Val	Lys
1				5					10					15	
Ser	Ser	Gly	Val	Ser	Gln	Ile	Val	Phe	Thr	Phe	Leu	Leu	Ile	Pro	Cys
		20						25					30		
Cys	Leu	Thr	Leu	Asn	Phe	Arg	Ala	Pro	Pro	Val	Ile	Pro	Asn	Val	Pro
		35					40					45			
Phe	Leu	Trp	Ala	Trp	Asn	Ala	Pro	Ser	Glu	Phe	Cys	Leu	Gly	Lys	Phe
	50				55					60					
Asp	Glu	Pro	Leu	Asp	Met	Ser	Leu	Phe	Ser	Phe	Ile	Gly	Ser	Pro	Arg
65				70				75						80	
Ile	Asn	Ala	Thr	Gly	Gln	Gly	Val	Thr	Ile	Phe	Tyr	Val	Asp	Arg	Leu
			85					90					95		
Gly	Tyr	Tyr	Pro	Tyr	Ile	Asp	Ser	Ile	Thr	Gly	Val	Thr	Val	Asn	Gly
			100					105					110		
Gly	Ile	Pro	Gln	Lys	Ile	Ser	Leu	Gln	Asp	His	Leu	Asp	Lys	Ala	Lys
		115					120					125			
Lys	Asp	Ile	Thr	Phe	Tyr	Met	Pro	Val	Asp	Asn	Leu	Gly	Met	Ala	Val
	130					135					140				
Ile	Asp	Trp	Glu	Glu	Trp	Arg	Pro	Thr	Trp	Ala	Arg	Asn	Trp	Lys	Pro
145					150					155					160
Lys	Asp	Val	Tyr	Lys	Asn	Arg	Ser	Ile	Glu	Leu	Val	Gln	Gln	Gln	Asn
				165					170					175	
Val	Gln	Leu	Ser	Leu	Thr	Glu	Ala	Thr	Glu	Lys	Ala	Lys	Gln	Glu	Phe
			180					185					190		
Glu	Lys	Ala	Gly	Lys	Asp	Phe	Leu	Val	Glu	Thr	Ile	Lys	Leu	Gly	Lys
		195					200					205			
Leu	Leu	Arg	Pro	Asn	His	Leu	Trp	Gly	Tyr	Tyr	Leu	Phe	Pro	Asp	Cys
	210					215					220				
Tyr	Asn	His	His	Tyr	Lys	Lys	Pro	Gly	Tyr	Asn	Gly	Ser	Cys	Phe	Asn
225					230					235					240
Val	Glu	Ile	Lys	Arg	Asn	Asp	Asp	Leu	Ser	Trp	Leu	Trp	Asn	Glu	Ser
				245					250					255	
Thr	Ala	Leu	Tyr	Pro	Ser	Ile	Tyr	Leu	Asn	Thr	Gln	Gln	Ser	Pro	Val
			260					265					270		
Ala	Ala	Thr	Leu	Tyr	Val	Arg	Asn	Arg	Val	Arg	Glu	Ala	Ile	Arg	Val
		275					280					285			
Ser	Lys	Ile	Pro	Asp	Ala	Lys	Ser	Pro	Leu	Pro	Val	Phe	Ala	Tyr	Thr
	290					295					300				
Arg	Ile	Val	Phe	Thr	Asp	Gln	Val	Leu	Lys	Phe	Leu	Ser	Gln	Asp	Glu
305					310					315					320
Leu	Val	Tyr	Thr	Phe	Gly	Glu	Thr	Val	Ala	Leu	Gly	Ala	Ser	Gly	Ile
				325					330					335	
Val	Ile	Trp	Gly	Thr	Leu	Ser	Ile	Met	Arg	Ser	Met	Lys	Ser	Cys	Leu
			340					345					350		
Leu	Leu	Asp	Asn	Tyr	Met	Glu	Thr	Ile	Leu	Asn	Pro	Tyr	Ile	Ile	Asn
		355					360					365			
Val	Thr	Leu	Ala	Ala	Lys	Met	Cys	Ser	Gln	Val	Leu	Cys	Gln	Glu	Gln
	370					375					380				
Gly	Val	Cys	Ile	Arg	Lys	Asn	Trp	Asn	Ser	Ser	Asp	Tyr	Leu	His	Leu
385					390					395					400
Asn	Pro	Asp	Asn	Phe	Ala	Ile	Gln	Leu	Glu	Lys	Gly	Gly	Lys	Phe	Thr
				405					410					415	
Val	Arg	Gly	Lys	Pro	Thr	Leu	Glu	Asp	Leu	Glu	Gln	Phe	Ser	Glu	Lys
			420					425					430		
Phe	Tyr	Cys	Ser	Cys	Tyr	Ser	Thr	Leu	Ser	Cys	Lys	Glu	Lys	Ala	Asp
		435					440					445			
Val	Lys	Asp	Thr	Asp	Ala	Val	Asp	Val	Cys	Ile	Ala	Asp	Gly	Val	Cys
	450					455					460				
Ile	Asp	Ala	Phe	Leu	Lys	Pro	Pro	Met	Glu	Thr	Glu	Glu	Pro	Gln	Ile
465					470					475					480
Phe	Tyr	Asn	Ala	Ser	Pro	Ser	Thr	Leu	Ser						
				485					490						

<210> 113
 <211> 487
 <212> PRT
 <213> Homo sapiens

<220>
 <223> HuPH20 precursor 1-487

<400> 113
 Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1 5 10 15
 Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
 20 25 30
 Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
 35 40 45
 Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
 50 55 60
 Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
 65 70 75 80
 Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
 85 90 95
 Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
 100 105 110
 Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
 115 120 125
 Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
 130 135 140
 Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
 145 150 155 160
 Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
 165 170 175
 Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
 180 185 190
 Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
 195 200 205
 Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
 210 215 220
 Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
 225 230 235 240
 Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
 245 250 255
 Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
 260 265 270
 Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
 275 280 285
 Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
 290 295 300
 Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
 305 310 315 320
 Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
 325 330 335
 Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
 340 345 350
 Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
 355 360 365
 Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
 370 375 380
 Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
 385 390 395 400
 Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
 405 410 415
 Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
 420 425 430
 Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
 435 440 445
 Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
 450 455 460
 Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
 465 470 475 480
 Phe Tyr Asn Ala Ser Pro Ser
 485

<210> 114
 <211> 485
 <212> PRT
 <213> Homo sapiens

<220>
 <223> HuPH20 precursor 1-485

<400> 114
 Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1 5 10 15
 Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
 20 25 30
 Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
 35 40 45
 Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
 50 55 60
 Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
 65 70 75 80
 Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
 85 90 95
 Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
 100 105 110
 Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
 115 120 125
 Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
 130 135 140
 Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
 145 150 155 160
 Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
 165 170 175
 Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
 180 185 190
 Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
 195 200 205
 Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
 210 215 220
 Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
 225 230 235 240
 Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
 245 250 255
 Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
 260 265 270
 Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
 275 280 285
 Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
 290 295 300
 Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
 305 310 315 320
 Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
 325 330 335
 Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
 340 345 350
 Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
 355 360 365
 Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
 370 375 380
 Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
 385 390 395 400
 Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
 405 410 415
 Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
 420 425 430
 Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
 435 440 445
 Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
 450 455 460
 Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
 465 470 475 480
 Phe Tyr Asn Ala Ser
 485

<210> 115
 <211> 484

<212> PRT

<213> Homo sapiens

<220>

<223> HuPH20 precursor 1-484

<400> 115

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Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1      5      10      15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
 20      25      30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
 35      40      45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
 50      55      60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
 65      70      75      80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
 85      90      95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
100      105      110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
115      120      125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
130      135      140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145      150      155      160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
165      170      175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
180      185      190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
195      200      205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
210      215      220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225      230      235      240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
245      250      255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
260      265      270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
275      280      285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
290      295      300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305      310      315      320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
325      330      335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
340      345      350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
355      360      365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
370      375      380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385      390      395      400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
405      410      415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
420      425      430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
435      440      445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
450      455      460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465      470      475      480
Phe Tyr Asn Ala

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<210> 116

<211> 476

<212> PRT

<213> Homo sapiens

<220>

<223> HuPH20 precursor 1-476

<400> 116

Met	Gly	Val	Leu	Lys	Phe	Lys	His	Ile	Phe	Phe	Arg	Ser	Phe	Val	Lys
1				5					10					15	
Ser	Ser	Gly	Val	Ser	Gln	Ile	Val	Phe	Thr	Phe	Leu	Leu	Ile	Pro	Cys
			20					25					30		
Cys	Leu	Thr	Leu	Asn	Phe	Arg	Ala	Pro	Pro	Val	Ile	Pro	Asn	Val	Pro
		35					40					45			
Phe	Leu	Trp	Ala	Trp	Asn	Ala	Pro	Ser	Glu	Phe	Cys	Leu	Gly	Lys	Phe
	50					55					60				
Asp	Glu	Pro	Leu	Asp	Met	Ser	Leu	Phe	Ser	Phe	Ile	Gly	Ser	Pro	Arg
65					70					75					80
Ile	Asn	Ala	Thr	Gly	Gln	Gly	Val	Thr	Ile	Phe	Tyr	Val	Asp	Arg	Leu
				85					90					95	
Gly	Tyr	Tyr	Pro	Tyr	Ile	Asp	Ser	Ile	Thr	Gly	Val	Thr	Val	Asn	Gly
			100					105					110		
Gly	Ile	Pro	Gln	Lys	Ile	Ser	Leu	Gln	Asp	His	Leu	Asp	Lys	Ala	Lys
		115					120					125			
Lys	Asp	Ile	Thr	Phe	Tyr	Met	Pro	Val	Asp	Asn	Leu	Gly	Met	Ala	Val
	130					135					140				
Ile	Asp	Trp	Glu	Glu	Trp	Arg	Pro	Thr	Trp	Ala	Arg	Asn	Trp	Lys	Pro
145					150					155					160
Lys	Asp	Val	Tyr	Lys	Asn	Arg	Ser	Ile	Glu	Leu	Val	Gln	Gln	Gln	Asn
				165					170					175	
Val	Gln	Leu	Ser	Leu	Thr	Glu	Ala	Thr	Glu	Lys	Ala	Lys	Gln	Glu	Phe
			180					185					190		
Glu	Lys	Ala	Gly	Lys	Asp	Phe	Leu	Val	Glu	Thr	Ile	Lys	Leu	Gly	Lys
	195					200						205			
Leu	Leu	Arg	Pro	Asn	His	Leu	Trp	Gly	Tyr	Tyr	Leu	Phe	Pro	Asp	Cys
	210					215					220				
Tyr	Asn	His	His	Tyr	Lys	Lys	Pro	Gly	Tyr	Asn	Gly	Ser	Cys	Phe	Asn
225					230					235					240
Val	Glu	Ile	Lys	Arg	Asn	Asp	Asp	Leu	Ser	Trp	Leu	Trp	Asn	Glu	Ser
				245					250					255	
Thr	Ala	Leu	Tyr	Pro	Ser	Ile	Tyr	Leu	Asn	Thr	Gln	Gln	Ser	Pro	Val
			260					265					270		
Ala	Ala	Thr	Leu	Tyr	Val	Arg	Asn	Arg	Val	Arg	Glu	Ala	Ile	Arg	Val
		275					280					285			
Ser	Lys	Ile	Pro	Asp	Ala	Lys	Ser	Pro	Leu	Pro	Val	Phe	Ala	Tyr	Thr
	290					295					300				
Arg	Ile	Val	Phe	Thr	Asp	Gln	Val	Leu	Lys	Phe	Leu	Ser	Gln	Asp	Glu
305					310					315					320
Leu	Val	Tyr	Thr	Phe	Gly	Glu	Thr	Val	Ala	Leu	Gly	Ala	Ser	Gly	Ile
				325					330					335	
Val	Ile	Trp	Gly	Thr	Leu	Ser	Ile	Met	Arg	Ser	Met	Lys	Ser	Cys	Leu
			340					345					350		
Leu	Leu	Asp	Asn	Tyr	Met	Glu	Thr	Ile	Leu	Asn	Pro	Tyr	Ile	Ile	Asn
		355					360					365			
Val	Thr	Leu	Ala	Ala	Lys	Met	Cys	Ser	Gln	Val	Leu	Cys	Gln	Glu	Gln
		370				375					380				
Gly	Val	Cys	Ile	Arg	Lys	Asn	Trp	Asn	Ser	Ser	Asp	Tyr	Leu	His	Leu
385					390					395					400
Asn	Pro	Asp	Asn	Phe	Ala	Ile	Gln	Leu	Glu	Lys	Gly	Gly	Lys	Phe	Thr
				405					410					415	
Val	Arg	Gly	Lys	Pro	Thr	Leu	Glu	Asp	Leu	Glu	Gln	Phe	Ser	Glu	Lys
			420					425					430		
Phe	Tyr	Cys	Ser	Cys	Tyr	Ser	Thr	Leu	Ser	Cys	Lys	Glu	Lys	Ala	Asp
		435					440					445			
Val	Lys	Asp	Thr	Asp	Ala	Val	Asp	Val	Cys	Ile	Ala	Asp	Gly	Val	Cys
	450					455					460				
Ile	Asp	Ala	Phe	Leu	Lys	Pro	Pro	Met	Glu	Thr	Glu				
465					470					475					

<210> 117

<211> 475

<212> PRT

<213> Homo sapiens

<220>

<223> HuPH20 precursor 1-475

<400> 117

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Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1      5      10      15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
 20      25      30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
 35      40      45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
 50      55      60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
 65      70      75      80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
 85      90      95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
100      105      110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
115      120      125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
130      135      140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145      150      155      160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
165      170      175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
180      185      190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
195      200      205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
210      215      220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225      230      235      240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
245      250      255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
260      265      270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
275      280      285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
290      295      300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305      310      315      320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
325      330      335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
340      345      350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
355      360      365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
370      375      380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385      390      395      400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
405      410      415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
420      425      430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
435      440      445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
450      455      460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr
465      470      475

