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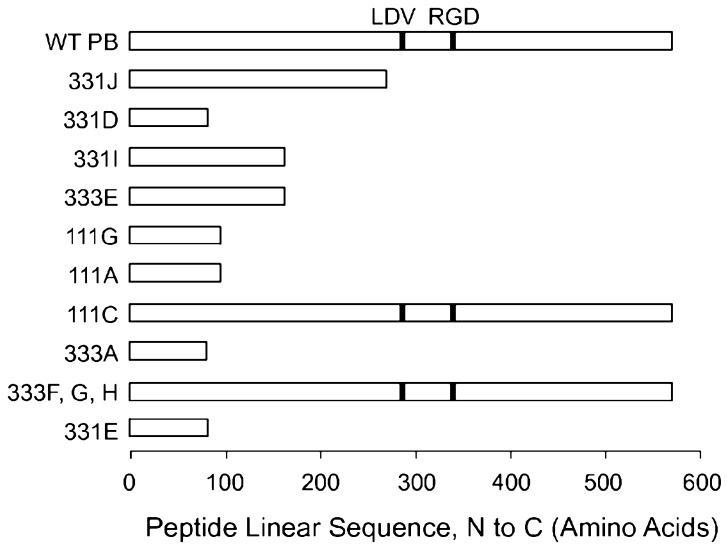
(54) Title: ISOLATING TRAFFIC-ENHANCING MUTANTS OF DRUG DELIVERY PROTEIN

(57) Abstract: The invention relates to methods for isolating traffic-enhancing mutants of drug delivery proteins. In one embodiment, the invention provides a carrier for delivering a therapeutic agent to an organelle, comprising a polypeptide encoded by a mutant penton base gene. In another embodiment, the invention provides a method of enhancing trafficking to a cell by administering a composition comprising a penton base (PB) protein with one or more mutations that enhance cellular entry.

Figure 7



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ISOLATING TRAFFIC-ENHANCING MUTANTS OF DRUG DELIVERY PROTEIN

GOVERNMENT RIGHTS

The invention was made with government support under Grant Nos. CA129822 and CA140995 awarded by the National Institutes of Health. The government has certain rights in the invention.

FIELD OF INVENTION

The invention provides methods and compositions for cell penetration function to improve drug delivery to specific organelles.

BACKGROUND OF THE INVENTION

Directed evolution has been used to generate pseudotyped adeno-associated virus (AAV) capsids with novel tropism, reduced non-specific delivery, and immune evasion (Kwon and Schaffer, 2008; Maheshri et al., 2006). Biopanning of phage display and whole viral libraries *in vitro* and *in vivo* with different selective pressures has generated proteins with desired properties such as improved ligand binding and uptake, immune interactions, or enzyme activities (Yuan et al., 2005). Thus, this methodology can be a powerful and more effective alternative to rational mutation for creating new protein variants with improved features. The most recently developed approach to this process employs error-prone PCR and staggered extension to create a library of variants that can be screened against different cell types to select out cell-specific targeted vectors (Zhao et al., 1998). To date, a process has not yet been developed to isolate protein variants with improved intracellular trafficking functions. This invention introduces a new procedure and new protein molecules with improved cell penetration functions for drug delivery resulting from the procedure.

SUMMARY OF THE INVENTION

Various embodiments include a method of enhancing trafficking to a cell, comprising providing a composition comprising a penton base (PB) protein with one or more mutations that enhance cellular entry and administering an effective dosage of the composition to the

cell. In one embodiment, the one or more mutations is 111C and/or 333F. In another embodiment, the composition targets the cytoplasm and/or nucleus of the cell. In another embodiment, the composition further comprises a therapeutic drug. In another embodiment, the cell is a tumor cell. In another embodiment, the one or more mutations is SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7,SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and/or SEQ ID NO: 20.

Other embodiments include a method for producing a drug delivery molecule that targets an organelle. The method includes the following steps: a) obtaining a polynucleotide encoding the penton base (PB) gene and generating mutants of the polynucleotide, b) cloning the mutant polynucleotide into a phage vector and generating a phage library comprising the phage vectors, c) transforming cells with the phage library, d) fractioning the transformed cells and harvesting the organelle from the transformed cells, e) amplifying the phages from the harvested organelles, f) transforming cells with the amplified phages from the harvested organelle, g) repeating steps (d), (e), (f), and (g), h) titering the phages from the harvested organelles from each round, i) selecting the phages with the highest titer and obtaining the sequences of the mutant polynucleotide from the phage, and j) producing polypeptides encoded by the sequences, where the polypeptides are the drug delivery molecules that target organelles. In another embodiment, the organelle is selecting from the group consisting of mitochondrion, Golgi apparatus, endoplasmic reticulum, nucleus, ribosomes, plasma membrane and cytosol. In another embodiment, the cells are mammalian or non-mammalian cells. In another embodiment, the mutants are generated using any one or more of PCR-based methods, chemical mutagenesis, ultraviolet-induced mutagenesis or a combination thereof. In another embodiment, the drug delivery molecule comprises a targeting domain, an endosomolytic ligand domain and a positively charged domain.

Other embodiments include a carrier for delivering a therapeutic agent to an organelle, comprising a polypeptide encoded by one or more penton base (PB) mutations that enhance cellular entry. In another embodiment, the one or more penton base (PB) mutations include 111C and/or 333F. In another embodiment, the carrier further comprises a polylysine motif. In another embodiment, the carrier further comprises a targeting domain of heregulin. In another embodiment, the one or more PB mutations comprises a C-terminal deletion.

Other embodiments include a therapeutic agent comprising a carrier for delivering a therapeutic agent to an organelle comprising a polypeptide encoded by one or more penton base (PB) mutations that enhance cellular entry and a therapeutic drug. In another embodiment, the therapeutic drug is a chemotherapeutic agent.