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<210> 118

<211> 474

<212> PRT

<213> Homo sapiens

<220>

<223> HuPH20 precursor 1-474

<400> 118

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Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1      5      10      15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys

```

			20					25					30		
Cys	Leu	Thr	Leu	Asn	Phe	Arg	Ala	Pro	Pro	Val	Ile	Pro	Asn	Val	Pro
		35					40					45			
Phe	Leu	Trp	Ala	Trp	Asn	Ala	Pro	Ser	Glu	Phe	Cys	Leu	Gly	Lys	Phe
	50					55					60				
Asp	Glu	Pro	Leu	Asp	Met	Ser	Leu	Phe	Ser	Phe	Ile	Gly	Ser	Pro	Arg
65					70					75					80
Ile	Asn	Ala	Thr	Gly	Gln	Gly	Val	Thr	Ile	Phe	Tyr	Val	Asp	Arg	Leu
				85					90					95	
Gly	Tyr	Tyr	Pro	Tyr	Ile	Asp	Ser	Ile	Thr	Gly	Val	Thr	Val	Asn	Gly
			100					105					110		
Gly	Ile	Pro	Gln	Lys	Ile	Ser	Leu	Gln	Asp	His	Leu	Asp	Lys	Ala	Lys
		115					120					125			
Lys	Asp	Ile	Thr	Phe	Tyr	Met	Pro	Val	Asp	Asn	Leu	Gly	Met	Ala	Val
	130					135					140				
Ile	Asp	Trp	Glu	Glu	Trp	Arg	Pro	Thr	Trp	Ala	Arg	Asn	Trp	Lys	Pro
145					150					155					160
Lys	Asp	Val	Tyr	Lys	Asn	Arg	Ser	Ile	Glu	Leu	Val	Gln	Gln	Gln	Asn
				165					170					175	
Val	Gln	Leu	Ser	Leu	Thr	Glu	Ala	Thr	Glu	Lys	Ala	Lys	Gln	Glu	Phe
			180					185					190		
Glu	Lys	Ala	Gly	Lys	Asp	Phe	Leu	Val	Glu	Thr	Ile	Lys	Leu	Gly	Lys
		195					200					205			
Leu	Leu	Arg	Pro	Asn	His	Leu	Trp	Gly	Tyr	Tyr	Leu	Phe	Pro	Asp	Cys
	210					215					220				
Tyr	Asn	His	His	Tyr	Lys	Lys	Pro	Gly	Tyr	Asn	Gly	Ser	Cys	Phe	Asn
225					230					235				240	
Val	Glu	Ile	Lys	Arg	Asn	Asp	Asp	Leu	Ser	Trp	Leu	Trp	Asn	Glu	Ser
				245					250					255	
Thr	Ala	Leu	Tyr	Pro	Ser	Ile	Tyr	Leu	Asn	Thr	Gln	Gln	Ser	Pro	Val
			260					265					270		
Ala	Ala	Thr	Leu	Tyr	Val	Arg	Asn	Arg	Val	Arg	Glu	Ala	Ile	Arg	Val
		275					280					285			
Ser	Lys	Ile	Pro	Asp	Ala	Lys	Ser	Pro	Leu	Pro	Val	Phe	Ala	Tyr	Thr
	290					295					300				
Arg	Ile	Val	Phe	Thr	Asp	Gln	Val	Leu	Lys	Phe	Leu	Ser	Gln	Asp	Glu
305					310					315					320
Leu	Val	Tyr	Thr	Phe	Gly	Glu	Thr	Val	Ala	Leu	Gly	Ala	Ser	Gly	Ile
				325					330					335	
Val	Ile	Trp	Gly	Thr	Leu	Ser	Ile	Met	Arg	Ser	Met	Lys	Ser	Cys	Leu
			340					345					350		
Leu	Leu	Asp	Asn	Tyr	Met	Glu	Thr	Ile	Leu	Asn	Pro	Tyr	Ile	Ile	Asn
		355					360					365			
Val	Thr	Leu	Ala	Ala	Lys	Met	Cys	Ser	Gln	Val	Leu	Cys	Gln	Glu	Gln
	370														

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<210> 119
<211> 473
<212> PRT
<213> Homo sapiens
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<220>
<223> HuPH20 precursor 1-473

<400> 119
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
1 5 10 15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
20 25 30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
35 40 45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe

50	55	60															
Asp	Glu	Pro	Leu	Asp	Met	Ser	Leu	Phe	Ser	Phe	Ile	Gly	Ser	Pro	Arg		
65	Ile	Asn	Ala	Thr	Gly	Gln	Gly	Val	Thr	Ile	Phe	Tyr	Val	Asp	Arg	Leu	
				85						90					95		
Gly	Tyr	Tyr	Pro	Tyr	Ile	Asp	Ser	Ile	Thr	Gly	Val	Thr	Val	Asn	Gly		
			100					105						110			
Gly	Ile	Pro	Gln	Lys	Ile	Ser	Leu	Gln	Asp	His	Leu	Asp	Lys	Ala	Lys		
		115					120					125					
Lys	Asp	Ile	Thr	Phe	Tyr	Met	Pro	Val	Asp	Asn	Leu	Gly	Met	Ala	Val		
	130					135					140						
Ile	Asp	Trp	Glu	Glu	Trp	Arg	Pro	Thr	Trp	Ala	Arg	Asn	Trp	Lys	Pro		
	145				150					155					160		
Lys	Asp	Val	Tyr	Lys	Asn	Arg	Ser	Ile	Glu	Leu	Val	Gln	Gln	Gln	Asn		
				165					170					175			
Val	Gln	Leu	Ser	Leu	Thr	Glu	Ala	Thr	Glu	Lys	Ala	Lys	Gln	Glu	Phe		
			180					185					190				
Glu	Lys	Ala	Gly	Lys	Asp	Phe	Leu	Val	Glu	Thr	Ile	Lys	Leu	Gly	Lys		
		195					200					205					
Leu	Leu	Arg	Pro	Asn	His	Leu	Trp	Gly	Tyr	Tyr	Leu	Phe	Pro	Asp	Cys		
	210					215					220						
Tyr	Asn	His	His	Tyr	Lys	Lys	Pro	Gly	Tyr	Asn	Gly	Ser	Cys	Phe	Asn		
	225				230					235					240		
Val	Glu	Ile	Lys	Arg	Asn	Asp	Asp	Leu	Ser	Trp	Leu	Trp	Asn	Glu	Ser		
				245					250					255			
Thr	Ala	Leu	Tyr	Pro	Ser	Ile	Tyr	Leu	Asn	Thr	Gln	Gln	Ser	Pro	Val		
			260					265					270				
Ala	Ala	Thr	Leu	Tyr	Val	Arg	Asn	Arg	Val	Arg	Glu	Ala	Ile	Arg	Val		
		275					280					285					
Ser	Lys	Ile	Pro	Asp	Ala	Lys	Ser	Pro	Leu	Pro	Val	Phe	Ala	Tyr	Thr		
	290					295					300						
Arg	Ile	Val	Phe	Thr	Asp	Gln	Val	Leu	Lys	Phe	Leu	Ser	Gln	Asp	Glu		
	305				310					315					320		
Leu	Val	Tyr	Thr	Phe	Gly	Glu	Thr	Val	Ala	Leu	Gly	Ala	Ser	Gly	Ile		
				325					330					335			
Val	Ile	Trp	Gly	Thr	Leu	Ser	Ile	Met	Arg	Ser	Met	Lys	Ser	Cys	Leu		
			340					345					350				
Leu	Leu	Asp	Asn	Tyr	Met	Glu	Thr	Ile	Leu	Asn	Pro	Tyr	Ile	Ile	Asn		
		355				360						365					
Val	Thr	Leu	Ala	Ala	Lys	Met	Cys	Ser	Gln	Val	Leu	Cys	Gln	Glu	Gln		
	370					375					380						
Gly	Val	Cys	Ile	Arg	Lys	Asn	Trp	Asn	Ser	Ser	Asp	Tyr	Leu	His	Leu		
	385				390					395					400		
Asn	Pro	Asp	Asn	Phe	Ala	Ile	Gln	Leu	Glu	Lys	Gly	Gly	Lys	Phe	Thr		
				405					410					415			
Val	Arg	Gly	Lys	Pro	Thr	Leu	Glu	Asp	Leu	Glu	Gln	Phe	Ser	Glu	Lys		
			420					425					430				
Phe	Tyr	Cys	Ser	Cys	Tyr	Ser	Thr	Leu	Ser	Cys	Lys	Glu	Lys	Ala	Asp		
		435					440					445					
Val	Lys	Asp	Thr	Asp	Ala	Val	Asp	Val	Cys	Ile	Ala	Asp	Gly	Val	Cys		
		450				455					460						
Ile	Asp	Ala	Phe	Leu	Lys	Pro	Pro	Met									
	465				470												

<210> 120

<211> 472

<212> PRT

<213> Homo sapiens

<220>

<223> HuPH20 precursor 1-472

<400> 120

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

3066SEQ PC1.TXT

				85					90				95				
Gly	Tyr	Tyr	Pro	Tyr	Ile	Asp	Ser	Ile	Thr	Gly	Val	Thr	Val	Asn	Gly		
			100					105					110				
Gly	Ile	Pro	Gln	Lys	Ile	Ser	Leu	Gln	Asp	His	Leu	Asp	Lys	Ala	Lys		
		115					120					125					
Lys	Asp	Ile	Thr	Phe	Tyr	Met	Pro	Val	Asp	Asn	Leu	Gly	Met	Ala	Val		
		130				135					140						
Ile	Asp	Trp	Glu	Glu	Trp	Arg	Pro	Thr	Trp	Ala	Arg	Asn	Trp	Lys	Pro		
				150						155					160		
Lys	Asp	Val	Tyr	Lys	Asn	Arg	Ser	Ile	Glu	Leu	Val	Gln	Gln	Gln	Asn		
				165					170					175			
Val	Gln	Leu	Ser	Leu	Thr	Glu	Ala	Thr	Glu	Lys	Ala	Lys	Gln	Glu	Phe		
			180					185					190				
Glu	Lys	Ala	Gly	Lys	Asp	Phe	Leu	Val	Glu	Thr	Ile	Lys	Leu	Gly	Lys		
		195					200					205					
Leu	Leu	Arg	Pro	Asn	His	Leu	Trp	Gly	Tyr	Tyr	Leu	Phe	Pro	Asp	Cys		
		210				215					220						
Tyr	Asn	His	His	Tyr	Lys	Lys	Pro	Gly	Tyr	Asn	Gly	Ser	Cys	Phe	Asn		
				230						235					240		
Val	Glu	Ile	Lys	Arg	Asn	Asp	Asp	Leu	Ser	Trp	Leu	Trp	Asn	Glu	Ser		
				245					250					255			
Thr	Ala	Leu	Tyr	Pro	Ser	Ile	Tyr	Leu	Asn	Thr	Gln	Gln	Ser	Pro	Val		
			260					265					270				
Ala	Ala	Thr	Leu	Tyr	Val	Arg	Asn	Arg	Val	Arg	Glu	Ala	Ile	Arg	Val		
		275					280					285					
Ser	Lys	Ile	Pro	Asp	Ala	Lys	Ser	Pro	Leu	Pro	Val	Phe	Ala	Tyr	Thr		
		290				295					300						
Arg	Ile	Val	Phe	Thr	Asp	Gln	Val	Leu	Lys	Phe	Leu	Ser	Gln	Asp	Glu		
				310						315					320		
Leu	Val	Tyr	Thr	Phe	Gly	Glu	Thr	Val	Ala	Leu	Gly	Ala	Ser	Gly	Ile		
				325					330					335			
Val	Ile	Trp	Gly	Thr	Leu	Ser	Ile	Met	Arg	Ser	Met	Lys	Ser	Cys	Leu		
			340					345					350				
Leu	Leu	Asp	Asn	Tyr	Met	Glu	Thr	Ile	Leu	Asn	Pro	Tyr	Ile	Ile	Asn		
		355					360					365					
Val	Thr	Leu	Ala	Ala	Lys	Met	Cys	Ser	Gln	Val	Leu	Cys	Gln	Glu	Gln		
		370				375					380						
Gly	Val	Cys	Ile	Arg	Lys	Asn	Trp	Asn	Ser	Ser	Asp	Tyr	Leu	His	Leu		
				390					395					400			
Asn	Pro	Asp	Asn	Phe	Ala	Ile	Gln	Leu	Glu	Lys	Gly	Gly	Lys	Phe	Thr		
				405					410					415			
Val	Arg	Gly	Lys	Pro	Thr	Leu	Glu	Asp	Leu	Glu	Gln	Phe	Ser	Glu	Lys		
			420					425					430				
Phe	Tyr	Cys	Ser	Cys	Tyr	Ser	Thr	Leu	Ser	Cys	Lys	Glu	Lys	Ala	Asp		
		435					440					445					
Val	Lys	Asp	Thr	Asp	Ala	Val	Asp	Val	Cys	Ile	Ala	Asp	Gly	Val	Cys		
		450				455					460						

Ile Asp Ala Phe Leu Lys Pro Pro
465 470

<210> 121

<211> 471

<212> PRT

<213> Homo sapiens

<220>

<223> HuPH20 precursor 1-471

<400> 121

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

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Lys	Asp	Ile	Thr	Phe	Tyr	Met	Pro	Val	Asp	Asn	Leu	Gly	Met	Ala	Val
115	130					120					125				
Ile	Asp	Trp	Glu	Glu	Trp	Arg	Pro	Thr	Trp	Ala	Arg	Asn	Trp	Lys	Pro
145					150					155					160
Lys	Asp	Val	Tyr	Lys	Asn	Arg	Ser	Ile	Glu	Leu	Val	Gln	Gln	Gln	Asn
				165					170						175
Val	Gln	Leu	Ser	Leu	Thr	Glu	Ala	Thr	Glu	Lys	Ala	Lys	Gln	Glu	Phe
			180					185					190		
Glu	Lys	Ala	Gly	Lys	Asp	Phe	Leu	Val	Glu	Thr	Ile	Lys	Leu	Gly	Lys
		195					200					205			
Leu	Leu	Arg	Pro	Asn	His	Leu	Trp	Gly	Tyr	Tyr	Leu	Phe	Pro	Asp	Cys
	210					215					220				
Tyr	Asn	His	His	Tyr	Lys	Lys	Pro	Gly	Tyr	Asn	Gly	Ser	Cys	Phe	Asn
225					230					235					240
Val	Glu	Ile	Lys	Arg	Asn	Asp	Asp	Leu	Ser	Trp	Leu	Trp	Asn	Glu	Ser
				245					250					255	
Thr	Ala	Leu	Tyr	Pro	Ser	Ile	Tyr	Leu	Asn	Thr	Gln	Gln	Ser	Pro	Val
			260					265					270		
Ala	Ala	Thr	Leu	Tyr	Val	Arg	Asn	Arg	Val	Arg	Glu	Ala	Ile	Arg	Val
		275					280					285			
Ser	Lys	Ile	Pro	Asp	Ala	Lys	Ser	Pro	Leu	Pro	Val	Phe	Ala	Tyr	Thr
	290					295					300				
Arg	Ile	Val	Phe	Thr	Asp	Gln	Val	Leu	Lys	Phe	Leu	Ser	Gln	Asp	Glu
305					310					315					320
Leu	Val	Tyr	Thr	Phe	Gly	Glu	Thr	Val	Ala	Leu	Gly	Ala	Ser	Gly	Ile
				325					330					335	
Val	Ile	Trp	Gly	Thr	Leu	Ser	Ile	Met	Arg	Ser	Met	Lys	Ser	Cys	Leu
			340					345					350		
Leu	Leu	Asp	Asn	Tyr	Met	Glu	Thr	Ile	Leu	Asn	Pro	Tyr	Ile	Ile	Asn
		355					360					365			
Val	Thr	Leu	Ala	Ala	Lys	Met	Cys	Ser	Gln	Val	Leu	Cys	Gln	Glu	Gln
		370				375					380				
Gly	Val	Cys	Ile	Arg	Lys	Asn	Trp	Asn	Ser	Ser	Asp	Tyr	Leu	His	Leu
385					390					395					400
Asn	Pro	Asp	Asn	Phe	Ala	Ile	Gln	Leu	Glu	Lys	Gly	Gly	Lys	Phe	Thr
				405					410					415	
Val	Arg	Gly	Lys	Pro	Thr	Leu	Glu	Asp	Leu	Glu	Gln	Phe	Ser	Glu	Lys
			420					425					430		
Phe	Tyr	Cys	Ser	Cys	Tyr	Ser	Thr	Leu	Ser	Cys	Lys	Glu	Lys	Ala	Asp
		435					440					445			
Val	Lys	Asp	Thr	Asp	Ala	Val	Asp	Val	Cys	Ile	Ala	Asp	Gly	Val	Cys
	450					455					460				
Ile	Asp	Ala	Phe	Leu	Lys	Pro									
465					470										

<210> 122
 <211> 470
 <212> PRT
 <213> Homo sapiens

<220>
 <223> HuPH20 precursor 1-470

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

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145					150											160
Lys	Asp	Val	Tyr	Lys	Asn	Arg	Ser	Ile	Glu	Leu	Val	Gln	Gln	Gln	Asn	
				165					170						175	
Val	Gln	Leu	Ser	Leu	Thr	Glu	Ala	Thr	Glu	Lys	Ala	Lys	Gln	Glu	Phe	
			180					185						190		
Glu	Lys	Ala	Gly	Lys	Asp	Phe	Leu	Val	Glu	Thr	Ile	Lys	Leu	Gly	Lys	
		195					200					205				
Leu	Leu	Arg	Pro	Asn	His	Leu	Trp	Gly	Tyr	Tyr	Leu	Phe	Pro	Asp	Cys	
	210					215					220					
Tyr	Asn	His	His	Tyr	Lys	Lys	Pro	Gly	Tyr	Asn	Gly	Ser	Cys	Phe	Asn	
225					230					235					240	
Val	Glu	Ile	Lys	Arg	Asn	Asp	Asp	Leu	Ser	Trp	Leu	Trp	Asn	Glu	Ser	
				245					250					255		
Thr	Ala	Leu	Tyr	Pro	Ser	Ile	Tyr	Leu	Asn	Thr	Gln	Gln	Ser	Pro	Val	
			260					265						270		
Ala	Ala	Thr	Leu	Tyr	Val	Arg	Asn	Arg	Val	Arg	Glu	Ala	Ile	Arg	Val	
		275					280					285				
Ser	Lys	Ile	Pro	Asp	Ala	Lys	Ser	Pro	Leu	Pro	Val	Phe	Ala	Tyr	Thr	
	290					295					300					
Arg	Ile	Val	Phe	Thr	Asp	Gln	Val	Leu	Lys	Phe	Leu	Ser	Gln	Asp	Glu	
305					310					315					320	
Leu	Val	Tyr	Thr	Phe	Gly	Glu	Thr	Val	Ala	Leu	Gly	Ala	Ser	Gly	Ile	
				325					330					335		
Val	Ile	Trp	Gly	Thr	Leu	Ser	Ile	Met	Arg	Ser	Met	Lys	Ser	Cys	Leu	
			340					345					350			
Leu	Leu	Asp	Asn	Tyr	Met	Glu	Thr	Ile	Leu	Asn	Pro	Tyr	Ile	Ile	Asn	
		355				360						365				
Val	Thr	Leu	Ala	Ala	Lys	Met	Cys	Ser	Gln	Val	Leu	Cys	Gln	Glu	Gln	
	370					375					380					
Gly	Val	Cys	Ile	Arg	Lys	Asn	Trp	Asn	Ser	Ser	Asp	Tyr	Leu	His	Leu	
385					390					395					400	
Asn	Pro	Asp	Asn	Phe	Ala	Ile	Gln	Leu	Glu	Lys	Gly	Gly	Lys	Phe	Thr	
				405					410					415		
Val	Arg	Gly	Lys	Pro	Thr	Leu	Glu	Asp	Leu	Glu	Gln	Phe	Ser	Glu	Lys	
			420					425					430			
Phe	Tyr	Cys	Ser	Cys	Tyr	Ser	Thr	Leu	Ser	Cys	Lys	Glu	Lys	Ala	Asp	
		435					440					445				
Val	Lys	Asp	Thr	Asp	Ala	Val	Asp	Val	Cys	Ile	Ala	Asp	Gly	Val	Cys	
	450					455					460					
Ile	Asp	Ala	Phe	Leu	Lys											
465					470											

<210> 123

<211> 469

<212> PRT

<213> Homo sapiens

<220>

<223> HuPH20 precursor 1-469

<400> 123

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

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180 185 190
 Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
 195 200 205
 Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
 210 215 220
 Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
 225 230 235 240
 Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
 245 250 255
 Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
 260 265 270
 Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
 275 280 285
 Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
 290 295 300
 Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
 305 310 315 320
 Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
 325 330 335
 Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
 340 345 350
 Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
 355 360 365
 Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
 370 375 380
 Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
 385 390 395 400
 Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
 405 410 415
 Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
 420 425 430
 Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
 435 440 445
 Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
 450 455 460
 Ile Asp Ala Phe Leu
 465

<210> 124

<211> 468

<212> PRT

<213> Homo sapiens

<220>

<223> HuPH20 precursor 1-468

<400> 124

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

210		215		220	
Tyr Asn His His Tyr Lys	Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn				
225 Val Glu Ile Lys Arg Asn	Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser				
245 Thr Ala Leu Tyr Pro Ser	Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val				
260 Ala Ala Thr Leu Tyr Val	Arg Asn Arg Val Arg Glu Ala Ile Arg Val				
275 Ser Lys Ile Pro Asp Ala	Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr				
290 Arg Ile Val Phe Thr Asp	Gln Val Leu Lys Phe Leu Ser Gln Asp Glu				
305 Leu Val Tyr Thr Phe Gly	Glu Thr Val Ala Leu Gly Ala Ser Gly Ile				
325 Val Ile Trp Gly Thr Leu	Ser Ile Met Arg Ser Met Lys Ser Cys Leu				
340 Leu Leu Asp Asn Tyr Met	Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn				
355 Val Thr Leu Ala Ala Lys	Met Cys Ser Gln Val Leu Cys Gln Glu Gln				
370 Gly Val Cys Ile Arg Lys	Asn Trp Asn Ser Ser Asp Tyr Leu His Leu				
385 Asn Pro Asp Asn Phe Ala	Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr				
405 Val Arg Gly Lys Pro Thr	Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys				
420 Phe Tyr Cys Ser Cys Tyr	Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp				
435 Val Lys Asp Thr Asp Ala	Val Asp Val Cys Ile Ala Asp Gly Val Cys				
450 Ile Asp Ala Phe					
465					

<210> 125

<211> 466

<212> PRT

<213> Homo sapiens

<220>

<223> HuPH20 precursor 1-466

<400> 125

Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys	
1 Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys	
20 Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro	
35 Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe	
50 Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg	
65 Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu	
85 Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly	
100 Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys	
115 Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val	
130 Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro	
145 Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn	
165 Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe	
180 Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys	
195 Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys	
210 Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn	
225 Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser	

Thr	Ala	Leu	Tyr	245	Pro	Ser	Ile	Tyr	Leu	250	Asn	Thr	Gln	Gln	Ser	255	Pro	Val
Ala	Ala	Thr	260	Leu	Tyr	Val	Arg	Asn	265	Arg	Val	Arg	Glu	Ala	270	Ile	Arg	Val
Ser	Lys	275	Ile	Pro	Asp	Ala	Lys	280	Ser	Pro	Leu	Pro	Val	285	Phe	Ala	Tyr	Thr
Arg	305	Ile	Val	Phe	Thr	Asp	Gln	310	Val	Leu	Lys	Phe	Leu	315	Ser	Gln	Asp	Glu
Leu	Val	Tyr	Thr	325	Phe	Gly	Glu	Thr	330	Val	Ala	Leu	Gly	Ala	Ser	Gly	335	Ile
Val	Ile	Trp	Gly	340	Thr	Leu	Ser	Ile	345	Met	Arg	Ser	Met	Lys	Ser	Cys	Leu	
Leu	Leu	Asp	Asn	355	Tyr	Met	Glu	Thr	360	Ile	Leu	Asn	Pro	365	Tyr	Ile	Ile	Asn
Val	Thr	Leu	Ala	Ala	Lys	Met	Cys	Ser	375	Gln	Val	Leu	Cys	380	Gln	Glu	Gln	
Gly	385	Val	Cys	Ile	Arg	Lys	Asn	Trp	390	Asn	Ser	Ser	Asp	395	Tyr	Leu	His	Leu
Asn	Pro	Asp	Asn	Phe	Ala	Ile	Gln	Leu	405	Glu	Lys	Gly	Gly	410	Lys	Phe	Thr	
Val	Arg	Gly	Lys	420	Pro	Thr	Leu	Glu	425	Asp	Leu	Glu	Gln	430	Phe	Ser	Glu	Lys
Phe	Tyr	Cys	Ser	Cys	Tyr	Ser	Thr	440	Leu	Ser	Cys	Lys	Glu	445	Lys	Ala	Asp	
Val	Lys	Asp	Thr	Asp	Ala	Val	Asp	455	Val	Cys	Ile	Ala	Asp	460	Gly	Val	Cys	
Ile	Asp																	
465																		

<210> 126

<211> 465

<212> PRT

<213> Homo sapiens

<220>

<223> HuPH20 precursor 1-465

<400> 126

Met	Gly	Val	Leu	Lys	Phe	Lys	His	Ile	Phe	10	Phe	Arg	Ser	Phe	15	Val	Lys	
1	Ser	Ser	Gly	Val	5	Ser	Gln	Ile	Val	25	Phe	Thr	Phe	Leu	30	Ile	Pro	Cys
Cys	Leu	Thr	Leu	Asn	Phe	Arg	Ala	Pro	Pro	40	Val	Ile	Pro	45	Asn	Val	Pro	
Phe	Leu	Trp	Ala	Trp	Asn	Ala	Pro	Ser	Glu	55	Phe	Cys	Leu	60	Gly	Lys	Phe	
Asp	Glu	Pro	Leu	Asp	Met	Ser	Leu	Phe	Ser	70	Phe	Ile	Gly	Ser	Pro	Arg		
Ile	Asn	Ala	Thr	Gly	Gln	Gly	Val	Thr	Ile	90	Phe	Tyr	Val	Asp	Arg	Leu		
Gly	Tyr	Tyr	Pro	Tyr	Ile	Asp	Ser	Ile	Thr	105	Gly	Val	Thr	110	Val	Asn	Gly	
Gly	Ile	Pro	Gln	Lys	Ile	Ser	Leu	Gln	Asp	120	His	Leu	Asp	125	Lys	Ala	Lys	
Lys	Asp	Ile	Thr	Phe	Tyr	Met	Pro	Val	Asp	135	Asn	Leu	Gly	140	Met	Ala	Val	
Ile	Asp	Trp	Glu	Glu	Trp	Arg	Pro	Thr	Trp	150	Ala	Arg	Asn	155	Trp	Lys	Pro	
Lys	Asp	Val	Tyr	Lys	Asn	Arg	Ser	Ile	Glu	165	Leu	Val	Gln	170	Gln	Gln	Asn	
Val	Gln	Leu	Ser	Leu	Thr	Glu	Ala	Thr	Glu	185	Lys	Ala	Lys	190	Gln	Glu	Phe	
Glu	Lys	Ala	Gly	Lys	Asp	Phe	Leu	Val	Glu	200	Thr	Ile	Lys	205	Leu	Gly	Lys	
Leu	Leu	Arg	Pro	Asn	His	Leu	Trp	Gly	Tyr	215	Tyr	Leu	Phe	220	Pro	Asp	Cys	
Tyr	Asn	His	His	Tyr	Lys	Lys	Pro	Gly	Tyr	230	Asn	Gly	Ser	235	Cys	Phe	Asn	
Val	Glu	Ile	Lys	Arg	Asn	Asp	Asp	Leu	Ser	245	Trp	Leu	Trp	250	Asn	Glu	Ser	
Thr	Ala	Leu	Tyr	Pro	Ser	Ile	Tyr	Leu	Asn	265	Thr	Gln	Gln	270	Ser	Pro	Val	
Ala	Ala	Thr	Leu	Tyr	Val	Arg	Asn	Arg	Val		Arg	Glu	Ala		Ile	Arg	Val	

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275 280
 Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
 290 295 300
 Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
 305 310 315 320
 Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
 325 330 335
 Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
 340 345 350
 Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
 355 360 365
 Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
 370 375 380
 Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
 385 390 395 400
 Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
 405 410 415
 Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
 420 425 430
 Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
 435 440 445
 Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
 450 455 460
 Ile
 465

<210> 127
 <211> 464
 <212> PRT
 <213> Homo sapiens

<220>
 <223> HuPH20 precursor 1-464

<400> 127
 Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1 5 10 15
 Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
 20 25 30
 Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
 35 40 45
 Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
 50 55 60
 Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
 65 70 75 80
 Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
 85 90 95
 Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
 100 105 110
 Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
 115 120 125
 Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
 130 135 140
 Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
 145 150 155 160
 Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
 165 170 175
 Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
 180 185 190
 Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
 195 200 205
 Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
 210 215 220
 Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
 225 230 235 240
 Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
 245 250 255
 Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
 260 265 270
 Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
 275 280 285
 Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
 290 295 300
 Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu

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305	Leu	Val	Tyr	Thr	Phe	310	Gly	Glu	Thr	Val	Ala	315	Leu	Gly	Ala	Ser	Gly	320	Ile
					325						330						335		
	Val	Ile	Trp	Gly	Thr	Leu	Ser	Ile	Met	Arg	Ser	Met	Lys	Ser	Cys	Leu			
			340						345						350				
	Leu	Leu	Asp	Asn	Tyr	Met	Glu	Thr	Ile	Leu	Asn	Pro	Tyr	Ile	Ile	Asn			
			355						360					365					
	Val	Thr	Leu	Ala	Ala	Lys	Met	Cys	Ser	Gln	Val	Leu	Cys	Gln	Glu	Gln			
			370				375						380						
	Gly	Val	Cys	Ile	Arg	Lys	Asn	Trp	Asn	Ser	Ser	Asp	Tyr	Leu	His	Leu			
			385			390					395					400			
	Asn	Pro	Asp	Asn	Phe	Ala	Ile	Gln	Leu	Glu	Lys	Gly	Gly	Lys	Phe	Thr			
					405						410				415				
	Val	Arg	Gly	Lys	Pro	Thr	Leu	Glu	Asp	Leu	Glu	Gln	Phe	Ser	Glu	Lys			
				420					425					430					
	Phe	Tyr	Cys	Ser	Cys	Tyr	Ser	Thr	Leu	Ser	Cys	Lys	Glu	Lys	Ala	Asp			
			435					440					445						
	Val	Lys	Asp	Thr	Asp	Ala	Val	Asp	Val	Cys	Ile	Ala	Asp	Gly	Val	Cys			
			450			455						460							

<210> 128
 <211> 462
 <212> PRT
 <213> Homo sapiens

<220>
 <223> HuPH20 precursor 1-462

<400> 128	Met	Gly	Val	Leu	Lys	Phe	Lys	His	Ile	Phe	Phe	Arg	Ser	Phe	Val	Lys
1	Ser	Ser	Gly	Val	Ser	Gln	Ile	Val	Phe	Thr	Phe	Leu	Leu	Ile	Pro	Cys
			20						25					30		
	Cys	Leu	Thr	Leu	Asn	Phe	Arg	Ala	Pro	Pro	Val	Ile	Pro	Asn	Val	Pro
			35					40					45			
	Phe	Leu	Trp	Ala	Trp	Asn	Ala	Pro	Ser	Glu	Phe	Cys	Leu	Gly	Lys	Phe
			50			55					60					
	Asp	Glu	Pro	Leu	Asp	Met	Ser	Leu	Phe	Ser	Phe	Ile	Gly	Ser	Pro	Arg
					70				75						80	
	Ile	Asn	Ala	Thr	Gly	Gln	Gly	Val	Thr	Ile	Phe	Tyr	Val	Asp	Arg	Leu
					85				90					95		
	Gly	Tyr	Tyr	Pro	Tyr	Ile	Asp	Ser	Ile	Thr	Gly	Val	Thr	Val	Asn	Gly
			100						105					110		
	Gly	Ile	Pro	Gln	Lys	Ile	Ser	Leu	Gln	Asp	His	Leu	Asp	Lys	Ala	Lys
			115					120					125			
	Lys	Asp	Ile	Thr	Phe	Tyr	Met	Pro	Val	Asp	Asn	Leu	Gly	Met	Ala	Val
			130				135					140				
	Ile	Asp	Trp	Glu	Glu	Trp	Arg	Pro	Thr	Trp	Ala	Arg	Asn	Trp	Lys	Pro
					150						155				160	
	Lys	Asp	Val	Tyr	Lys	Asn	Arg	Ser	Ile	Glu	Leu	Val	Gln	Gln	Gln	Asn
					165					170					175	
	Val	Gln	Leu	Ser	Leu	Thr	Glu	Ala	Thr	Glu	Lys	Ala	Lys	Gln	Glu	Phe
					180				185					190		
	Glu	Lys	Ala	Gly	Lys	Asp	Phe	Leu	Val	Glu	Thr	Ile	Lys	Leu	Gly	Lys
			195					200					205			
	Leu	Leu	Arg	Pro	Asn	His	Leu	Trp	Gly	Tyr	Tyr	Leu	Phe	Pro	Asp	Cys
			210			215						220				
	Tyr	Asn	His	His	Tyr	Lys	Pro	Gly	Tyr	Asn	Gly	Ser	Cys	Phe	Asn	
					230					235					240	
	Val	Glu	Ile	Lys	Arg	Asn	Asp	Asp	Leu	Ser	Trp	Leu	Trp	Asn	Glu	Ser
					245				250					255		
	Thr	Ala	Leu	Tyr	Pro	Ser	Ile	Tyr	Leu	Asn	Thr	Gln	Gln	Ser	Pro	Val
					260				265					270		
	Ala	Ala	Thr	Leu	Tyr	Val	Arg	Asn	Arg	Val	Arg	Glu	Ala	Ile	Arg	Val
			275					280					285			
	Ser	Lys	Ile	Pro	Asp	Ala	Lys	Ser	Pro	Leu	Pro	Val	Phe	Ala	Tyr	Thr
					290		295					300				
	Arg	Ile	Val	Phe	Thr	Asp	Gln	Val	Leu	Lys	Phe	Leu	Ser	Gln	Asp	Glu
					310						315				320	
	Leu	Val	Tyr	Thr	Phe	Gly	Glu	Thr	Val	Ala	Leu	Gly	Ala	Ser	Gly	Ile
					325					330					335	
	Val	Ile	Trp	Gly	Thr	Leu	Ser	Ile	Met	Arg	Ser	Met	Lys	Ser	Cys	Leu
			340						345					350		
	Leu	Leu	Asp	Asn	Tyr	Met	Glu	Thr	Ile	Leu	Asn	Pro	Tyr	Ile	Ile	Asn


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      355      360      365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
      370      375      380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
      385      390      395
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
      405      410      415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
      420      425      430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
      435      440      445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly
      450      455      460

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<210> 129

<211> 460

<212> PRT

<213> Homo sapiens

<220>

<223> HuPH20 precursor 1-460

<400> 129

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Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
  1      5      10      15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
      20      25      30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
      35      40      45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
      50      55      60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
      65      70      75      80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
      85      90      95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
      100      105      110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
      115      120      125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
      130      135      140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
      145      150      155      160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
      165      170      175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
      180      185      190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
      195      200      205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
      210      215      220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
      225      230      235      240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
      245      250      255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
      260      265      270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
      275      280      285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
      290      295      300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
      305      310      315      320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
      325      330      335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
      340      345      350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
      355      360      365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
      370      375      380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
      385      390      395      400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr

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405
 Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
 410
 420
 Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
 430
 435
 Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala
 440
 445
 450
 455
 460

<210> 130
 <211> 458
 <212> PRT
 <213> Homo sapiens

<220>
 <223> HuPH20 precursor 1-458

<400> 130
 Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1 5 10 15
 Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
 20 25 30
 Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
 35 40 45
 Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
 50 55 60
 Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
 65 70 75 80
 Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
 85 90 95
 Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
 100 105 110
 Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
 115 120 125
 Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
 130 135 140
 Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
 145 150 155 160
 Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
 165 170 175
 Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
 180 185 190
 Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
 195 200 205
 Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
 210 215 220
 Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
 225 230 235 240
 Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
 245 250 255
 Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
 260 265 270
 Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
 275 280 285
 Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
 290 295 300
 Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
 305 310 315 320
 Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
 325 330 335
 Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
 340 345 350
 Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
 355 360 365
 Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
 370 375 380
 Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
 385 390 395 400
 Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
 405 410 415
 Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
 420 425 430
 Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
 435 440 445
 Val Lys Asp Thr Asp Ala Val Asp Val Cys

450

455

<210> 131
 <211> 456
 <212> PRT
 <213> Homo sapiens

<220>
 <223> HuPH20 precursor 1-456

<400> 131
 Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1 5 10 15
 Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
 20 25 30
 Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
 35 40 45
 Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
 50 55 60
 Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
 65 70 75 80
 Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
 85 90 95
 Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
 100 105 110
 Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
 115 120 125
 Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
 130 135 140
 Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
 145 150 155 160
 Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
 165 170 175
 Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
 180 185 190
 Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
 195 200 205
 Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
 210 215 220
 Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
 225 230 235 240
 Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
 245 250 255
 Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
 260 265 270
 Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
 275 280 285
 Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
 290 295 300
 Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
 305 310 315 320
 Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
 325 330 335
 Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
 340 345 350
 Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
 355 360 365
 Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
 370 375 380
 Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
 385 390 395 400
 Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
 405 410 415
 Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
 420 425 430
 Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
 435 440 445
 Val Lys Asp Thr Asp Ala Val Asp
 450 455

<210> 132
 <211> 454
 <212> PRT

<213> Homo sapiens

<220>

<223> HuPH20 precursor 1-454

<400> 132

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Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1      5      10      15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
      20      25      30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
      35      40      45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
      50      55      60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
      65      70      75      80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
      85      90      95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
      100      105      110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
      115      120      125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
      130      135      140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
      145      150      155      160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
      165      170      175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
      180      185      190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
      195      200      205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
      210      215      220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
      225      230      235      240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
      245      250      255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
      260      265      270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
      275      280      285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
      290      295      300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
      305      310      315      320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
      325      330      335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
      340      345      350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
      355      360      365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
      370      375      380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
      385      390      395      400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
      405      410      415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
      420      425      430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
      435      440      445
Val Lys Asp Thr Asp Ala
      450

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<210> 133

<211> 452

<212> PRT

<213> Homo sapiens

<220>

<223> HuPH20 precursor 1-452

<400> 133

3066SEQ PC1.TXT

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Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1      5      10      15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
 20      25      30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
 35      40      45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
 50      55      60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
 65      70      75      80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
 85      90      95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
100      105      110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
115      120      125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
130      135      140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145      150      155      160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
165      170      175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
180      185      190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
195      200      205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
210      215      220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225      230      235      240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
245      250      255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
260      265      270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
275      280      285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
290      295      300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305      310      315      320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
325      330      335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
340      345      350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
355      360      365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
370      375      380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385      390      395      400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
405      410      415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
420      425      430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
435      440      445
Val Lys Asp Thr
450

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<210> 134

<211> 450

<212> PRT

<213> Homo sapiens

<220>

<223> HuPH20 precursor 1-450

<400> 134

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

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3066SEQ PC1.TXT

Phe	Leu	Trp	Ala	Trp	Asn	Ala	Pro	Ser	Glu	Phe	Cys	Leu	Gly	Lys	Phe
50						55					60				
Asp	Glu	Pro	Leu	Asp	Met	Ser	Leu	Phe	Ser	Phe	Ile	Gly	Ser	Pro	Arg
65					70					75					80
Ile	Asn	Ala	Thr	Gly	Gln	Gly	Val	Thr	Ile	Phe	Tyr	Val	Asp	Arg	Leu
				85					90					95	
Gly	Tyr	Tyr	Pro	Tyr	Ile	Asp	Ser	Ile	Thr	Gly	Val	Thr	Val	Asn	Gly
			100					105					110		
Gly	Ile	Pro	Gln	Lys	Ile	Ser	Leu	Gln	Asp	His	Leu	Asp	Lys	Ala	Lys
		115					120					125			
Lys	Asp	Ile	Thr	Phe	Tyr	Met	Pro	Val	Asp	Asn	Leu	Gly	Met	Ala	Val
	130					135					140				
Ile	Asp	Trp	Glu	Glu	Trp	Arg	Pro	Thr	Trp	Ala	Arg	Asn	Trp	Lys	Pro
145					150					155					160
Lys	Asp	Val	Tyr	Lys	Asn	Arg	Ser	Ile	Glu	Leu	Val	Gln	Gln	Gln	Asn
				165					170					175	
Val	Gln	Leu	Ser	Leu	Thr	Glu	Ala	Thr	Glu	Lys	Ala	Lys	Gln	Glu	Phe
			180					185					190		
Glu	Lys	Ala	Gly	Lys	Asp	Phe	Leu	Val	Glu	Thr	Ile	Lys	Leu	Gly	Lys
		195					200					205			
Leu	Leu	Arg	Pro	Asn	His	Leu	Trp	Gly	Tyr	Tyr	Leu	Phe	Pro	Asp	Cys
	210					215					220				
Tyr	Asn	His	His	Tyr	Lys	Lys	Pro	Gly	Tyr	Asn	Gly	Ser	Cys	Phe	Asn
225					230					235					240
Val	Glu	Ile	Lys	Arg	Asn	Asp	Asp	Leu	Ser	Trp	Leu	Trp	Asn	Glu	Ser
				245					250					255	
Thr	Ala	Leu	Tyr	Pro	Ser	Ile	Tyr	Leu	Asn	Thr	Gln	Gln	Ser	Pro	Val
			260					265					270		
Ala	Ala	Thr	Leu	Tyr	Val	Arg	Asn	Arg	Val	Arg	Glu	Ala	Ile	Arg	Val
		275					280					285			
Ser	Lys	Ile	Pro	Asp	Ala	Lys	Ser	Pro	Leu	Pro	Val	Phe	Ala	Tyr	Thr
	290					295					300				
Arg	Ile	Val	Phe	Thr	Asp	Gln	Val	Leu	Lys	Phe	Leu	Ser	Gln	Asp	Glu
305					310					315					320
Leu	Val	Tyr	Thr	Phe	Gly	Glu	Thr	Val	Ala	Leu	Gly	Ala	Ser	Gly	Ile
				325					330					335	
Val	Ile	Trp	Gly	Thr	Leu	Ser	Ile	Met	Arg	Ser	Met	Lys	Ser	Cys	Leu
			340					345					350		
Leu	Leu	Asp	Asn	Tyr	Met	Glu	Thr	Ile	Leu	Asn	Pro	Tyr	Ile	Ile	Asn
		355					360					365			
Val	Thr	Leu	Ala	Ala	Lys	Met	Cys	Ser	Gln	Val	Leu	Cys	Gln	Glu	Gln
		370					375					380			
Gly	Val	Cys	Ile	Arg	Lys	Asn	Trp	Asn	Ser	Ser	Asp	Tyr	Leu	His	Leu
385					390					395					400
Asn	Pro	Asp	Asn	Phe	Ala	Ile	Gln	Leu	Glu	Lys	Gly	Gly	Lys	Phe	Thr
				405					410					415	
Val	Arg	Gly	Lys	Pro	Thr	Leu	Glu	Asp	Leu	Glu	Gln	Phe	Ser	Glu	Lys
			420					425					430		
Phe	Tyr	Cys	Ser	Cys	Tyr	Ser	Thr	Leu	Ser	Cys	Lys	Glu	Lys	Ala	Asp
		435					440					445			
Val	Lys														
	450														

<210> 135
 <211> 508
 <212> PRT
 <213> Homo sapiens

<220>
 <223> HuPH20 precursor 1-508

<400> 135

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

3066SEQ PC1.TXT

Gly	Tyr	Tyr	Pro	Tyr	Ile	Asp	Ser	Ile	Thr	Gly	Val	Thr	Val	Asn	Gly
			100					105						110	
Gly	Ile	Pro	Gln	Lys	Ile	Ser	Leu	Gln	Asp	His	Leu	Asp	Lys	Ala	Lys
		115					120					125			
Lys	Asp	Ile	Thr	Phe	Tyr	Met	Pro	Val	Asp	Asn	Leu	Gly	Met	Ala	Val
	130					135					140				
Ile	Asp	Trp	Glu	Glu	Trp	Arg	Pro	Thr	Trp	Ala	Arg	Asn	Trp	Lys	Pro
	145				150					155					160
Lys	Asp	Val	Tyr	Lys	Asn	Arg	Ser	Ile	Glu	Leu	Val	Gln	Gln	Gln	Asn
				165					170					175	
Val	Gln	Leu	Ser	Leu	Thr	Glu	Ala	Thr	Glu	Lys	Ala	Lys	Gln	Glu	Phe
			180					185					190		
Glu	Lys	Ala	Gly	Lys	Asp	Phe	Leu	Val	Glu	Thr	Ile	Lys	Leu	Gly	Lys
		195					200					205			
Leu	Leu	Arg	Pro	Asn	His	Leu	Trp	Gly	Tyr	Tyr	Leu	Phe	Pro	Asp	Cys
	210					215					220				