Various other embodiments include a method of producing a carrier without proliferative activity, comprising a) obtaining a polynucleotide encoding the receptor binding domain of heregulin (Her) and generating mutants in the polynucleotide, b) cloning the mutant Her polynucleotides into phage vectors and generating a phage library comprising the phage vectors, c) transforming MDA-MB-435 cells with the phage library in the presence of mitotic inhibitors, d) fractioning the transformed cell and extracting the membrane fraction of the MDA-MB-435 cells, e) harvesting membrane phages from the membrane fraction, f) transforming the membrane phages into MDA-MB-435 cells in the presence of mitotic inhibitors, g) repeating steps (d), (e), and (f), h) monitoring MDA-MB-435 cell proliferation in each round and selecting the membrane phages with the lowest MDA-MB-435 cell proliferation, i) obtaining the sequences of the Her polynucleotide mutants in the selected membrane phages, and j) producing polypeptides encoded by the Her sequences and penton base gene, where the polypeptides are the carrier without proliferative activity.

In one embodiment, the present invention provides a method of delivering a therapeutic agent to a subcellular location in a cell, comprising: administering a composition comprising a carrier polypeptide to the cell, the carrier polypeptide comprising a penton base polypeptide comprising one or more mutations that enhance subcellular localization of the carrier polypeptide in the cell, wherein the one or more mutations is one or more point mutations within the penton base polypeptide sequence or a carboxy-terminal truncation of the penton base polypeptide sequence.

In another embodiment, the present invention provides a method of delivering a therapeutic agent to a subcellular location in a cell, comprising: administering a composition comprising a carrier polypeptide to the cell, the carrier polypeptide comprising a penton base polypeptide, wherein: the penton base polypeptide comprises one or more point mutations in SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID

NO: 8, SEQ ID NO: 12, or SEQ ID NO: 20; or the penton base polypeptide consists of the penton base polypeptide recited in SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 14, SEQ ID NO: 16, or SEQ ID NO: 18.

In another embodiment, the present invention provides a method for producing a drug delivery molecule that targets an organelle comprising:

a. obtaining a polynucleotide encoding a penton base gene and generating mutants of the polynucleotide;

b. cloning the mutant polynucleotide into a phage vector and generating a phage library comprising the phage vectors;

c. transforming cells with the phage library;

d. fractioning the transformed cells and harvesting the organelle from the transformed cells;

e. amplifying the phages from the harvested organelles;

f. transforming cells with the amplified phages from the harvested organelle;

g. repeating steps (d), (e), and (f);

h. titering the phages from the harvested organelles from each round;

i. selecting the phages with the highest titer and obtaining the sequences of the mutant polynucleotide from the phage; and

j. producing a mutant polypeptide encoded by one of the sequences,

wherein the mutant polypeptide is the drug delivery molecule that target organelle.

In a further embodiment, the present invention provides a carrier polypeptide for delivering a therapeutic agent to a subcellular location in a cell, comprising a penton base polypeptide comprising one or more mutations that enhance subcellular localization of the carrier polypeptide in the cell; wherein the one or more mutations is one or more point mutations within the penton base polypeptide sequence or a carboxy-terminal truncation of the penton base polypeptide sequence.

In yet another embodiment, the present invention provides a method of producing a carrier polypeptide comprising a mutant Her targeting domain without proliferative activity, comprising:

(a) obtaining a polynucleotide encoding the receptor binding domain of heregulin (Her) and generating mutants in the polynucleotide;

(b) cloning the mutant Her polynucleotides into phage vectors and generating a phage library comprising the phage vectors;

(c) transforming cells with the phage library in the presence of mitotic inhibitors;

(d) fractioning the transformed cells and extracting the membrane fraction of the cells;

(e) harvesting phages from the membrane fraction;

(f) transforming the phages into cells in the presence of mitotic inhibitors;

(g) repeating steps (d), (e), and (f);

(h) monitoring cell proliferation in each round and selecting the phages from the membrane fraction with the lowest cell proliferation;

(i) obtaining the sequence of at least one of the mutant Her polynucleotides in the selected phages from the membrane fraction; and

(j) producing polypeptides encoded by one of the at least one mutant Her sequences and a penton base gene,

wherein the carrier polypeptide does not have proliferative activity.

Other embodiments include a carrier for delivering therapeutics to the nucleus, comprising a polypeptide encoded by mutant Her sequences. In another embodiment, the carrier further comprises a polypeptide encoding penton base (PB) protein and a polylysine motif. In another embodiment, the PB protein is a mutant penton base protein.

In a further embodiment of the present invention, there is provided a carrier polypeptide for delivering a therapeutic agent to a subcellular location in a cell,

comprising a penton base polypeptide, wherein: the penton base polypeptide comprises one or more point mutations in SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 12, or SEQ ID NO: 20; or the penton base polypeptide consists of the penton base polypeptide recited in SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 14, SEQ ID NO: 16, or SEQ ID NO: 18.

Other embodiments include a therapeutic agent comprising a carrier and a therapeutic drug.

BRIEF DESCRIPTION OF FIGURES

Figures 1 depicts, in accordance with an embodiment herein, the biopanning strategy.

Figure 2 depicts, in accordance with an embodiment herein, a PCR-based random mutagenesis of the penton base gene. The PCR product (just above the 1600bp band)

[Text continued on page 4]

contains 100ng DNA, based on densitometry analysis, giving us a total yield of ~4800ng. As the initial target DNA was 97ng, we have a yield/initial ratio of ~50 and a duplication number of ~5.6, which when extrapolated against the standard curve provided in the manufacturer's protocol (Genemorph II, Strategene, La Jolla, CA, USA), corresponds to a mutation rate of ~8/kb.

Figure 3 depicts, in accordance with an embodiment herein, isolation of phage displaying penton base variants with enhanced partitioning in nuclear compartment. A T7 phage library displaying randomly mutagenized PB was added to 1x10^6 HeLa cells at 1x10^8 pfu following the conditions described in the text for cell binding and uptake. Cells were harvested by trypsinization to remove any surface-bound phage, then fractionated using a commercial fractionation kit (Qproteome Cell Compartment Kit; Qiagen Inc., Valencia, CA, USA). Phage was PEG-precipitated overnight from each fraction following standard procedures, then resuspended in TB bacterial media and added to BLT5403 bacteria to amplify the isolated phage. Amplified phage was titered by plaque assay, then re-panned on HeLa using the same titer and conditions as described earlier. The phage obtained from cytosolic fractions underwent 3 rounds of cytosolic biopanning, whereas that obtained from nuclear fractions underwent 2 rounds.

Figure 4 depicts, in accordance with an embodiment herein, the alignment of trafficking variants isolated from biopanning.