Tyr	Asn	His	His	Tyr	Lys	Lys	Pro	Gly	Tyr	Asn	Gly	Ser	Cys	Phe	Asn
	225				230					235					240
Val	Glu	Ile	Lys	Arg	Asn	Asp	Asp	Leu	Ser	Trp	Leu	Trp	Asn	Glu	Ser
				245					250					255	
Thr	Ala	Leu	Tyr	Pro	Ser	Ile	Tyr	Leu	Asn	Thr	Gln	Gln	Ser	Pro	Val
			260					265					270		
Ala	Ala	Thr	Leu	Tyr	Val	Arg	Asn	Arg	Val	Arg	Glu	Ala	Ile	Arg	Val
		275					280					285			
Ser	Lys	Ile	Pro	Asp	Ala	Lys	Ser	Pro	Leu	Pro	Val	Phe	Ala	Tyr	Thr
	290					295					300				
Arg	Ile	Val	Phe	Thr	Asp	Gln	Val	Leu	Lys	Phe	Leu	Ser	Gln	Asp	Glu
	305				310					315					320
Leu	Val	Tyr	Thr	Phe	Gly	Glu	Thr	Val	Ala	Leu	Gly	Ala	Ser	Gly	Ile
				325					330					335	
Val	Ile	Trp	Gly	Thr	Leu	Ser	Ile	Met	Arg	Ser	Met	Lys	Ser	Cys	Leu
			340					345					350		
Leu	Leu	Asp	Asn	Tyr	Met	Glu	Thr	Ile	Leu	Asn	Pro	Tyr	Ile	Ile	Asn
		355					360					365			
Val	Thr	Leu	Ala	Ala	Lys	Met	Cys	Ser	Gln	Val	Leu	Cys	Gln	Glu	Gln
		370				375						380			
Gly	Val	Cys	Ile	Arg	Lys	Asn	Trp	Asn	Ser	Ser	Asp	Tyr	Leu	His	Leu
	385				390					395					400
Asn	Pro	Asp	Asn	Phe	Ala	Ile	Gln	Leu	Glu	Lys	Gly	Gly	Lys	Phe	Thr
				405					410					415	
Val	Arg	Gly	Lys	Pro	Thr	Leu	Glu	Asp	Leu	Glu	Gln	Phe	Ser	Glu	Lys
			420					425					430		
Phe	Tyr	Cys	Ser	Cys	Tyr	Ser	Thr	Leu	Ser	Cys	Lys	Glu	Lys	Ala	Asp
		435					440					445			
Val	Lys	Asp	Thr	Asp	Ala	Val	Asp	Val	Cys	Ile	Ala	Asp	Gly	Val	Cys
	450					455					460				
Ile	Asp	Ala	Phe	Leu	Lys	Pro	Pro	Met	Glu	Thr	Glu	Glu	Pro	Gln	Ile
	465				470					475					480
Phe	Tyr	Asn	Ala	Ser	Pro	Ser	Thr	Leu	Ser	Ala	Thr	Met	Phe	Ile	Val
				485					490					495	
Ser	Ile	Leu	Phe	Leu	Ile	Ile	Ser	Ser	Val	Ala	Ser				
			500					505							

<210> 136
 <211> 506
 <212> PRT
 <213> Homo sapiens

<220>
 <223> HuPH20 precursor 1-506

<400> 136

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

3066SEQ PC1.TXT

Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
 100 105 110
 Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
 115 120 125
 Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
 130 135 140
 Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
 145 150 155
 Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
 165 170 175
 Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
 180 185 190
 Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
 195 200 205
 Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
 210 215 220
 Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
 225 230 235
 Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
 245 250 255
 Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
 260 265 270
 Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
 275 280 285
 Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
 290 295 300
 Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
 305 310 315
 Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
 325 330 335
 Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
 340 345 350
 Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
 355 360 365
 Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
 370 375 380
 Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
 385 390 395
 Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
 405 410 415
 Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
 420 425 430
 Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
 435 440 445
 Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
 450 455 460
 Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
 465 470 475 480
 Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
 485 490 495
 Ser Ile Leu Phe Leu Ile Ile Ser Ser Val
 500 505

<210> 137

<211> 504

<212> PRT

<213> Homo sapiens

<220>

<223> HuPH20 precursor 1-504

<400> 137

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

3066SEQ PC1.TXT

Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
 100 105 110
 Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
 115 120 125
 Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
 130 135 140
 Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
 145 150 155
 Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
 165 170 175
 Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
 180 185 190
 Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
 195 200 205
 Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
 210 215 220
 Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
 225 230 235
 Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
 245 250 255
 Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
 260 265 270
 Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
 275 280 285
 Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
 290 295 300
 Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
 305 310 315
 Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
 325 330 335
 Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
 340 345 350
 Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
 355 360 365
 Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
 370 375 380
 Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
 385 390 395
 Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
 405 410 415
 Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
 420 425 430
 Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
 435 440 445
 Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
 450 455 460
 Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
 465 470 475 480
 Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
 485 490 495
 Ser Ile Leu Phe Leu Ile Ile Ser
 500

<210> 138

<211> 502

<212> PRT

<213> Homo sapiens

<220>

<223> HuPH20 precursor 1-502

<400> 138

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

3066SEQ PC1.TXT

Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
 100 105 110
 Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
 115 120 125
 Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
 130 135 140
 Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
 145 150 155 160
 Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
 165 170 175
 Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
 180 185 190
 Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
 195 200 205
 Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
 210 215 220
 Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
 225 230 235 240
 Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
 245 250 255
 Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
 260 265 270
 Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
 275 280 285
 Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
 290 295 300
 Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
 305 310 315 320
 Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
 325 330 335
 Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
 340 345 350
 Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
 355 360 365
 Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
 370 375 380
 Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
 385 390 395 400
 Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
 405 410 415
 Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
 420 425 430
 Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
 435 440 445
 Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
 450 455 460
 Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
 465 470 475 480
 Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
 485 490 495
 Ser Ile Leu Phe Leu Ile
 500

<210> 139

<211> 500

<212> PRT

<213> Homo sapiens

<220>

<223> HuPH20 precursor 1-500

<400> 139

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

3066SEQ PC1.TXT

Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
 100 105 110
 Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
 115 120 125
 Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
 130 135 140
 Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
 145 150 155
 Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
 165 170 175
 Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
 180 185 190
 Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
 195 200 205
 Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
 210 215 220
 Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
 225 230 235
 Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
 245 250 255
 Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
 260 265 270
 Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
 275 280 285
 Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
 290 295 300
 Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
 305 310 315
 Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
 325 330 335
 Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
 340 345 350
 Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
 355 360 365
 Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
 370 375 380
 Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
 385 390 395
 Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
 405 410 415
 Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
 420 425 430
 Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
 435 440 445
 Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
 450 455 460
 Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
 465 470 475 480
 Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
 485 490 495
 Ser Ile Leu Phe
 500

<210> 140

<211> 498

<212> PRT

<213> Homo sapiens

<220>

<223> HuPH20 precursor 1-498

<400> 140

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

Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
 100 105 110
 Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
 115 120 125
 Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
 130 135 140
 Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
 145 150 155 160
 Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
 165 170 175
 Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
 180 185 190
 Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
 195 200 205
 Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
 210 215 220
 Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
 225 230 235 240
 Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
 245 250 255
 Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
 260 265 270
 Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
 275 280 285
 Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
 290 295 300
 Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
 305 310 315 320
 Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
 325 330 335
 Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
 340 345 350
 Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
 355 360 365
 Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
 370 375 380
 Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
 385 390 395 400
 Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
 405 410 415
 Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
 420 425 430
 Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
 435 440 445
 Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
 450 455 460
 Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
 465 470 475 480
 Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
 485 490 495
 Ser Ile

<210> 141

<211> 496

<212> PRT

<213> Homo sapiens

<220>

<223> HuPH20 precursor 1-496

<400> 141

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

3066SEQ PC1.TXT

Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
 100 105 110
 Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
 115 120 125
 Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
 130 135 140
 Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
 145 150 155
 Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
 160 165 170 175
 Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
 180 185 190
 Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
 195 200 205
 Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
 210 215 220
 Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
 225 230 235 240
 Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
 245 250 255
 Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
 260 265 270
 Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
 275 280 285
 Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
 290 295 300
 Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
 305 310 315 320
 Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
 325 330 335
 Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
 340 345 350
 Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
 355 360 365
 Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
 370 375 380
 Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
 385 390 395 400
 Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
 405 410 415
 Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
 420 425 430
 Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
 435 440 445
 Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
 450 455 460
 Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
 465 470 475 480
 Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
 485 490 495

<210> 142

<211> 494

<212> PRT

<213> Homo sapiens

<220>

<223> HuPH20 precursor 1-494

<400> 142

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

3066SEQ PC1.TXT

Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
 115 120 125
 Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
 130 135 140
 Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
 145 150 155 160
 Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
 165 170 175
 Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
 180 185 190
 Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
 195 200 205
 Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
 210 215 220
 Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
 225 230 235 240
 Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
 245 250 255
 Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
 260 265 270
 Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
 275 280 285
 Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
 290 295 300
 Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
 305 310 315 320
 Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
 325 330 335
 Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
 340 345 350
 Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
 355 360 365
 Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
 370 375 380
 Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
 385 390 395 400
 Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
 405 410 415
 Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
 420 425 430
 Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
 435 440 445
 Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
 450 455 460
 Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
 465 470 475 480
 Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe
 485 490

<210> 143
 <211> 492
 <212> PRT
 <213> Homo sapiens

<220>
 <223> HuPH20 precursor 1-492

<400> 143
 Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1 5 10 15
 Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
 20 25 30
 Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
 35 40 45
 Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
 50 55 60
 Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
 65 70 75 80
 Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
 85 90 95
 Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
 100 105 110
 Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
 115 120 125

Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
 130 135 140
 Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
 145 150 155 160
 Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
 165 170 175
 Val Gln Leu Ser Leu Thr Glu Ala Thr Lys Ala Lys Gln Glu Phe
 180 185 190
 Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
 195 200 205
 Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
 210 215 220
 Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
 225 230 235 240
 Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
 245 250 255
 Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
 260 265 270
 Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
 275 280 285
 Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
 290 295 300
 Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
 305 310 315 320
 Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
 325 330 335
 Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
 340 345 350
 Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
 355 360 365
 Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
 370 375 380
 Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
 385 390 395 400
 Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
 405 410 415
 Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
 420 425 430
 Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
 435 440 445
 Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
 450 455 460
 Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
 465 470 475 480
 Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr
 485 490

<210> 144
 <211> 488
 <212> PRT
 <213> Homo sapiens

<220>
 <223> HuPH20 precursor 1-488

<400> 144
 Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1 5 10 15
 Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
 20 25 30
 Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
 35 40 45
 Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
 50 55 60
 Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
 65 70 75 80
 Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
 85 90 95
 Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
 100 105 110
 Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
 115 120 125
 Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
 130 135 140

3066SEQ PC1.TXT

Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
 145 150 155 160
 Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
 165 170 175
 Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
 180 185 190
 Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
 195 200 205
 Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
 210 215 220
 Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
 225 230 235 240
 Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
 245 250 255
 Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
 260 265 270
 Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
 275 280 285
 Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
 290 295 300
 Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
 305 310 315 320
 Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
 325 330 335
 Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
 340 345 350
 Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
 355 360 365
 Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
 370 375 380
 Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
 385 390 395 400
 Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
 405 410 415
 Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
 420 425 430
 Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
 435 440 445
 Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
 450 455 460
 Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
 465 470 475 480
 Phe Tyr Asn Ala Ser Pro Ser Thr
 485

<210> 145

<211> 486

<212> PRT

<213> Homo sapiens

<220>

<223> HuPH20 precursor 1-486

<400> 145

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

3066SEQ PC1.TXT

Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Asn
165 170 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
180 185 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
195 200 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
210 215 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225 230 235 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
245 250 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
260 265 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
275 280 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
290 295 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305 310 315 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
325 330 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
340 345 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
355 360 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
370 375 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385 390 395 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
405 410 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
420 425 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
435 440 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
450 455 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465 470 475 480
Phe Tyr Asn Ala Ser Pro
485

<210> 146

<211> 472

<212> PRT

<213> Homo sapiens

<220>

<223> mature HuPH20 36-507

<400> 146

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

3066SEQ PC1.TXT

Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
 180 185 190
 His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
 195 200 205
 Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
 210 215 220
 Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
 225 230 235 240
 Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
 245 250 255
 Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
 260 265 270
 Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
 275 280 285
 Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
 290 295 300
 Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
 305 310 315 320
 Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
 325 330 335
 Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
 340 345 350
 Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
 355 360 365
 Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
 370 375 380
 Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
 385 390 395 400
 Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
 405 410 415
 Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
 420 425 430
 Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
 435 440 445
 Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val Ser Ile Leu
 450 455 460
 Phe Leu Ile Ile Ser Ser Val Ala
 465 470

<210> 147

<211> 470

<212> PRT

<213> Homo sapiens

<220>

<223> mature HuPH20 36-505

<400> 147

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

3066SEQ PC1.TXT

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

<210> 148

<211> 468

<212> PRT

<213> Homo sapiens

<220>

<223> mature HuPH20 36-503

<400> 148

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

3066SEQ PC1.TXT

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

<210> 149

<211> 466

<212> PRT

<213> Homo sapiens

<220>

<223> mature HuPH20 36-501

<400> 149

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

3066SEQ PC1.TXT

Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
 275 280 285
 Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
 290 295 300
 Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
 305 310 315 320
 Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
 325 330 335
 Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
 340 345 350
 Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
 355 360 365
 Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
 370 375 380
 Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
 385 390 395 400
 Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
 405 410 415
 Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
 420 425 430
 Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
 435 440 445
 Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val Ser Ile Leu
 450 455 460
 Phe Leu
 465

<210> 150
 <211> 464
 <212> PRT
 <213> Homo sapiens

<220>
 <223> mature HuPH20 36-499

<400> 150
 Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1 5 10 15
 Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
 20 25 30
 Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
 35 40 45
 Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
 50 55 60
 Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
 65 70 75 80
 Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
 85 90 95
 Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
 100 105 110
 Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
 115 120 125
 Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
 130 135 140
 Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
 145 150 155 160
 Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
 165 170 175
 Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
 180 185 190
 His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
 195 200 205
 Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
 210 215 220
 Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
 225 230 235 240
 Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
 245 250 255
 Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
 260 265 270
 Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
 275 280 285
 Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
 290 295 300

Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
 305 310 315 320
 Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
 325 330 335
 Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
 340 345 350
 Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
 355 360 365
 Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
 370 375 380
 Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
 385 390 395 400
 Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
 405 410 415
 Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
 420 425 430
 Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
 435 440 445
 Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val Ser Ile Leu
 450 455 460

<210> 151

<211> 462

<212> PRT

<213> Homo sapiens

<220>

<223> esHuPH20 36-497

<400> 151

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

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

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Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
      355      360      365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
      370      375      380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
      385      390      395      400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
      405      410      415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
      420      425      430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
      435      440      445
Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val Ser
      450      455      460

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 <213> Homo sapiens

<220>
 <223> esHuPH20 36-495

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Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
      20      25      30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
      35      40      45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
      50      55      60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
      65      70      75      80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
      85      90      95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
      100      105      110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
      115      120      125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
      130      135      140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
      145      150      155      160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
      165      170      175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
      180      185      190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
      195      200      205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
      210      215      220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
      225      230      235      240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
      245      250      255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
      260      265      270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
      275      280      285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
      290      295      300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
      305      310      315      320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
      325      330      335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
      340      345      350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
      355      360      365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
      370      375      380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
      385      390      395      400

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3066SEQ PC1.TXT

Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
 405 410 415
 Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
 420 425 430
 Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
 435 440 445
 Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile
 450 455 460

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 <212> PRT
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<220>
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<400> 153
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 Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
 20 25 30
 Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
 35 40 45
 Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
 50 55 60
 Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
 65 70 75 80
 Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
 85 90 95
 Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
 100 105 110
 Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
 115 120 125
 Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
 130 135 140
 Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
 145 150 155 160
 Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
 165 170 175
 Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
 180 185 190
 His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
 195 200 205
 Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
 210 215 220

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

Ala Ser Pro Ser Thr Leu Ser Ala Thr Met
450 455

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<212> PRT
<213> Homo sapiens

<220>
<223> esHuPH20 36-491

<400> 154
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
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Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
20 25 30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
35 40 45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
50 55 60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65 70 75 80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
85 90 95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
100 105 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
115 120 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
130 135 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145 150 155 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
165 170 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
180 185 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
195 200 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
210 215 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225 230 235 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
245 250 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
260 265 270

Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
275 280 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
290 295 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305 310 315 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
325 330 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
340 345 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
355 360 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
370 375 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385 390 395 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
405 410 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
420 425 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
435 440 445
Ala Ser Pro Ser Thr Leu Ser Ala
450 455

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<211> 454

<212> PRT
 <213> Homo sapiens

<220>
 <223> mature HuPH20 36-489

<400> 155
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 Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
 20 25 30
 Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
 35 40 45
 Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
 50 55 60
 Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
 65 70 75 80
 Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
 85 90 95
 Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
 100 105 110
 Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
 115 120 125
 Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
 130 135 140
 Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
 145 150 155 160
 Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
 165 170 175
 Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
 180 185 190
 His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
 195 200 205
 Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
 210 215 220
 Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
 225 230 235 240
 Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
 245 250 255
 Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
 260 265 270
 Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
 275 280 285
 Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
 290 295 300
 Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
 305 310 315 320
 Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
 325 330 335
 Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
 340 345 350
 Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
 355 360 365
 Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
 370 375 380
 Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
 385 390 395 400
 Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
 405 410 415
 Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
 420 425 430
 Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
 435 440 445
 Ala Ser Pro Ser Thr Leu
 450

<210> 156
 <211> 455
 <212> PRT
 <213> Homo sapiens

<220>
 <223> mature HuPH20 36-490

<400> 156

Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1 5 10 15
 Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
 20 25 30
 Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
 35 40 45
 Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
 50 55 60
 Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
 65 70 75 80
 Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
 85 90 95
 Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
 100 105 110
 Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
 115 120 125
 Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
 130 135 140
 Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
 145 150 155 160
 Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
 165 170 175
 Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
 180 185 190
 His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
 195 200 205
 Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
 210 215 220
 Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
 225 230 235 240
 Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
 245 250 255
 Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
 260 265 270
 Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
 275 280 285
 Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
 290 295 300
 Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
 305 310 315 320
 Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
 325 330 335
 Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
 340 345 350
 Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
 355 360 365

Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
 370 375 380
 Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
 385 390 395 400
 Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
 405 410 415
 Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
 420 425 430
 Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
 435 440 445
 Ala Ser Pro Ser Thr Leu Ser
 450 455

<210> 157

<211> 452

<212> PRT

<213> Homo sapiens

<220>

<223> mature HuPH20 36-487

<400> 157

Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
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 Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
 20 25 30
 Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala

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Thr	Gly	35	Gln	Gly	Val	Thr	Ile	40	Phe	Tyr	Val	Asp	Arg	45	Leu	Gly	Tyr	Tyr
Pro	Tyr	50	Ile	Asp	Ser	Ile	Thr	55	Gly	Val	Thr	Val	Asn	60	Gly	Gly	Ile	Pro
65	Gln	Lys	Ile	Ser	85	Gln	Asp	His	Leu	Asp	90	Lys	Ala	Lys	Lys	Asp	95	Ile
Thr	Phe	Tyr	Met	100	Pro	Val	Asp	Asn	105	Gly	Met	Ala	Val	110	Ile	Asp	Trp	
Glu	Glu	Trp	Arg	Pro	Thr	Trp	Ala	Arg	120	Asn	Trp	Lys	Pro	125	Lys	Asp	Val	
Tyr	Lys	Asn	Arg	Ser	Ile	Glu	Leu	Val	135	Gln	Gln	Gln	Asn	140	Val	Gln	Leu	
Ser	145	Leu	Thr	Glu	Ala	Thr	150	Glu	Lys	Ala	Lys	Gln	Glu	155	Phe	Glu	Lys	Ala
Gly	Lys	Asp	Phe	Leu	Val	Glu	Thr	Ile	165	Lys	Leu	Gly	Lys	170	Leu	Leu	Arg	
Pro	Asn	His	180	Trp	Gly	Tyr	Tyr	Leu	185	Phe	Pro	Asp	Cys	190	Tyr	Asn	His	
His	Tyr	Lys	195	Lys	Pro	Gly	Tyr	Asn	200	Gly	Ser	Cys	Phe	205	Asn	Val	Glu	Ile
Lys	Arg	Asn	210	Asp	Asp	Leu	Ser	Trp	215	Leu	Trp	Asn	Glu	220	Ser	Thr	Ala	Leu
Tyr	225	Pro	Ser	Ile	Tyr	Leu	Asn	Thr	230	Gln	Gln	Ser	Pro	235	Val	Ala	Ala	Thr
Leu	Tyr	Val	Arg	Asn	245	Arg	Val	Arg	250	Glu	Ala	Ile	Arg	255	Val	Ser	Lys	Ile
Pro	Asp	Ala	Lys	Ser	Pro	Leu	Pro	Val	260	Phe	Ala	Tyr	Thr	265	Arg	Ile	Val	
Phe	Thr	Asp	Gln	Val	Leu	Lys	Phe	Leu	270	Ser	Gln	Asp	Glu	275	Leu	Val	Tyr	
Thr	Phe	Gly	Glu	Thr	Val	Ala	280	Gly	Ala	Ser	Gly	Ile	Val	285	Ile	Trp		
Gly	305	Thr	Leu	Ser	Ile	Met	310	Arg	Ser	Met	Lys	Ser	Cys	315	Leu	Leu	Leu	Asp
Asn	Tyr	Met	Glu	Thr	Ile	Leu	Asn	Pro	320	Tyr	Ile	Ile	Asn	325	Val	Thr	Leu	
Ala	Ala	Lys	Met	Cys	Ser	Gln	Val	Leu	330	Cys	Gln	Glu	Gln	335	Gly	Val	Cys	
Ile	Arg	Lys	Asn	Trp	Asn	Ser	Ser	Asp	340	Tyr	Leu	His	Leu	345	Asn	Pro	Asp	
Asn	Phe	Ala	Ile	Gln	Leu	Glu	Lys	Gly	350	Gly	Lys	Phe	Thr	355	Val	Arg	Gly	
Lys	360	Pro	Thr	Leu	Glu	Asp	Leu	Glu	365	Gln	Phe	Ser	Glu	370	Lys	Phe	Tyr	Cys
Ser	Cys	Tyr	Ser	Thr	Leu	Ser	Cys	Lys	375	Glu	Lys	Ala	Asp	380	Val	Lys	Asp	
Thr	Asp	Ala	Val	Asp	Val	Cys	Ile	Ala	385	Asp	Gly	Val	Cys	390	Ile	Asp	Ala	
Phe	Leu	Lys	Pro	Pro	Met	Glu	Thr	Glu	400	Glu	Pro	Gln	Ile	405	Phe	Tyr	Asn	
Ala	Ser	Pro	Ser						410					415				
									420					425				
									430					435				
									440					445				
									450									

<210> 158

<211> 450

<212> PRT

<213> Homo sapiens

<220>

<223> Mature HuPH20 36-485

<400> 158

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