Figure 5 depicts, in accordance with an embodiment herein, full-length mutant, 111C, exhibits enhanced trafficking to cytoplasm and nucleus. Figure 5(A) depicts protein was precipitated from each fraction by acetone precipitation and pellets were resuspended in 40uL desalting buffer, followed by analysis via SDS-PAGE and Western blotting. Figure 5(B) depicts analysis of band densities using Image J show an increase of 111C in both cytosolic and nuclear fractions compared to the wild-type protein. Trafficking and Cell fractionation assay: Adherent HeLa cells growing in flasks were detached with 5mL 1XPBS+2mM EDTA at 37C incubation with agitation for 50 min and transferred to 15 mL conical tubes. The density of the cells were measured, and cells were distributed into separate tubes at 5x106 cells per tube. Cells were washed with 1X PBS + Ca2+ + Mg2+ three times to remove the EDTA, and cell pellets were resuspended in 0.7 ml Buffer A (20mM HEPES, pH 7.4; 2mM MgCl2; 3% BSA in DMEM). Wild-type (WT) or mutant (111C, 333F) penton base protein

(450 ug) was added to separate cell aliquots and mixtures were incubated at 4C for 2hrs with agitation to promote receptor binding, followed by incubation at 37C for 2h with agiatation to promote internalization of receptor-bound protein. Control treatments received no protein (NP). Cells were then collected and processed for subcellular fractionation using the Qproteome Cell Compartment assay kit (Qiagen).

Figure 6 depicts, in accordance with an embodiment herein, PB mutants, 111C and 333F, exhibit enhanced nuclear entry compared to wild-type PB. Figure 6(A) depicts intracellular trafficking and immunocytochemistry. Procedure followed the established protocols detailed in Gene Therapy (2006) 13, 821–836. Anti-penton base antibody (Ad5 antibody) was used at 1:500 dilution. Alexa-Fluor 488 Goat Anti-Rabbit was used at 1:400 dilution (2nd antibody). Phalloidin was used at 1:100 dilution, and DAPI used at 300nM. Green, wild-type (WT) or mutant (111C, 333F) PB; Red, actin; Blue, nucleus. Figure 6(B) depicts quantification of protein trafficking. Sixteen cells from each treatment represented in the left panel was selected for quantification. The counts were based on the histogram of each image in Adobe Photoshop. Bars represent green pixel counts within the 80-255 window in the green channel.

Figure 7 depicts, in accordance with an embodiment herein, an updated Alignment of PB Mutants. New peptide lengths are based on amino acid sequences translated from nucleic acid sequences of mutant clones. Sequences are provided in Figures 8-17 herein.

Figure 8 depicts, in accordance with an embodiment herein, nucleic Acid and peptide sequence of mutated PB from Fraction 111 Clone A (111A).

Figure 9 depicts, in accordance with an embodiment herein, nucleic acid and predicted amino acid sequence of mutated PB from Fraction 111 Clone C (111C).

Figure 10 depicts, in accordance with an embodiment herein, nucleic acid and peptide sequence of mutated PB from Fraction 111 Clone G (111G).

Figure 11 depicts, in accordance with an embodiment herein, nucleic acid and peptide sequence of mutated PB from Fraction 331 Clone E (331E).

Figure 12 depicts, in accordance with an embodiment herein, nucleic acid and peptide sequence of mutated PB from Fraction 331 Clone I (331I).

Figure 13 depicts, in accordance with an embodiment herein, nucleic acid and peptide sequence of mutated PB from Fraction 331 Clone J (331J).

Figure 14 depicts, in accordance with an embodiment herein, nucleic acid and peptide sequence of mutated PB from Fraction 333 Clone A (333A).

Figure 15 depicts, in accordance with an embodiment herein, nucleic acid and peptide sequence of mutated PB from Fraction 333 Clone D (333D).

Figure 16 depicts, in accordance with an embodiment herein, nucleic acid and peptide sequence of mutated PB from Fraction 333 Clone E (333E).

Figure 17 depicts, in accordance with an embodiment herein, nucleic acid and peptide sequence of mutated PB from Franction 333, clones F, G, and H (333F, 333G, 333H).

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the abbreviation "PB" means penton base.

A variety of reagents have been developed to deliver therapeutic genes and drugs to diseased cells, and include liposomes, synthetic polymers, peptides, proteins, viruses, and viral nanoparticles (Medina-Kauwe, 2006; MedinaKauwe et al., 2005). Typically, the particles formed by these reagents require modifications to facilitate delivery of gene or drug payloads. Such modifications include appending targeting ligands or antibodies, membrane penetrating agents, and/or intracellular targeting (such as nuclear targeting) agents. These modifications are usually introduced through rational design and thus each new variant generated by such modification requires empirical testing. This can be time consuming and labor intensive, and runs the risk of yielding suboptimal activity.

Past and present attempts to improve cell membrane penetration and/or intracellular trafficking have used rational design to conjugate cell penetration or intracellular targeting peptides to drug carriers. Such an approach requires empirical testing of each molecule.

Numerous types of cell penetration peptides have been tested for enhanced gene and drug delivery, and include AntP, TAT, GALA, honey bee melittin, and similar peptides (Medina-Kauwe, 2006; Medina-Kauwe et al., 2005). Likewise, nuclear targeting activity has been added to gene delivery agents via appendage of different poly-basic domains such as the SV40 NLS, HMG-1, protamine, and similar peptides or proteins (MedinaKauwe, 2006; Medina-Kauwe et al., 2005). While each of these peptides possess the capacity to penetrate cell membranes or target intracellular compartments, including the nucleus, their activities are altered when covalently coupled to another molecule or expressed as a fusion protein to another molecule. Moreover, empirical testing of each different variant of these peptides is time consuming and labor intensive. Therefore, while rational design and empirical testing is the existing solution to this problem, this approach, has its limitations.

The invention described herein circumvents the time and effort required for rational design and empirical testing of cell penetration/intracellular trafficking proteins/peptides by using selective pressure to isolate protein mutants that have acquired improved cell penetration, intracellular trafficking, and/or subcellular targeting. The protein mutants derived from this process have unique advantages over the parent proteins or existing gene/drug delivery proteins currently in use because of the improved features acquired through artificial evolution/selective pressure. Finally, the specific proteins isolated by the process described below will provide an advantage over existing cell penetrating peptides because of their improved membrane lysis and trafficking features. Therefore, they can be used to augment gene and drug delivery, and thus enhance therapeutic efficacy of particles used in nanomedicine.