				85					3066SEQ	PC1.TXT			95		
Thr	Phe	Tyr	Met	Pro	Val	Asp	Asn	Leu	Gly	Met	Ala	Val	Ile	Asp	Trp
			100					105					110		
Glu	Glu	Trp	Arg	Pro	Thr	Trp	Ala	Arg	Asn	Trp	Lys	Pro	Lys	Asp	Val
			115				120					125			
Tyr	Lys	Asn	Arg	Ser	Ile	Glu	Leu	Val	Gln	Gln	Gln	Asn	Val	Gln	Leu
			130			135					140				
Ser	Leu	Thr	Glu	Ala	Thr	Glu	Lys	Ala	Lys	Gln	Glu	Phe	Glu	Lys	Ala
			145		150				155						160
Gly	Lys	Asp	Phe	Leu	Val	Glu	Thr	Ile	Lys	Leu	Gly	Lys	Leu	Leu	Arg
			165						170					175	
Pro	Asn	His	Leu	Trp	Gly	Tyr	Tyr	Leu	Phe	Pro	Asp	Cys	Tyr	Asn	His
			180					185					190		
His	Tyr	Lys	Lys	Pro	Gly	Tyr	Asn	Gly	Ser	Cys	Phe	Asn	Val	Glu	Ile
			195				200					205			
Lys	Arg	Asn	Asp	Asp	Leu	Ser	Trp	Leu	Trp	Asn	Glu	Ser	Thr	Ala	Leu
			210			215					220				
Tyr	Pro	Ser	Ile	Tyr	Leu	Asn	Thr	Gln	Gln	Ser	Pro	Val	Ala	Ala	Thr
			225		230					235					240
Leu	Tyr	Val	Arg	Asn	Arg	Val	Arg	Glu	Ala	Ile	Arg	Val	Ser	Lys	Ile
			245						250					255	
Pro	Asp	Ala	Lys	Ser	Pro	Leu	Pro	Val	Phe	Ala	Tyr	Thr	Arg	Ile	Val
			260					265					270		
Phe	Thr	Asp	Gln	Val	Leu	Lys	Phe	Leu	Ser	Gln	Asp	Glu	Leu	Val	Tyr
			275				280					285			
Thr	Phe	Gly	Glu	Thr	Val	Ala	Leu	Gly	Ala	Ser	Gly	Ile	Val	Ile	Trp
			290			295					300				
Gly	Thr	Leu	Ser	Ile	Met	Arg	Ser	Met	Lys	Ser	Cys	Leu	Leu	Leu	Asp
			305		310					315					320
Asn	Tyr	Met	Glu	Thr	Ile	Leu	Asn	Pro	Tyr	Ile	Ile	Asn	Val	Thr	Leu
			325						330					335	
Ala	Ala	Lys	Met	Cys	Ser	Gln	Val	Leu	Cys	Gln	Glu	Gln	Gly	Val	Cys
			340					345					350		
Ile	Arg	Lys	Asn	Trp	Asn	Ser	Ser	Asp	Tyr	Leu	His	Leu	Asn	Pro	Asp
			355				360					365			
Asn	Phe	Ala	Ile	Gln	Leu	Glu	Lys	Gly	Gly	Lys	Phe	Thr	Val	Arg	Gly
			370			375					380				
Lys	Pro	Thr	Leu	Glu	Asp	Leu	Glu	Gln	Phe	Ser	Glu	Lys	Phe	Tyr	Cys
			385		390					395				400	
Ser	Cys	Tyr	Ser	Thr	Leu	Ser	Cys	Lys	Glu	Lys	Ala	Asp	Val	Lys	Asp
			405						410					415	
Thr	Asp	Ala	Val	Asp	Val	Cys	Ile	Ala	Asp	Gly	Val	Cys	Ile	Asp	Ala
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<210> 159
<211> 449
<212> PRT
<213> Homo sapiens
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<220>
<223> Mature HuPH20 36-484

<400>	159															
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Ala	Trp	Asn	Ala	Pro	Ser	Glu	Phe	Cys	Leu	Gly	Lys	Phe	Asp	Glu	Pro	
			20					25					30			
Leu	Asp	Met	Ser	Leu	Phe	Ser	Phe	Ile	Gly	Ser	Pro	Arg	Ile	Asn	Ala	
		35					40					45				
Thr	Gly	Gln	Gly	Val	Thr	Ile	Phe	Tyr	Val	Asp	Arg	Leu	Gly	Tyr	Tyr	
	50					55					60					
Pro	Tyr	Ile	Asp	Ser	Ile	Thr	Gly	Val	Thr	Val	Asn	Gly	Gly	Ile	Pro	
65					70					75					80	
Gln	Lys	Ile	Ser	Leu	Gln	Asp	His	Leu	Asp	Lys	Ala	Lys	Lys	Asp	Ile	
				85					90					95		
Thr	Phe	Tyr	Met	Pro	Val	Asp	Asn	Leu	Gly	Met	Ala	Val	Ile	Asp	Trp	
			100					105					110			
Glu	Glu	Trp	Arg	Pro	Thr	Trp	Ala	Arg	Asn	Trp	Lys	Pro	Lys	Asp	Val	
		115					120					125				
Tyr	Lys	Asn	Arg	Ser	Ile	Glu	Leu	Val	Gln	Gln	Gln	Asn	Val	Gln	Leu	

3066SEQ PC1.TXT

130 135 140
 Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
 145 150 155 160
 Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
 165 170 175
 Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
 180 185 190
 His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
 195 200 205
 Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
 210 215 220
 Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
 225 230 235 240
 Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
 245 250 255
 Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
 260 265 270
 Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
 275 280 285
 Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
 290 295 300
 Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
 305 310 315 320
 Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
 325 330 335
 Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
 340 345 350
 Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
 355 360 365
 Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
 370 375 380
 Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
 385 390 395 400
 Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
 405 410 415
 Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
 420 425 430
 Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
 435 440 445
 Ala

<210> 160

<211> 441

<212> PRT

<213> Homo sapiens

<220>

<223> Mature HuPH20 36-476

<400> 160

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

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

<210> 161

<211> 440

<212> PRT

<213> Homo sapiens

<220>

<223> Mature HuPH20 36-475

<400> 161

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

3066SEQ PC1.TXT

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

<210> 162
 <211> 439
 <212> PRT
 <213> Homo sapiens

<220>
 <223> Mature HuPH20 36-474

<400> 162

Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1 5 10 15
 Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
 20 25 30
 Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
 35 40 45
 Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
 50 55 60
 Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
 65 70 75 80
 Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
 85 90 95
 Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
 100 105 110
 Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
 115 120 125
 Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
 130 135 140
 Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
 145 150 155 160
 Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
 165 170 175
 Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
 180 185 190
 His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
 195 200 205
 Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
 210 215 220
 Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
 225 230 235 240
 Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
 245 250 255
 Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
 260 265 270
 Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
 275 280 285
 Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
 290 295 300
 Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp

3066SEQ PC1.TXT

305 310 315 320
 Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
 325 330 335
 Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
 340 345 350
 Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
 355 360 365
 Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
 370 375 380
 Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
 385 390 395 400
 Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
 405 410 415
 Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
 420 425 430
 Phe Leu Lys Pro Pro Met Glu
 435

<210> 163
 <211> 438
 <212> PRT
 <213> Homo sapiens

<220>
 <223> Mature HuPH20 36-473

<400> 163
 Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1 5 10 15
 Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
 20 25 30
 Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
 35 40 45
 Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
 50 55 60
 Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
 65 70 75 80
 Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
 85 90 95
 Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
 100 105 110
 Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
 115 120 125
 Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
 130 135 140
 Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
 145 150 155 160
 Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
 165 170 175
 Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
 180 185 190
 His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
 195 200 205
 Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
 210 215 220
 Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
 225 230 235 240
 Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
 245 250 255
 Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
 260 265 270
 Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
 275 280 285
 Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
 290 295 300
 Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
 305 310 315 320
 Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
 325 330 335
 Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
 340 345 350
 Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
 355 360 365
 Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly

370 375 380
 Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
 385 390 395 400
 Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
 405 410 415
 Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
 420 425 430
 Phe Leu Lys Pro Pro Met
 435

<210> 164
 <211> 437
 <212> PRT
 <213> Homo sapiens

<220>
 <223> Mature HuPH20 36-472

<400> 164
 Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1 5 10 15
 Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
 20 25 30
 Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
 35 40 45
 Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
 50 55 60
 Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
 65 70 75 80
 Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
 85 90 95
 Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
 100 105 110
 Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
 115 120 125
 Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
 130 135 140
 Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
 145 150 155 160
 Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
 165 170 175
 Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
 180 185 190
 His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
 195 200 205
 Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
 210 215 220
 Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
 225 230 235 240
 Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
 245 250 255
 Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
 260 265 270
 Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
 275 280 285
 Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
 290 295 300
 Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
 305 310 315 320
 Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
 325 330 335
 Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
 340 345 350
 Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
 355 360 365
 Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
 370 375 380
 Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
 385 390 395 400
 Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
 405 410 415
 Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
 420 425 430
 Phe Leu Lys Pro Pro

435

<210> 165
 <211> 436
 <212> PRT
 <213> Homo sapiens

<220>
 <223> Mature HuPH20 36-471

<400> 165
 Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1 5 10 15
 Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
 20 25 30
 Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
 35 40 45
 Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
 50 55 60
 Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
 65 70 75 80
 Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
 85 90 95
 Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
 100 105 110
 Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
 115 120 125
 Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
 130 135 140
 Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
 145 150 155 160
 Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
 165 170 175
 Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
 180 185 190
 His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
 195 200 205
 Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
 210 215 220
 Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
 225 230 235 240
 Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
 245 250 255
 Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
 260 265 270
 Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
 275 280 285
 Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
 290 295 300
 Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
 305 310 315 320
 Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
 325 330 335
 Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
 340 345 350
 Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
 355 360 365
 Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
 370 375 380
 Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
 385 390 395 400
 Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
 405 410 415
 Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
 420 425 430
 Phe Leu Lys Pro
 435

<210> 166
 <211> 435
 <212> PRT
 <213> Homo sapiens

<220>

<223> Mature HuPH20 36-470

<400> 166

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Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1      5      10      15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
 20      25      30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
 35      40      45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
 50      55      60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
 65      70      75      80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
 85      90      95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
100      105      110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
115      120      125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
130      135      140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145      150      155      160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
165      170      175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
180      185      190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
195      200      205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
210      215      220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225      230      235      240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
245      250      255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
260      265      270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
275      280      285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
290      295      300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305      310      315      320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
325      330      335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
340      345      350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
355      360      365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
370      375      380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385      390      395      400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
405      410      415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
420      425      430
Phe Leu Lys
435

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<210> 167

<211> 434

<212> PRT

<213> Homo sapiens

<220>

<223> Mature HuPH20 36-469

<400> 167

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Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1      5      10      15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
 20      25      30

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3066SEQ PC1.TXT

Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
 35 40 45
 Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
 50 55 60
 Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
 65 70 75 80
 Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
 85 90 95
 Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
 100 105 110
 Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
 115 120 125
 Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
 130 135 140
 Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
 145 150 155 160
 Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
 165 170 175
 Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
 180 185 190
 His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
 195 200 205
 Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
 210 215 220
 Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
 225 230 235 240
 Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
 245 250 255
 Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
 260 265 270
 Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
 275 280 285
 Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
 290 295 300
 Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
 305 310 315 320
 Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
 325 330 335
 Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
 340 345 350
 Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
 355 360 365
 Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
 370 375 380
 Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
 385 390 395 400
 Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
 405 410 415
 Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
 420 425 430
 Phe Leu

<210> 168

<211> 433

<212> PRT

<213> Homo sapiens

<220>

<223> Mature HuPH20 36-468

<400> 168

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

Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
 100 105 110
 Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
 115 120 125
 Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
 130 135 140
 Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
 145 150 155 160
 Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
 165 170 175
 Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
 180 185 190
 His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
 195 200 205
 Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
 210 215 220
 Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
 225 230 235 240
 Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
 245 250 255
 Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
 260 265 270
 Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
 275 280 285
 Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
 290 295 300
 Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
 305 310 315 320
 Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
 325 330 335
 Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
 340 345 350
 Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
 355 360 365
 Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
 370 375 380
 Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
 385 390 395 400
 Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
 405 410 415
 Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
 420 425 430
 Phe

<210> 169

<211> 431

<212> PRT

<213> Homo sapiens

<220>

<223> Mature HuPH20 36-466

<400> 169

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

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Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
 165 170 175
 Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
 180 185 190
 His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
 195 200 205
 Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
 210 215 220
 Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
 225 230 235 240
 Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
 245 250 255
 Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
 260 265 270
 Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
 275 280 285
 Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
 290 295 300
 Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
 305 310 315 320
 Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