The invention provides a method for producing a drug delivery molecule that targets an organelle. The method includes the steps of (a) obtaining a polynucleotide encoding the penton base gene and generating mutants of the polynucleotide; (b) cloning the mutant polynucleotide into a phage vector and generating a phage library comprising the phage vectors; (c) transforming cells with the phage library; (d) fractioning the transformed cells and harvesting the organell from the transformed cells; (e) amplifying the phages from the harvested organelles; (f) transforming cells with the amplified phages from the harvested organelle; (g) repeating steps (d), (e), (f), and (g); (h) titering the phages from the harvested organelles from each round; (i) selecting the phages with the highest titer and obtaining the sequences of the mutant polynucleotide from the phage; and (j) producing polypeptides

encoded by the sequences, wherein the polypeptides are the drug delivery molecules that target organelles.

In some embodiments, the organelle is selecting from the group consisting of mitochondrion, Golgi apparatus, endoplasmic reticulum, nucleus, ribosomes, plasma membrane and cytosol. The cells may be mammalian or non-mammalian cells. Mutants may be generated using any random mutagenesis methods or targeted mutagenesis methods. Examples of methods that may be used to generate mutants include but are not limited to any one or more of PCR-based methods, chemical mutagenesis, ultraviolet-induced mutagenesis or a combination thereof. Mutations in the penton base gene may be any one or more of insertions, deletions, substitutions or a combination thereof.

In an embodiment of the invention, the drug delivery molecule comprises a targeting domain, an endosomolytic ligand domain and a positively charged domain.

The invention also provides a carrier for delivering a therapeutic agent to an organelle, comprising a polypeptide encoded by a mutant penton base gene. The mutation in the penton base gene may be isolated by a) obtaining a polynucleotide encoding the penton base gene and generating mutants of the polynucleotide; (b) cloning the mutant polynucleotide into a phage vector and generating a phage library comprising the phage vectors; (c) transforming cells with the phage library; (d) fractioning the transformed cells and harvesting the organell from the transformed cells; (e) amplifying the phages from the harvested organelles; (f) transforming cells with the amplified phages from the harvested organelle; (g) repeating steps (d), (e), (f), and (g); (h) titering the phages from the harvested organelles from each round; (i) selecting the phages with the highest titer and obtaining the sequences of the mutant polynucleotide from the phage; and (j) producing polypeptides encoded by the sequences, wherein the polypeptides are the drug delivery molecules that target organelles. The carrier further comprises a polylysine motif and a targeting domain, for example the targeting domain of heregulin.

The invention further provides a therapeutic agent comprising the carrier described above and a therapeutic drug. A therapeutic drug may be any drug that, for example, treats, inhibits, prevents, mitigates the effects of, reduce the severity of, reduce the likelihood of developing, slow the progression of and/or cure, a disease. Diseases targeted by the therapeutic agents

include but are not limited to carcinomas, sarcomas, lymphomas, leukemia, germ cell tumors, blastomas, antigens expressed on various immune cells, and antigens expressed on cells associated with various hematologic diseases, autoimmune diseases, and/or inflammatory diseases. Therapeutic agents may be a chemotherapeutic agent.

The invention also provides a method of producing a carrier without proliferative activity. The method comprises (a) obtaining a polynucleotide encoding the receptor binding domain of heregulin (Her) and generating mutants in the polynucleotide; (b) cloning the mutant Her polynucleotides into phage vectors and generating a phage library comprising the phage vectors; (c) transforming MDA-MB-435 cells with the phage library in the presence of mitotic inhibitors; (for example taxol); (d)fractioning the transformed cell and extracting the membrane fraction of the MDA-MB-435 cells; (e) harvesting membrane phages from the membrane fraction; (f) transforming the membrane phages into MDA-MB-435 cells in the presence of mitotic inhibitors; (g) repeating steps (d), (e), and (f); (h) monitoring MDA-MB-435 cell proliferation in each round and selecting the membrane phages with the lowest MDA-MB-435 cell proliferation; (i) obtaining the sequences of the Her polynucleotide mutants in the selected membrane phages; and (j) producing polypeptides encoded by the Her sequences and the penton base gene, wherein the polypeptides are the carrier without proliferative activity. Mutations in the Her gene may be any one or more of insertions, deletions, substitutions or a combination thereof.

The invention further provides a carrier for delivering therapeutics to the nucleus, comprising a polypeptide encoded by the mutant Her sequences wherein the mutant Her is obtained according to the method described above. The carrier further comprises a polypeptide encoding penton base protein and a polylysine motif. The penton base protein may be a mutant protein.

EXAMPLES

Example 1

Isolation of traffic-enhancing mutants.

Previously, a recombinant adenovirus penton base protein was developed to target and

deliver a variety of therapeutic molecules to tumor cells in vitro and in vivo (Agadjanian et al., 2012; Agadjanian et al., 2009; Agadjanian et al., 2006; Medina-Kauwe et al., 2001a; Medina-Kauwe et al., 2001b; Rentsendorj et al., 2008). Currently, the penton base recombinant protein (the same one that comprises the 'PB' domain of HerPBK10, used to target therapeutics to HER2+ tumor cells; (Agadjanian et al., 2012; Agadjanian et al., 2009; Agadjanian et al., 2006; MedinaKauwe et al., 2001b; Rentsendorj et al., 2008) proceeds through multiple cell entry routes after cell binding, some but not all of which support membrane penetration and entry into the cytosol (Rentsendorj et al., 2006). To improve upon this function and enhance the delivery and penetration of therapeutics into cell targets, a directed evolution approach was used to isolate penton base variants with enhanced cell penetration activity by using nuclear accumulation as a readout, as endosomal escape enables entry into the nucleus (Rentsendorj et al., 2006).

Procedures: This two-step process involves: 1. Creation of a mutant library through random mutagenesis, and 2. Introduction of a selective pressure to isolate variants with improved function, depending on the screening process. Here, isolation of phage that survive entry into the cytosol and/or nucleus will serve as the selective pressure, which is expected to yield variants with enhanced cell penetration activity (Summarized in Fig. 1). Accordingly, the inventors have randomly mutagenized the penton base gene and generated a library of mutants cloned into a K. T7 phage vector. Based on previously established directed evolution studies (Cherry et al., 1999; Shafikhani et al., 1997; Wan et al., 1998; You and Arnold, 1996), the inventors aimed for a mutation frequency of 1-4 amino acid changes (or 2-7 nucleotide changes) per gene. Based on the product yield using a specialized error-prone polymerase chain reaction (PCR) method (GeneMorphII Random Mutagenesis Kit; Stratagene, La Jolla, CA, USA), the inventors achieved an estimated mutation frequency of ~8 nucleotides/kb or 13.6 nucleotides per penton base gene (Fig. 2). The inventors inserted this product into a T7-Select phage vector and packaged recombinant phage to produce an amplified library titer of $5x10^{10}$ pfu/mL.

The library was panned onto HeLa cells (which express integrin receptors for binding and uptake of the PB protein). Phage were incubated on the cells at 4°C for 1h to promote receptor binding but not uptake, then cells were washed and incubated for 2h at 37°C to promoted internalization. After uptake, harvested cells underwent fractionation to isolate cytosolic and nuclear fractions. Phage amplified from each fraction then underwent

repeated biopanning, and corresponding sequential fractions were extracted from cell harvests (i.e. nuclear phage isolated from round 1 were amplified and added back to cells, followed by repeat isolation of nuclear fractions to re-obtain nuclear phage). After either two or three rounds of biopanning, phage isolated from each repeat fraction was titered to determine the relative enrichment of nuclear/cytosolic phage from the mutagenized library compared to phage displaying wild-type penton base.

Results: The non-mutagenized parent phage, T7-PB, yielded a nuclear/cytosolic phage titer ratio of less than 1, indicating that the proportion of phage arriving at the nucleus by 2h was less than the proportion of phage that remained in the cytoplasm (Fig. 3). In contrast, after 2 rounds of biopanning and isolation of nuclear phage (3-3a), a shift was observed toward higher nuclear accumulation compared to cytoplasmic retention, with a significant increase compared to T7-PB (P=0.05) (Fig. 3). Even phage isolated from 3 rounds of cytosolic fraction panning (1-1-1) showed a relative, though not highly significant (P=0.07), increase in nuclear partitioning compared to T7-PB (Fig. 3).

After three rounds of biopanning and isolation of cytosolic and nuclear mutants, the inventors sequenced clones picked randomly from each enriched population and found that the majority of clones isolated from both cytosolic and nuclear fractions encoded carboxy-[C-] terminal truncated protein (Fig. 4). The 287LDV and 340RGD integrin binding motifs located near the middle of the wild-type penton base (wt PB) linear sequence were not retained in most of the truncated clones. One of the truncated mutants contains the LDV but not RGD motif, whereas the remaining truncations lack both LDV and RGD motifs. The full-length clones isolated from the biopanning retain both LDV and RGD motifs but also contain several point mutations that introduce potential function-altering amino acid changes (Fig. 4). Among these are a Leu60Trp replacement in cytosolic fraction clone 111C; and Lys375Glu, Val449Met, and Pro469Ser amino acid changes in nuclear fraction clone 333F. To test the ability of each isolated variant to impart enhanced cytosolic and/or nuclear penetration, the intracellular trafficking of each will be compared to the parent protein by immunofluorescence and confocal microscopy, and confirmed by subcellular fractionation. Specifically, as the full-length mutants (111C and 333F) and mutant 331J retain the integrin binding motifs, these variants are tested in comparison to wt PB, as they are predicted to enter cells via integrin binding and uptake. Meanwhile, as the remaining truncated variants lack any receptor-binding motifs, these will be inserted into HerPBK10 to replace the PB

domain, and tested in comparison to parental HerPBK10, which enters cells via human epidermal growth factor receptor (HER) binding and uptake.

Example 2

Isolation of receptor-binding and endocytosis mutants with blunted signaling.

Rationale: The tumor-targeted cell penetration protein, HerPBK10, is specifically directed to the human epidermal growth factor receptor (HER) via inclusion of the receptor binding domain of heregulin, designated here as the 'Her' domain of HerPBK10 (Medina-Kauwe et al., 2001b). However, ligation of heregulin receptors can induce signaling that may result in tumor cell proliferation, differentiation, and in some cases, apoptosis, depending on several factors including receptor heterodimer ratio, ligand subtype, cell type, and presence of certain intracellular molecules (Aguilar et al., 1999; Lewis et al., 1996; Weinstein et al., 1998). The possibility of inducing adverse effects such as tumor progression leads to examining whether the selective pressure introduced by mitotic inhibitors can select receptor binding and endocytosis mutants that lack proliferative signaling. The inventors proposed testing this approach by generating a phage library displaying Her variants and screen the library for internalizing Her species lacking proliferative signaling by isolating internalized phage from quiescent cells, and re-panning on non-proliferating human breast cancer cells.

Procedure: A library of Her sequences containing different mutations distributed across the coding sequence will be produced by error-prone PCR and staggered extension, which entails repeated cycles of denaturation and brief annealing/extension after initial priming of template sequences (Zhao et al., 1998). The resulting library will be inserted into the appropriate vector arms for transfer into T7Select bacteriophage (which is developed to display whole proteins), and recombinant phage produced following manufacturer's instructions (Novagen, Gibbstown, NJ, USA). Based on biopanning of evolved AAV capsids, an initial titer of 10^12 phage will be added to MDA-MB-435 cells maintained in media containing mitotic inhibitor such as taxol, and at about 30-45 min later (the time required for binding and internalization; Medina-Kauwe et al., 2000) the cells will be harvested by trypsin/EDTA (to remove non-internalized phage) and membrane-extracted to isolate the vesicle fraction of crude virus (Qproteome Plasma Membrane Protein Kit,

Qiagen Inc., Valencia, CA, USA), which can then be isolated by CsCl banding. Three to four rounds of selection (i.e. adding isolated virus to fresh cells and repeating membrane extraction) will be performed and isolated virus will be characterized in the following ways. First, fresh cells receiving enriched virus will be fixed and processed for immunofluorescence using an anti-phage antibody (Sigma-Aldrich, St. Louis, MO, USA) to confirm that the isolated phage still internalize. Separate cells treated in parallel will be assessed for proliferation rate in comparison to mock and untreated cells, by metabolic assay. Second, the Her sequence from isolated phage will be excised and inserted into a bacterial expression vector for recombinant protein production (Medina-Kauwe et al., 2001a), then mutant Her tested for internalization and proliferative activity in MDA-MB-435 human breast cancer cells as described earlier, in comparison to parental Her as well as mock and untreated cells. The mutant clones will be sequenced to identify mutated regions, and inserted back into the HerPBK10 expression cassette, replacing parental 'Her'.