 325 330 335
 Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
 340 345 350
 Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
 355 360 365
 Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
 370 375 380
 Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
 385 390 395 400
 Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
 405 410 415
 Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp
 420 425 430

<210> 170

<211> 430

<212> PRT

<213> Homo sapiens

<220>

<223> Mature HuPH20 36-465

<400> 170

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

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Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
 245 250 255
 Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
 260 265 270
 Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
 275 280 285
 Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
 290 295 300
 Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
 305 310 315 320
 Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
 325 330 335
 Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
 340 345 350
 Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
 355 360 365
 Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
 370 375 380
 Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
 385 390 395 400
 Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
 405 410 415
 Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile
 420 425 430

<210> 171

<211> 429

<212> PRT

<213> Homo sapiens

<220>

<223> Mature HuPH20 36-464

<400> 171

Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1 5 10 15
 Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
 20 25 30
 Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
 35 40 45
 Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
 50 55 60
 Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
 65 70 75 80
 Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
 85 90 95
 Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
 100 105 110
 Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
 115 120 125
 Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
 130 135 140
 Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
 145 150 155 160
 Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
 165 170 175
 Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
 180 185 190
 His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
 195 200 205
 Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
 210 215 220
 Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
 225 230 235 240
 Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
 245 250 255
 Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
 260 265 270
 Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
 275 280 285
 Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
 290 295 300
 Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
 305 310 315 320

Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
 325 330 335
 Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
 340 345 350
 Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
 355 360 365
 Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
 370 375 380
 Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
 385 390 395 400
 Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
 405 410 415
 Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
 420 425

<210> 172

<211> 427

<212> PRT

<213> Homo sapiens

<220>

<223> Mature HuPH20 36-462

<400> 172

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

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

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Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
 405 410 415
 Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly
 420 425

<210> 173
 <211> 425
 <212> PRT
 <213> Homo sapiens

<220>
 <223> Mature HuPH20 36-460

<400> 173
 Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
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 Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
 20 25 30
 Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
 35 40 45
 Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
 50 55 60
 Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
 65 70 75 80
 Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
 85 90 95
 Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
 100 105 110
 Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
 115 120 125
 Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
 130 135 140
 Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
 145 150 155 160
 Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
 165 170 175
 Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
 180 185 190
 His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
 195 200 205
 Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
 210 215 220
 Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
 225 230 235 240
 Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
 245 250 255

Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
 260 265 270
 Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
 275 280 285
 Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
 290 295 300
 Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
 305 310 315 320
 Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
 325 330 335
 Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
 340 345 350
 Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
 355 360 365
 Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
 370 375 380
 Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
 385 390 395 400
 Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
 405 410 415
 Thr Asp Ala Val Asp Val Cys Ile Ala
 420 425

<210> 174
 <211> 423
 <212> PRT
 <213> Homo sapiens

<220>

<223> Mature HuPH20 36-458

<400> 174

Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1 5 10 15
 Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
 20 25 30
 Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
 35 40 45
 Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
 50 55 60
 Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
 65 70 75 80
 Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
 85 90 95
 Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
 100 105 110
 Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
 115 120 125
 Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
 130 135 140
 Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
 145 150 155 160
 Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
 165 170 175
 Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
 180 185 190
 His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
 195 200 205
 Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
 210 215 220
 Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
 225 230 235 240
 Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
 245 250 255
 Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
 260 265 270
 Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
 275 280 285
 Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
 290 295 300
 Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
 305 310 315 320
 Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
 325 330 335
 Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
 340 345 350
 Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
 355 360 365
 Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
 370 375 380
 Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
 385 390 395 400
 Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
 405 410 415
 Thr Asp Ala Val Asp Val Cys
 420

<210> 175

<211> 421

<212> PRT

<213> Homo sapiens

<220>

<223> Mature HuPH20 36-456

<400> 175

Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
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 Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
 20 25 30
 Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala

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Thr	Gly	Gln	Gly	Val	Thr	Ile	Phe	Tyr	Val	Asp	Arg	Leu	Gly	Tyr	Tyr
50		35				40					45				
Pro	Tyr	Ile	Asp	Ser	Ile	Thr	Gly	Val	Thr	Val	Asn	Gly	Gly	Ile	Pro
65					70					75					80
Gln	Lys	Ile	Ser	Leu	Gln	Asp	His	Leu	Asp	Lys	Ala	Lys	Lys	Asp	Ile
				85					90					95	
Thr	Phe	Tyr	Met	Pro	Val	Asp	Asn	Leu	Gly	Met	Ala	Val	Ile	Asp	Trp
			100					105					110		
Glu	Glu	Trp	Arg	Pro	Thr	Trp	Ala	Arg	Asn	Trp	Lys	Pro	Lys	Asp	Val
		115					120					125			
Tyr	Lys	Asn	Arg	Ser	Ile	Glu	Leu	Val	Gln	Gln	Gln	Asn	Val	Gln	Leu
	130					135					140				
Ser	Leu	Thr	Glu	Ala	Thr	Glu	Lys	Ala	Lys	Gln	Glu	Phe	Glu	Lys	Ala
145					150					155					160
Gly	Lys	Asp	Phe	Leu	Val	Glu	Thr	Ile	Lys	Leu	Gly	Lys	Leu	Leu	Arg
				165					170					175	
Pro	Asn	His	Leu	Trp	Gly	Tyr	Tyr	Leu	Phe	Pro	Asp	Cys	Tyr	Asn	His
			180					185					190		
His	Tyr	Lys	Lys	Pro	Gly	Tyr	Asn	Gly	Ser	Cys	Phe	Asn	Val	Glu	Ile
		195					200					205			
Lys	Arg	Asn	Asp	Asp	Leu	Ser	Trp	Leu	Trp	Asn	Glu	Ser	Thr	Ala	Leu
	210					215					220				
Tyr	Pro	Ser	Ile	Tyr	Leu	Asn	Thr	Gln	Gln	Ser	Pro	Val	Ala	Ala	Thr
225					230					235					240
Leu	Tyr	Val	Arg	Asn	Arg	Val	Arg	Glu	Ala	Ile	Arg	Val	Ser	Lys	Ile
				245					250					255	
Pro	Asp	Ala	Lys	Ser	Pro	Leu	Pro	Val	Phe	Ala	Tyr	Thr	Arg	Ile	Val
			260					265					270		
Phe	Thr	Asp	Gln	Val	Leu	Lys	Phe	Leu	Ser	Gln	Asp	Glu	Leu	Val	Tyr
		275					280					285			
Thr	Phe	Gly	Glu	Thr	Val	Ala	Leu	Gly	Ala	Ser	Gly	Ile	Val	Ile	Trp
	290				295						300				
Gly	Thr	Leu	Ser	Ile	Met	Arg	Ser	Met	Lys	Ser	Cys	Leu	Leu	Leu	Asp
305					310					315					320
Asn	Tyr	Met	Glu	Thr	Ile	Leu	Asn	Pro	Tyr	Ile	Ile	Asn	Val	Thr	Leu
				325					330					335	
Ala	Ala	Lys	Met	Cys	Ser	Gln	Val	Leu	Cys	Gln	Glu	Gln	Gly	Val	Cys
			340					345					350		
Ile	Arg	Lys	Asn	Trp	Asn	Ser	Ser	Asp	Tyr	Leu	His	Leu	Asn	Pro	Asp
		355					360					365			
Asn	Phe	Ala	Ile	Gln	Leu	Glu	Lys	Gly	Gly	Lys	Phe	Thr	Val	Arg	Gly
	370					375					380				
Lys	Pro	Thr	Leu	Glu	Asp	Leu	Glu	Gln	Phe	Ser	Glu	Lys	Phe	Tyr	Cys
385					390					395					400
Ser	Cys	Tyr	Ser	Thr	Leu	Ser	Cys	Lys	Glu	Lys	Ala	Asp	Val	Lys	Asp
				405					410					415	
Thr	Asp	Ala	Val	Asp											
			420												

<210> 176

<211> 419

<212> PRT

<213> Homo sapiens

<220>

<223> Mature HuPH20 36-454

<400> 176

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

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      115      120      125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
130      135      140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145      150      155      160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
165      170      175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
180      185      190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
195      200      205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
210      215      220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225      230      235
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
245      250      255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
260      265      270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
275      280      285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
290      295      300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305      310      315      320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
325      330      335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
340      345      350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
355      360      365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
370      375      380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385      390      395      400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
405      410      415
Thr Asp Ala

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<210> 177
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<220>

<223> Mature HuPH20 36-452

<400> 177

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

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3066SEQ PC1.TXT

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

<210> 178

<211> 415

<212> PRT

<213> Homo sapiens

<220>

<223> Mature HuPH20 36-450

<400> 178

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

3066SEQ PC1.TXT

Thr	Phe	275	Gly	Glu	Thr	Val	Ala	280	Leu	Gly	Ala	Ser	Gly	285	Ile	Val	Ile	Trp
	290						295						300					
Gly	Thr	Leu	Ser	Ile	Met	Arg	Ser	Met	Lys	Ser	Cys	Leu	Leu	Leu	Asp			
305				310						315					320			
Asn	Tyr	Met	Glu	Thr	Ile	Leu	Asn	Pro	Tyr	Ile	Ile	Asn	Val	Thr	Leu			
			325						330					335				
Ala	Ala	Lys	Met	Cys	Ser	Gln	Val	Leu	Cys	Gln	Glu	Gln	Gly	Val	Cys			
			340					345					350					
Ile	Arg	Lys	Asn	Trp	Asn	Ser	Ser	Asp	Tyr	Leu	His	Leu	Asn	Pro	Asp			
		355					360					365						
Asn	Phe	Ala	Ile	Gln	Leu	Glu	Lys	Gly	Gly	Lys	Phe	Thr	Val	Arg	Gly			
	370					375					380							
Lys	Pro	Thr	Leu	Glu	Asp	Leu	Glu	Gln	Phe	Ser	Glu	Lys	Phe	Tyr	Cys			
385					390					395					400			
Ser	Cys	Tyr	Ser	Thr	Leu	Ser	Cys	Lys	Glu	Lys	Ala	Asp	Val	Lys				
				405					410					415				

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<211> 473

<212> PRT

<213> Homo sapiens

<220>

<223> Mature HuPH20 36-508

<400> 179

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			20					25					30		
Leu	Asp	Met	Ser	Leu	Phe	Ser	Phe	Ile	Gly	Ser	Pro	Arg	Ile	Asn	Ala
		35					40					45			
Thr	Gly	Gln	Gly	Val	Thr	Ile	Phe	Tyr	Val	Asp	Arg	Leu	Gly	Tyr	Tyr
	50				55					60					
Pro	Tyr	Ile	Asp	Ser	Ile	Thr	Gly	Val	Thr	Val	Asn	Gly	Gly	Ile	Pro
65					70				75						80
Gln	Lys	Ile	Ser	Leu	Gln	Asp	His	Leu	Asp	Lys	Ala	Lys	Lys	Asp	Ile
			85					90						95	
Thr	Phe	Tyr	Met	Pro	Val	Asp	Asn	Leu	Gly	Met	Ala	Val	Ile	Asp	Trp
			100					105					110		
Glu	Glu	Trp	Arg	Pro	Thr	Trp	Ala	Arg	Asn	Trp	Lys	Pro	Lys	Asp	Val
		115					120					125			
Tyr	Lys	Asn	Arg	Ser	Ile	Glu	Leu	Val	Gln	Gln	Gln	Asn	Val	Gln	Leu
	130					135					140				
Ser	Leu	Thr	Glu	Ala	Thr	Glu	Lys	Ala	Lys	Gln	Glu	Phe	Glu	Lys	Ala
145					150					155					160
Gly	Lys	Asp	Phe	Leu	Val	Glu	Thr	Ile	Lys	Leu	Gly	Lys	Leu	Leu	Arg
				165					170					175	
Pro	Asn	His	Leu	Trp	Gly	Tyr	Tyr	Leu	Phe	Pro	Asp	Cys	Tyr	Asn	His
			180					185					190		
His	Tyr	Lys	Lys	Pro	Gly	Tyr	Asn	Gly	Ser	Cys	Phe	Asn	Val	Glu	Ile
		195					200					205			
Lys	Arg	Asn	Asp	Asp	Leu	Ser	Trp	Leu	Trp	Asn	Glu	Ser	Thr	Ala	Leu
	210					215					220				
Tyr	Pro	Ser	Ile	Tyr	Leu	Asn	Thr	Gln	Gln	Ser	Pro	Val	Ala	Ala	Thr
225					230					235					240
Leu	Tyr	Val	Arg	Asn	Arg	Val	Arg	Glu	Ala	Ile	Arg	Val	Ser	Lys	Ile
				245					250					255	
Pro	Asp	Ala	Lys	Ser	Pro	Leu	Pro	Val	Phe	Ala	Tyr	Thr	Arg	Ile	Val
			260					265					270		
Phe	Thr	Asp	Gln	Val	Leu	Lys	Phe	Leu	Ser	Gln	Asp	Glu	Leu	Val	Tyr
		275					280					285			
Thr	Phe	Gly	Glu	Thr	Val	Ala	Leu	Gly	Ala	Ser	Gly	Ile	Val	Ile	Trp
	290					295					300				
Gly	Thr	Leu	Ser	Ile	Met	Arg	Ser	Met	Lys	Ser	Cys	Leu	Leu	Leu	Asp
305				310						315					320
Asn	Tyr	Met	Glu	Thr	Ile	Leu	Asn	Pro	Tyr	Ile	Ile	Asn	Val	Thr	Leu
			325						330					335	
Ala	Ala	Lys	Met	Cys	Ser	Gln	Val	Leu	Cys	Gln	Glu	Gln	Gly	Val	Cys
			340					345					350		
Ile	Arg	Lys	Asn	Trp	Asn	Ser	Ser	Asp	Tyr	Leu	His	Leu	Asn	Pro	Asp
		355				360						365			
Asn	Phe	Ala	Ile	Gln	Leu	Glu	Lys	Gly	Gly	Lys	Phe	Thr	Val	Arg	Gly

370	375	380															
Lys	Pro	Thr	Leu	Glu	Asp	Leu	Glu	Gln	Phe	Ser	Glu	Lys	Phe	Tyr	Cys		
385					390					395					400		
Ser	Cys	Tyr	Ser	Thr	Leu	Ser	Cys	Lys	Glu	Lys	Ala	Asp	Val	Lys	Asp		
				405					410					415			
Thr	Asp	Ala	Val	Asp	Val	Cys	Ile	Ala	Asp	Gly	Val	Cys	Ile	Asp	Ala		
			420					425					430				
Phe	Leu	Lys	Pro	Pro	Met	Glu	Thr	Glu	Glu	Pro	Gln	Ile	Phe	Tyr	Asn		
		435					440					445					
Ala	Ser	Pro	Ser	Thr	Leu	Ser	Ala	Thr	Met	Phe	Ile	Val	Ser	Ile	Leu		
	450					455					460						
Phe	Leu	Ile	Ile	Ser	Ser	Val	Ala	Ser									
465					470												

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<211> 471

<212> PRT

<213> Homo sapiens

<220>

<223> Mature HuPH20 36-506

<400> 180

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Ala	Trp	Asn	Ala	Pro	Ser	Glu	Phe	Cys	Leu	Gly	Lys	Phe	Asp	Glu	Pro		
			20					25					30				
Leu	Asp	Met	Ser	Leu	Phe	Ser	Phe	Ile	Gly	Ser	Pro	Arg	Ile	Asn	Ala		
		35					40					45					
Thr	Gly	Gln	Gly	Val	Thr	Ile	Phe	Tyr	Val	Asp	Arg	Leu	Gly	Tyr	Tyr		
	50					55					60						
Pro	Tyr	Ile	Asp	Ser	Ile	Thr	Gly	Val	Thr	Val	Asn	Gly	Gly	Ile	Pro		
65					70				75					80			
Gln	Lys	Ile	Ser	Leu	Gln	Asp	His	Leu	Asp	Lys	Ala	Lys	Lys	Asp	Ile		
			85						90					95			
Thr	Phe	Tyr	Met	Pro	Val	Asp	Asn	Leu	Gly	Met	Ala	Val	Ile	Asp	Trp		
			100					105					110				
Glu	Glu	Trp	Arg	Pro	Thr	Trp	Ala	Arg	Asn	Trp	Lys	Pro	Lys	Asp	Val		
		115					120					125					
Tyr	Lys	Asn	Arg	Ser	Ile	Glu	Leu	Val	Gln	Gln	Gln	Asn	Val	Gln	Leu		
	130					135					140						
Ser	Leu	Thr	Glu	Ala	Thr	Glu	Lys	Ala	Lys	Gln	Glu	Phe	Glu	Lys	Ala		
145					150					155					160		
Gly	Lys	Asp	Phe	Leu	Val	Glu	Thr	Ile	Lys	Leu	Gly	Lys	Leu	Leu	Arg		
			165						170					175			
Pro	Asn	His	Leu	Trp	Gly	Tyr	Tyr	Leu	Phe	Pro	Asp	Cys	Tyr	Asn	His		
			180					185					190				
His	Tyr	Lys	Lys	Pro	Gly	Tyr	Asn	Gly	Ser	Cys	Phe	Asn	Val	Glu	Ile		
		195					200					205					
Lys	Arg	Asn	Asp	Asp	Leu	Ser	Trp	Leu	Trp	Asn	Glu	Ser	Thr	Ala	Leu		
	210					215					220						
Tyr	Pro	Ser	Ile	Tyr	Leu	Asn	Thr	Gln	Gln	Ser	Pro	Val	Ala	Ala	Thr		
225					230					235					240		
Leu	Tyr	Val	Arg	Asn	Arg	Val	Arg	Glu	Ala	Ile	Arg	Val	Ser	Lys	Ile		
			245						250					255			
Pro	Asp	Ala	Lys	Ser	Pro	Leu	Pro	Val	Phe	Ala	Tyr	Thr	Arg	Ile	Val		
		260						265					270				
Phe	Thr	Asp	Gln	Val	Leu	Lys	Phe	Leu	Ser	Gln	Asp	Glu	Leu	Val	Tyr		
		275					280					285					
Thr	Phe	Gly	Glu	Thr	Val	Ala	Leu	Gly	Ala	Ser	Gly	Ile	Val	Ile	Trp		
	290					295					300						
Gly	Thr	Leu	Ser	Ile	Met	Arg	Ser	Met	Lys	Ser	Cys	Leu	Leu	Leu	Asp		
305					310					315					320		
Asn	Tyr	Met	Glu	Thr	Ile	Leu	Asn	Pro	Tyr	Ile	Ile	Asn	Val	Thr	Leu		
				325					330					335			
Ala	Ala	Lys	Met	Cys	Ser	Gln	Val	Leu	Cys	Gln	Glu	Gln	Gly	Val	Cys		
		340						345					350				
Ile	Arg	Lys	Asn	Trp	Asn	Ser	Ser	Asp	Tyr	Leu	His	Leu	Asn	Pro	Asp		
		355					360					365					
Asn	Phe	Ala	Ile	Gln	Leu	Glu	Lys	Gly	Gly	Lys	Phe	Thr	Val	Arg	Gly		
	370					375					380						
Lys	Pro	Thr	Leu	Glu	Asp	Leu	Glu	Gln	Phe	Ser	Glu	Lys	Phe	Tyr	Cys		
385					390					395					400		
Ser	Cys	Tyr	Ser	Thr	Leu	Ser	Cys	Lys	Glu	Lys	Ala	Asp	Val	Lys	Asp		

				405					410					415	
Thr	Asp	Ala	Val	Asp	Val	Cys	Ile	Ala	Asp	Gly	Val	Cys	Ile	Asp	Ala
			420					425					430		
Phe	Leu	Lys	Pro	Pro	Met	Glu	Thr	Glu	Glu	Pro	Gln	Ile	Phe	Tyr	Asn
		435					440					445			
Ala	Ser	Pro	Ser	Thr	Leu	Ser	Ala	Thr	Met	Phe	Ile	Val	Ser	Ile	Leu
		450				455					460				
Phe	Leu	Ile	Ile	Ser	Ser	Val									
465					470										

<210> 181

<211> 469

<212> PRT

<213> Homo sapiens

<220>

<223> Mature HuPH20 36-504

<400> 181

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Ala	Trp	Asn	Ala	Pro	Ser	Glu	Phe	Cys	Leu	Gly	Lys	Phe	Asp	Glu	Pro
			20					25					30		
Leu	Asp	Met	Ser	Leu	Phe	Ser	Phe	Ile	Gly	Ser	Pro	Arg	Ile	Asn	Ala
		35					40					45			
Thr	Gly	Gln	Gly	Val	Thr	Ile	Phe	Tyr	Val	Asp	Arg	Leu	Gly	Tyr	Tyr
	50				55					60					
Pro	Tyr	Ile	Asp	Ser	Ile	Thr	Gly	Val	Thr	Val	Asn	Gly	Gly	Ile	Pro
65					70					75					80
Gln	Lys	Ile	Ser	Leu	Gln	Asp	His	Leu	Asp	Lys	Ala	Lys	Lys	Asp	Ile
				85					90					95	
Thr	Phe	Tyr	Met	Pro	Val	Asp	Asn	Leu	Gly	Met	Ala	Val	Ile	Asp	Trp
			100					105					110		
Glu	Glu	Trp	Arg	Pro	Thr	Trp	Ala	Arg	Asn	Trp	Lys	Pro	Lys	Asp	Val
		115					120					125			
Tyr	Lys	Asn	Arg	Ser	Ile	Glu	Leu	Val	Gln	Gln	Gln	Asn	Val	Gln	Leu
	130					135					140				