The various methods and techniques described above provide a number of ways to carry out the application. Of course, it is to be understood that not necessarily all objectives or advantages described can be achieved in accordance with any particular embodiment described herein. Thus, for example, those skilled in the art will recognize that the methods can be performed in a manner that achieves or optimizes one advantage or group of advantages as taught herein without necessarily achieving other objectives or advantages as taught or suggested herein. A variety of alternatives are mentioned herein. It is to be understood that some preferred embodiments specifically include one, another, or several features, while others specifically exclude one, another, or several features, while still others mitigate a particular feature by inclusion of one, another, or several advantageous features.

Furthermore, the skilled artisan will recognize the applicability of various features from different embodiments. Similarly, the various elements, features and steps discussed above, as well as other known equivalents for each such element, feature or step, can be employed in various combinations by one of ordinary skill in this art to perform methods in accordance with the principles described herein. Among the various elements, features, and steps some will be specifically included and others specifically excluded in diverse embodiments.

Although the application has been disclosed in the context of certain embodiments and examples, it will be understood by those skilled in the art that the embodiments of the

application extend beyond the specifically disclosed embodiments to other alternative embodiments and/or uses and modifications and equivalents thereof.

In some embodiments, the terms "a" and "an" and "the" and similar references used in the context of describing a particular embodiment of the application (especially in the context of certain of the following claims) can be construed to cover both the singular and the plural. The recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (for example, "such as") provided with respect to certain embodiments herein is intended merely to better illuminate the application and does not pose a limitation on the scope of the application otherwise claimed. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the application.

Preferred embodiments of this application are described herein, including the best mode known to the inventors for carrying out the application. Variations on those preferred embodiments will become apparent to those of ordinary skill in the art upon reading the foregoing description. It is contemplated that skilled artisans can employ such variations as appropriate, and the application can be practiced otherwise than specifically described herein. Accordingly, many embodiments of this application include all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the application unless otherwise indicated herein or otherwise clearly contradicted by context.

All patents, patent applications, publications of patent applications, and other material, such as articles, books, specifications, publications, documents, things, and/or the like, referenced herein are hereby incorporated herein by this reference in their entirety for all purposes, excepting any prosecution file history associated with same, any of same that is inconsistent with or in conflict with the present document, or any of same that may have a limiting affect as to the broadest scope of the claims now or later associated with the present document. By

way of example, should there be any inconsistency or conflict between the description, definition, and/or the use of a term associated with any of the incorporated material and that associated with the present document, the description, definition, and/or the use of the term in the present document shall prevail.

In closing, it is to be understood that the embodiments of the application disclosed herein are illustrative of the principles of the embodiments of the application. Other modifications that can be employed can be within the scope of the application. Thus, by way of example, but not of limitation, alternative configurations of the embodiments of the application can be utilized in accordance with the teachings herein. Accordingly, embodiments of the present application are not limited to that precisely as shown and described.

Throughout the specification and claims, unless the context requires otherwise, the word "comprise" or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

CLAIMS

WHAT IS CLAIMED IS:

1. A method of delivering a therapeutic agent to a subcellular location in a cell, comprising:
administering a composition comprising a carrier polypeptide to the cell, the carrier polypeptide comprising a penton base polypeptide, wherein:
the penton base polypeptide comprises one or more point mutations in SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 12, or SEQ ID NO: 20; or
the penton base polypeptide consists of the penton base polypeptide recited in SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 14, SEQ ID NO: 16, or SEQ ID NO: 18.

2. The method of claim 1, wherein the penton base polypeptide comprises the one or more point mutations in SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 12, or SEQ ID NO: 20.

3. The method of claim 2, wherein the penton base polypeptide comprises the penton base polypeptide recited in SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 12, or SEQ ID NO: 20.

4. The method of claim 1, wherein the penton base polypeptide consists of the penton base polypeptide recited in SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 14, SEQ ID NO: 16, or SEQ ID NO: 18.

5. The method of claim 1, wherein the carrier polypeptide comprises the penton base polypeptide recited in SEQ ID NO: 4 or SEQ ID NO: 20.

6. The method of any one of claims 1-5, wherein the composition further comprises a therapeutic drug.

7. The method of any one of claims 1-6, wherein the cell is a tumor cell.

8. A method for producing a drug delivery molecule that targets an organelle comprising:
   a. obtaining a polynucleotide encoding a penton base gene and generating mutants of the polynucleotide;
   b. cloning the mutant polynucleotide into a phage vector and generating a phage library comprising the phage vectors;
   c. transforming cells with the phage library;
   d. fractioning the transformed cells and harvesting the organelle from the transformed cells;
   e. amplifying the phages from the harvested organelles;
   f. transforming cells with the amplified phages from the harvested organelle;
   g. repeating steps (d), (e), and (f);
   h. titering the phages from the harvested organelles from each round;
   i. selecting the phages with the highest titer and obtaining the sequences of the mutant polynucleotide from the phage; and
   j. producing a mutant polypeptide encoded by one of the sequences,
   wherein the mutant polypeptide is the drug delivery molecule that target organelle.

9. The method of claim 8, wherein the organelle is selecting from the group consisting of mitochondrion, Golgi apparatus, endoplasmic reticulum, nucleus, ribosomes, plasma membrane and cytosol.

10. The method of claim 8 or 9, wherein the cells are mammalian or non-mammalian cells.

11. The method of any one of claims 8-10, wherein mutants are generated using any one or more of PCR-based methods, chemical mutagenesis, ultraviolet-induced mutagenesis or a combination thereof.

12. The method of any one of claims 8-11, wherein the drug delivery molecule comprises a targeting domain, an endosomolytic ligand domain and a positively charged domain.