Ser	Leu	Thr	Glu	Ala	Thr	Glu	Lys	Ala	Lys	Gln	Glu	Phe	Glu	Lys	Ala
	145				150					155					160
Gly	Lys	Asp	Phe	Leu	Val	Glu	Thr	Ile	Lys	Leu	Gly	Lys	Leu	Leu	Arg
				165					170					175	
Pro	Asn	His	Leu	Trp	Gly	Tyr	Tyr	Leu	Phe	Pro	Asp	Cys	Tyr	Asn	His
			180					185					190		
His	Tyr	Lys	Lys	Pro	Gly	Tyr	Asn	Gly	Ser	Cys	Phe	Asn	Val	Glu	Ile
		195					200					205			
Lys	Arg	Asn	Asp	Asp	Leu	Ser	Trp	Leu	Trp	Asn	Glu	Ser	Thr	Ala	Leu
	210					215					220				
Tyr	Pro	Ser	Ile	Tyr	Leu	Asn	Thr	Gln	Gln	Ser	Pro	Val	Ala	Ala	Thr
225					230					235					240
Leu	Tyr	Val	Arg	Asn	Arg	Val	Arg	Glu	Ala	Ile	Arg	Val	Ser	Lys	Ile
				245					250					255	
Pro	Asp	Ala	Lys	Ser	Pro	Leu	Pro	Val	Phe	Ala	Tyr	Thr	Arg	Ile	Val
			260					265					270		
Phe	Thr	Asp	Gln	Val	Leu	Lys	Phe	Leu	Ser	Gln	Asp	Glu	Leu	Val	Tyr
		275					280					285			
Thr	Phe	Gly	Glu	Thr	Val	Ala	Leu	Gly	Ala	Ser	Gly	Ile	Val	Ile	Trp
	290					295					300				
Gly	Thr	Leu	Ser	Ile	Met	Arg	Ser	Met	Lys	Ser	Cys	Leu	Leu	Leu	Asp
	305				310					315					320
Asn	Tyr	Met	Glu	Thr	Ile	Leu	Asn	Pro	Tyr	Ile	Ile	Asn	Val	Thr	Leu
				325					330					335	
Ala	Ala	Lys	Met	Cys	Ser	Gln	Val	Leu	Cys	Gln	Glu	Gln	Gly	Val	Cys
			340					345					350		
Ile	Arg	Lys	Asn	Trp	Asn	Ser	Ser	Asp	Tyr	Leu	His	Leu	Asn	Pro	Asp
		355					360					365			
Asn	Phe	Ala	Ile	Gln	Leu	Glu	Lys	Gly	Gly	Lys	Phe	Thr	Val	Arg	Gly
	370				375						380				
Lys	Pro	Thr	Leu	Glu	Asp	Leu	Glu	Gln	Phe	Ser	Glu	Lys	Phe	Tyr	Cys
385					390					395					400
Ser	Cys	Tyr	Ser	Thr	Leu	Ser	Cys	Lys	Glu	Lys	Ala	Asp	Val	Lys	Asp
				405					410					415	
Thr	Asp	Ala	Val	Asp	Val	Cys	Ile	Ala	Asp	Gly	Val	Cys	Ile	Asp	Ala
			420					425						430	
Phe	Leu	Lys	Pro	Pro	Met	Glu	Thr	Glu	Glu	Pro	Gln	Ile	Phe	Tyr	Asn

435 440 445
 Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val Ser Ile Leu
 450 455 460
 Phe Leu Ile Ile Ser
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<210> 182
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 <212> PRT
 <213> Homo sapiens

<220>
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<400> 182
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 Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
 20 25 30
 Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
 35 40 45
 Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
 50 55 60
 Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
 65 70 75 80
 Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
 85 90 95
 Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
 100 105 110
 Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
 115 120 125
 Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
 130 135 140
 Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
 145 150 155 160
 Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
 165 170 175
 Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
 180 185 190
 His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
 195 200 205
 Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
 210 215 220
 Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
 225 230 235 240
 Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
 245 250 255
 Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
 260 265 270
 Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
 275 280 285
 Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
 290 295 300
 Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
 305 310 315 320
 Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
 325 330 335
 Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
 340 345 350
 Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
 355 360 365
 Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
 370 375 380
 Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
 385 390 395 400
 Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
 405 410 415
 Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
 420 425 430
 Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
 435 440 445
 Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val Ser Ile Leu
 450 455 460
 Phe Leu Ile

465

<210> 183
 <211> 465
 <212> PRT
 <213> Homo sapiens

<220>
 <223> Mature HuPH20 36-500

<400> 183

Leu	Asn	Phe	Arg	Ala	Pro	Pro	Val	Ile	Pro	Asn	Val	Pro	Phe	Leu	Trp
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Ala	Trp	Asn	Ala	Pro	Ser	Glu	Phe	Cys	Leu	Gly	Lys	Phe	Asp	Glu	Pro
		20						25					30		
Leu	Asp	Met	Ser	Leu	Phe	Ser	Phe	Ile	Gly	Ser	Pro	Arg	Ile	Asn	Ala
		35					40					45			
Thr	Gly	Gln	Gly	Val	Thr	Ile	Phe	Tyr	Val	Asp	Arg	Leu	Gly	Tyr	Tyr
	50					55				60					
Pro	Tyr	Ile	Asp	Ser	Ile	Thr	Gly	Val	Thr	Val	Asn	Gly	Gly	Ile	Pro
65					70				75					80	
Gln	Lys	Ile	Ser	Leu	Gln	Asp	His	Leu	Asp	Lys	Ala	Lys	Lys	Asp	Ile
			85					90					95		
Thr	Phe	Tyr	Met	Pro	Val	Asp	Asn	Leu	Gly	Met	Ala	Val	Ile	Asp	Trp
			100					105					110		
Glu	Glu	Trp	Arg	Pro	Thr	Trp	Ala	Arg	Asn	Trp	Lys	Pro	Lys	Asp	Val
		115					120					125			
Tyr	Lys	Asn	Arg	Ser	Ile	Glu	Leu	Val	Gln	Gln	Gln	Asn	Val	Gln	Leu
	130					135					140				
Ser	Leu	Thr	Glu	Ala	Thr	Glu	Lys	Ala	Lys	Gln	Glu	Phe	Glu	Lys	Ala
145					150					155					160
Gly	Lys	Asp	Phe	Leu	Val	Glu	Thr	Ile	Lys	Leu	Gly	Lys	Leu	Leu	Arg
			165					170					175		
Pro	Asn	His	Leu	Trp	Gly	Tyr	Tyr	Leu	Phe	Pro	Asp	Cys	Tyr	Asn	His
			180					185					190		
His	Tyr	Lys	Lys	Pro	Gly	Tyr	Asn	Gly	Ser	Cys	Phe	Asn	Val	Glu	Ile
	195						200					205			
Lys	Arg	Asn	Asp	Asp	Leu	Ser	Trp	Leu	Trp	Asn	Glu	Ser	Thr	Ala	Leu
	210					215					220				
Tyr	Pro	Ser	Ile	Tyr	Leu	Asn	Thr	Gln	Gln	Ser	Pro	Val	Ala	Ala	Thr
225					230					235					240
Leu	Tyr	Val	Arg	Asn	Arg	Val	Arg	Glu	Ala	Ile	Arg	Val	Ser	Lys	Ile
			245						250					255	
Pro	Asp	Ala	Lys	Ser	Pro	Leu	Pro	Val	Phe	Ala	Tyr	Thr	Arg	Ile	Val
		260						265					270		
Phe	Thr	Asp	Gln	Val	Leu	Lys	Phe	Leu	Ser	Gln	Asp	Glu	Leu	Val	Tyr
		275					280					285			
Thr	Phe	Gly	Glu	Thr	Val	Ala	Leu	Gly	Ala	Ser	Gly	Ile	Val	Ile	Trp
	290					295					300				
Gly	Thr	Leu	Ser	Ile	Met	Arg	Ser	Met	Lys	Ser	Cys	Leu	Leu	Leu	Asp
305					310					315					320
Asn	Tyr	Met	Glu	Thr	Ile	Leu	Asn	Pro	Tyr	Ile	Ile	Asn	Val	Thr	Leu
				325					330					335	
Ala	Ala	Lys	Met	Cys	Ser	Gln	Val	Leu	Cys	Gln	Glu	Gln	Gly	Val	Cys
			340					345					350		
Ile	Arg	Lys	Asn	Trp	Asn	Ser	Ser	Asp	Tyr	Leu	His	Leu	Asn	Pro	Asp
		355					360					365			
Asn	Phe	Ala	Ile	Gln	Leu	Glu	Lys	Gly	Gly	Lys	Phe	Thr	Val	Arg	Gly
	370					375					380				
Lys	Pro	Thr	Leu	Glu	Asp	Leu	Glu	Gln	Phe	Ser	Glu	Lys	Phe	Tyr	Cys
385					390					395					400
Ser	Cys	Tyr	Ser	Thr	Leu	Ser	Cys	Lys	Glu	Lys	Ala	Asp	Val	Lys	Asp
			405						410					415	
Thr	Asp	Ala	Val	Asp	Val	Cys	Ile	Ala	Asp	Gly	Val	Cys	Ile	Asp	Ala
		420						425					430		
Phe	Leu	Lys	Pro	Pro	Met	Glu	Thr	Glu	Glu	Pro	Gln	Ile	Phe	Tyr	Asn
		435					440					445			
Ala	Ser	Pro	Ser	Thr	Leu	Ser	Ala	Thr	Met	Phe	Ile	Val	Ser	Ile	Leu
	450					455					460				
Phe															
465															

<210> 184

<211> 463
 <212> PRT
 <213> Homo sapiens

<220>
 <223> Mature HuPH20 36-498

<400> 184
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 1 5 10 15
 Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
 20 25 30
 Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
 35 40 45
 Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
 50 55 60
 Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
 65 70 75 80
 Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
 85 90 95
 Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
 100 105 110
 Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
 115 120 125
 Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
 130 135 140
 Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
 145 150 155 160
 Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
 165 170 175
 Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
 180 185 190
 His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
 195 200 205
 Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
 210 215 220
 Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
 225 230 235 240
 Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
 245 250 255
 Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
 260 265 270
 Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
 275 280 285
 Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
 290 295 300
 Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
 305 310 315 320
 Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
 325 330 335
 Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
 340 345 350
 Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
 355 360 365
 Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
 370 375 380
 Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
 385 390 395 400
 Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
 405 410 415
 Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
 420 425 430
 Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
 435 440 445
 Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val Ser Ile
 450 455 460

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 <213> Homo sapiens

<220>
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<400> 185

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Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1      5      10      15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
 20      25      30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
 35      40      45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
 50      55      60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
 65      70      75      80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
 85      90      95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
100      105      110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
115      120      125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
130      135      140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145      150      155      160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
165      170      175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
180      185      190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
195      200      205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
210      215      220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225      230      235      240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
245      250      255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
260      265      270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
275      280      285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
290      295      300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305      310      315      320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
325      330      335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
340      345      350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
355      360      365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
370      375      380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385      390      395      400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
405      410      415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
420      425      430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
435      440      445
Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
450      455      460

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<210> 186

<211> 459

<212> PRT

<213> Homo sapiens

<220>

<223> esHuPH20 36-494

<400> 186

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Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
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Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
 20      25      30

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Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
 35 40 45
 Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
 50 55 60
 Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
 65 70 75 80
 Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
 85 90 95
 Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
 100 105 110
 Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
 115 120 125
 Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
 130 135 140
 Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
 145 150 155 160
 Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
 165 170 175
 Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
 180 185 190
 His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
 195 200 205
 Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
 210 215 220
 Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
 225 230 235 240
 Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
 245 250 255
 Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
 260 265 270
 Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
 275 280 285
 Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
 290 295 300
 Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
 305 310 315 320
 Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
 325 330 335
 Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
 340 345 350
 Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
 355 360 365
 Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
 370 375 380
 Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
 385 390 395 400
 Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
 405 410 415
 Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
 420 425 430
 Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
 435 440 445
 Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe
 450 455

<210> 187
 <211> 457
 <212> PRT
 <213> Homo sapiens

<220>
 <223> esHuPH20 36-492

<400> 187
 Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1 5 10 15
 Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
 20 25 30
 Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
 35 40 45
 Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
 50 55 60
 Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
 65 70 75 80

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Gln	Lys	Ile	Ser	Leu	Gln	Asp	His	Leu	Asp	Lys	Ala	Lys	Lys	Asp	Ile
			85						90					95	
Thr	Phe	Tyr	Met	Pro	Val	Asp	Asn	Leu	Gly	Met	Ala	Val	Ile	Asp	Trp
			100					105					110		
Glu	Glu	Trp	Arg	Pro	Thr	Trp	Ala	Arg	Asn	Trp	Lys	Pro	Lys	Asp	Val
		115					120					125			
Tyr	Lys	Asn	Arg	Ser	Ile	Glu	Leu	Val	Gln	Gln	Gln	Asn	Val	Gln	Leu
		130				135					140				
Ser	Leu	Thr	Glu	Ala	Thr	Glu	Lys	Ala	Lys	Gln	Glu	Phe	Glu	Lys	Ala
		145			150					155					160
Gly	Lys	Asp	Phe	Leu	Val	Glu	Thr	Ile	Lys	Leu	Gly	Lys	Leu	Leu	Arg
			165						170					175	
Pro	Asn	His	Leu	Trp	Gly	Tyr	Tyr	Leu	Phe	Pro	Asp	Cys	Tyr	Asn	His
			180					185					190		
His	Tyr	Lys	Lys	Pro	Gly	Tyr	Asn	Gly	Ser	Cys	Phe	Asn	Val	Glu	Ile
		195					200					205			
Lys	Arg	Asn	Asp	Asp	Leu	Ser	Trp	Leu	Trp	Asn	Glu	Ser	Thr	Ala	Leu
		210				215					220				
Tyr	Pro	Ser	Ile	Tyr	Leu	Asn	Thr	Gln	Gln	Ser	Pro	Val	Ala	Ala	Thr
		225			230					235					240
Leu	Tyr	Val	Arg	Asn	Arg	Val	Arg	Glu	Ala	Ile	Arg	Val	Ser	Lys	Ile
			245						250					255	
Pro	Asp	Ala	Lys	Ser	Pro	Leu	Pro	Val	Phe	Ala	Tyr	Thr	Arg	Ile	Val
		260						265					270		
Phe	Thr	Asp	Gln	Val	Leu	Lys	Phe	Leu	Ser	Gln	Asp	Glu	Leu	Val	Tyr
		275					280					285			
Thr	Phe	Gly	Glu	Thr	Val	Ala	Leu	Gly	Ala	Ser	Gly	Ile	Val	Ile	Trp
		290			295						300				
Gly	Thr	Leu	Ser	Ile	Met	Arg	Ser	Met	Lys	Ser	Cys	Leu	Leu	Leu	Asp
				310						315					320
Asn	Tyr	Met	Glu	Thr	Ile	Leu	Asn	Pro	Tyr	Ile	Ile	Asn	Val	Thr	Leu
			325						330					335	
Ala	Ala	Lys	Met	Cys	Ser	Gln	Val	Leu	Cys	Gln	Glu	Gln	Gly	Val	Cys
			340					345					350		
Ile	Arg	Lys	Asn	Trp	Asn	Ser	Ser	Asp	Tyr	Leu	His	Leu	Asn	Pro	Asp
		355					360					365			
Asn	Phe	Ala	Ile	Gln	Leu	Glu	Lys	Gly	Gly	Lys	Phe	Thr	Val	Arg	Gly
		370				375					380				
Lys	Pro	Thr	Leu	Glu	Asp	Leu	Glu	Gln	Phe	Ser	Glu	Lys	Phe	Tyr	Cys
				390						395					400
Ser	Cys	Tyr	Ser	Thr	Leu	Ser	Cys	Lys	Glu	Lys	Ala	Asp	Val	Lys	Asp
				405					410					415	
Thr	Asp	Ala	Val	Asp	Val	Cys	Ile	Ala	Asp	Gly	Val	Cys	Ile	Asp	Ala
			420					425					430		
Phe	Leu	Lys	Pro	Pro	Met	Glu	Thr	Glu	Glu	Pro	Gln	Ile	Phe	Tyr	Asn
		435					440					445			
Ala	Ser	Pro	Ser	Thr	Leu	Ser	Ala	Thr							
		450				455									

<210> 188
 <211> 453
 <212> PRT
 <213> Homo sapiens

<220>
 <223> Mature HuPH20 36-488

<400> 188

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

Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
 130 135 140
 Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
 145 150 155 160
 Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
 165 170 175
 Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
 180 185 190
 His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
 195 200 205
 Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
 210 215 220
 Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
 225 230 235 240
 Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
 245 250 255
 Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
 260 265 270
 Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
 275 280 285
 Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
 290 295 300
 Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
 305 310 315 320
 Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
 325 330 335
 Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
 340 345 350
 Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
 355 360 365
 Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
 370 375 380
 Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
 385 390 395 400
 Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
 405 410 415
 Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
 420 425 430
 Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
 435 440 445
 Ala Ser Pro Ser Thr
 450

<210> 189

<211> 451

<212> PRT

<213> Homo sapiens

<220>

<223> Mature HuPH20 36-486

<400> 189

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

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Pro	Asn	His	Leu	Trp	Gly	Tyr	Tyr	Leu	Phe	Pro	Asp	Cys	Tyr	Asn	His
			180					185					190		
His	Tyr	Lys	Lys	Pro	Gly	Tyr	Asn	Gly	Ser	Cys	Phe	Asn	Val	Glu	Ile
		195					200					205			
Lys	Arg	Asn	Asp	Asp	Leu	Ser	Trp	Leu	Trp	Asn	Glu	Ser	Thr	Ala	Leu
	210					215					220				
Tyr	Pro	Ser	Ile	Tyr	Leu	Asn	Thr	Gln	Gln	Ser	Pro	Val	Ala	Ala	Thr
	225				230					235					240
Leu	Tyr	Val	Arg	Asn	Arg	Val	Arg	Glu	Ala	Ile	Arg	Val	Ser	Lys	Ile
			245						250					255	
Pro	Asp	Ala	Lys	Ser	Pro	Leu	Pro	Val	Phe	Ala	Tyr	Thr	Arg	Ile	Val
		260						265					270		
Phe	Thr	Asp	Gln	Val	Leu	Lys	Phe	Leu	Ser	Gln	Asp	Glu	Leu	Val	Tyr
		275					280					285			
Thr	Phe	Gly	Glu	Thr	Val	Ala	Leu	Gly	Ala	Ser	Gly	Ile	Val	Ile	Trp
	290				295						300				
Gly	Thr	Leu	Ser	Ile	Met	Arg	Ser	Met	Lys	Ser	Cys	Leu	Leu	Leu	Asp
	305				310					315					320
Asn	Tyr	Met	Glu	Thr	Ile	Leu	Asn	Pro	Tyr	Ile	Ile	Asn	Val	Thr	Leu
			325						330					335	
Ala	Ala	Lys	Met	Cys	Ser	Gln	Val	Leu	Cys	Gln	Glu	Gln	Gly	Val	Cys
		340						345					350		
Ile	Arg	Lys	Asn	Trp	Asn	Ser	Ser	Asp	Tyr	Leu	His	Leu	Asn	Pro	Asp
		355					360					365			
Asn	Phe	Ala	Ile	Gln	Leu	Glu	Lys	Gly	Gly	Lys	Phe	Thr	Val	Arg	Gly
	370					375					380				
Lys	Pro	Thr	Leu	Glu	Asp	Leu	Glu	Gln	Phe	Ser	Glu	Lys	Phe	Tyr	Cys
	385				390					395					400
Ser	Cys	Tyr	Ser	Thr	Leu	Ser	Cys	Lys	Glu	Lys	Ala	Asp	Val	Lys	Asp
			405						410					415	
Thr	Asp	Ala	Val	Asp	Val	Cys	Ile	Ala	Asp	Gly	Val	Cys	Ile	Asp	Ala
		420						425					430		
Phe	Leu	Lys	Pro	Pro	Met	Glu	Thr	Glu	Glu	Pro	Gln	Ile	Phe	Tyr	Asn
		435					440					445			
Ala	Ser	Pro													
	450														