13. A carrier polypeptide for delivering a therapeutic agent to a subcellular location in a cell, comprising a penton base polypeptide, wherein:

the penton base polypeptide comprises one or more point mutations in SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 12, or SEQ ID NO: 20; or the penton base polypeptide consists of the penton base polypeptide recited in SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 14, SEQ ID NO: 16, or SEQ ID NO: 18.

14. The carrier polypeptide of claim 13, wherein the penton base polypeptide comprises the one or more point mutations in SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 12, or SEQ ID NO: 20.

15. The carrier polypeptide of claim 14, wherein the penton base polypeptide comprises the penton base polypeptide recited in SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 12, or SEQ ID NO: 20.

16. The carrier polypeptide of claim 13, wherein the penton base polypeptide consists of the penton base polypeptide recited in SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 14, SEQ ID NO: 16, or SEQ ID NO: 18.

17. The carrier of claim 13, wherein the carrier polypeptide comprises the penton base polypeptide recited in SEQ ID NO: 4 or SEQ ID NO: 20.

18. The carrier polypeptide of any one of claims 13-17, further comprising a polylysine motif.

19. The carrier polypeptide of any one of claims 13-18, further comprising a targeting domain of heregulin.

20. A therapeutic agent comprising the carrier polypeptide of any one of claims 13-19 and a therapeutic drug.

21. The therapeutic agent of claim 20, wherein the therapeutic drug is a chemotherapeutic agent.

Figure 1

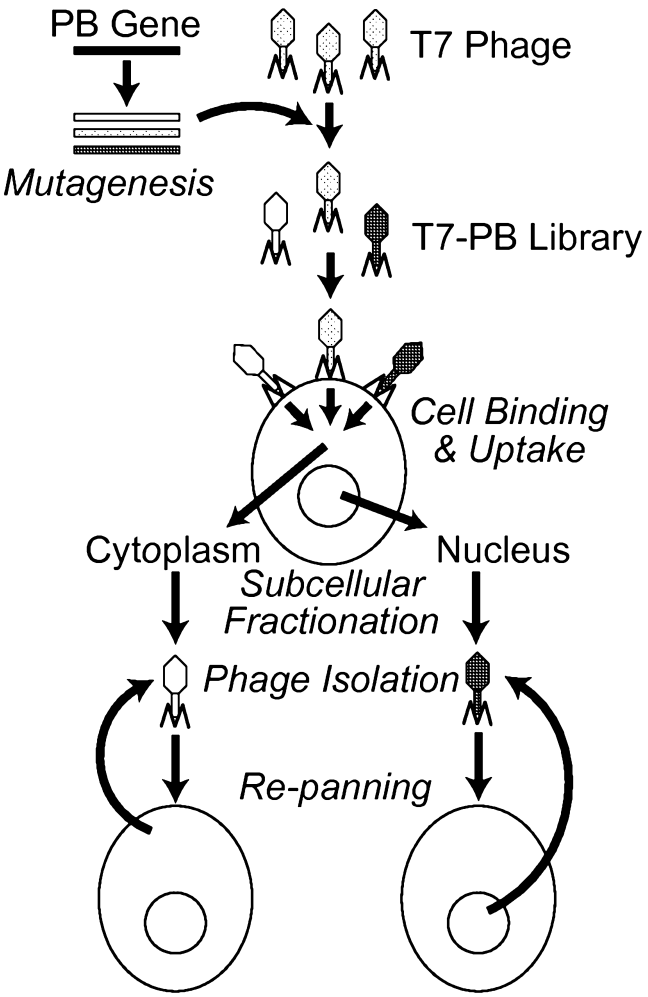


Figure 2

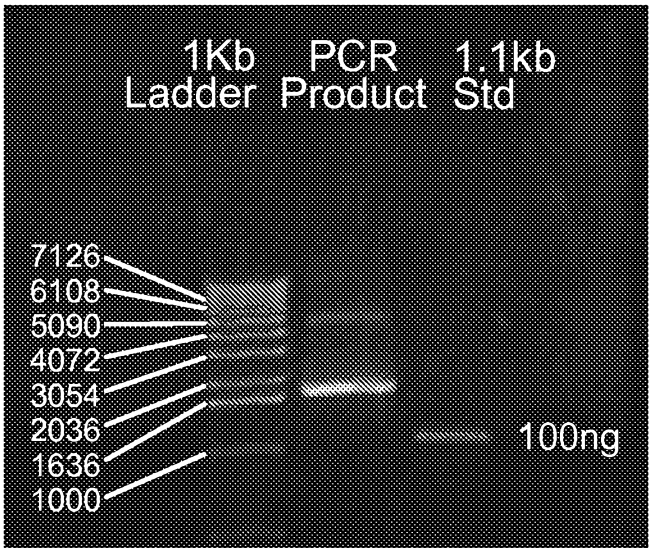


Figure 3

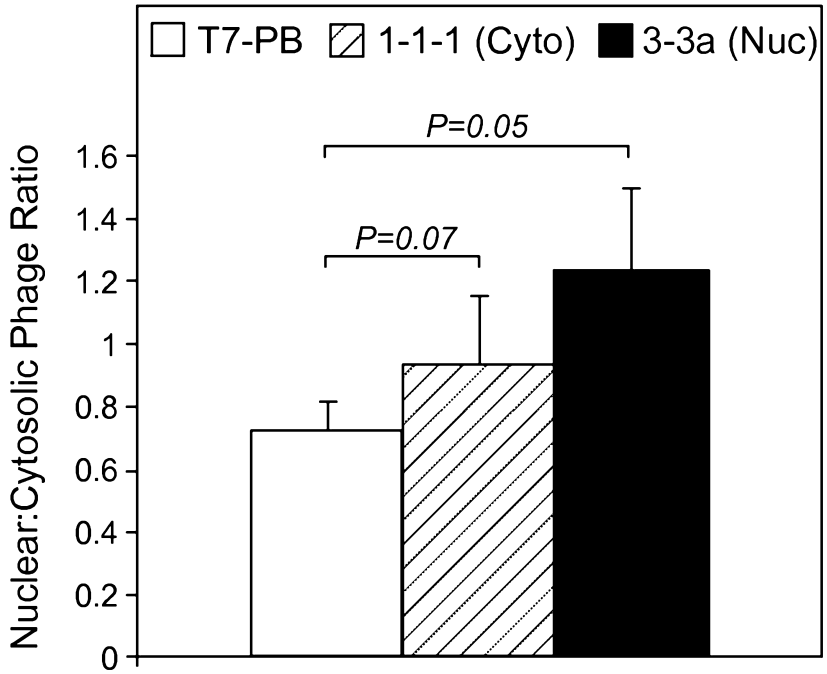


Figure 4

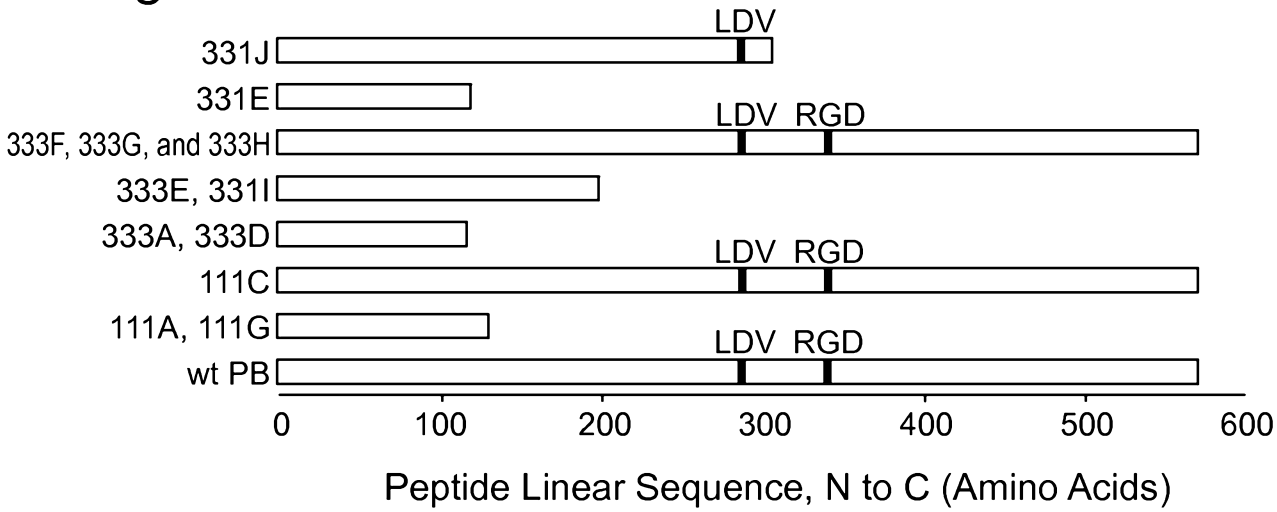


Figure 5

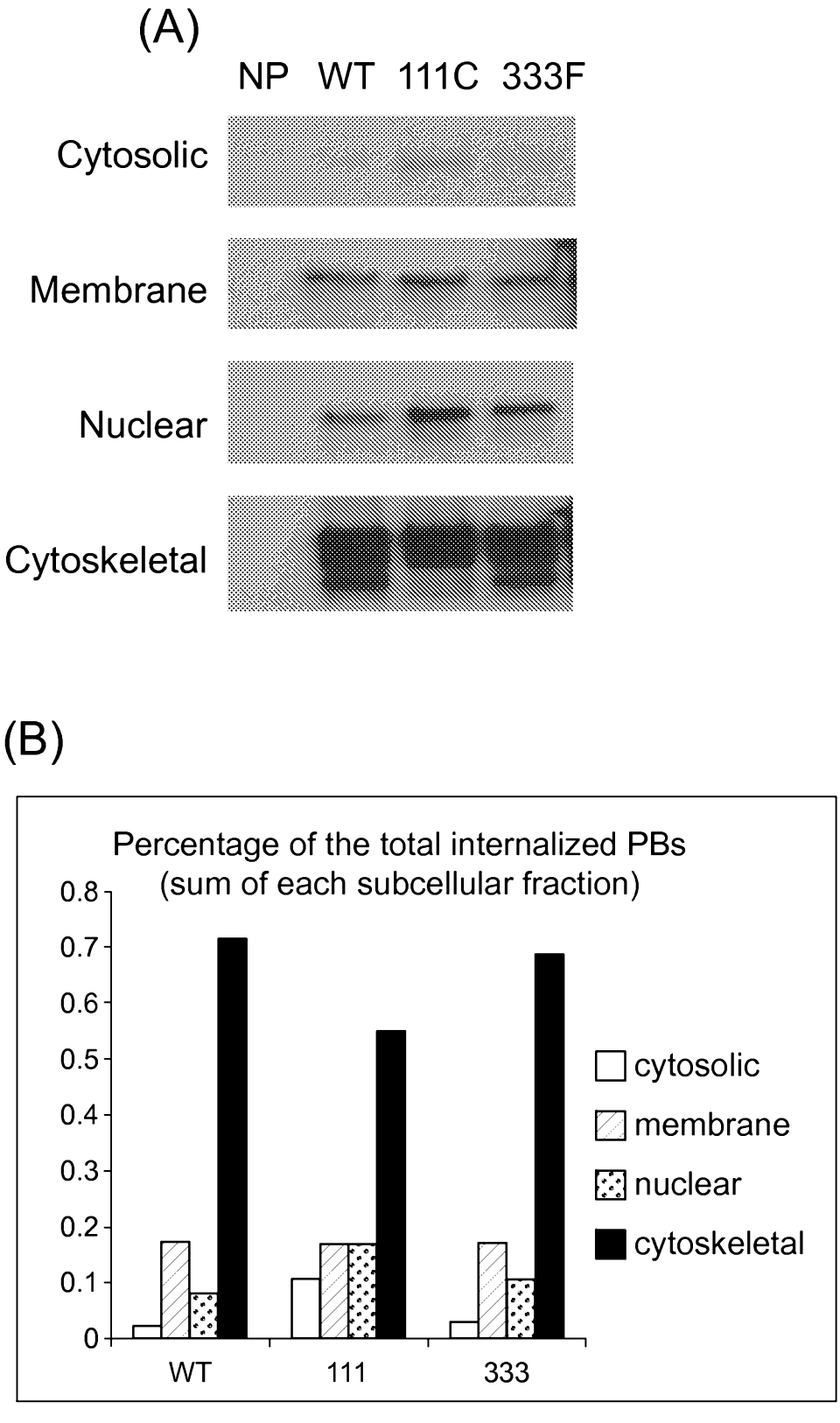
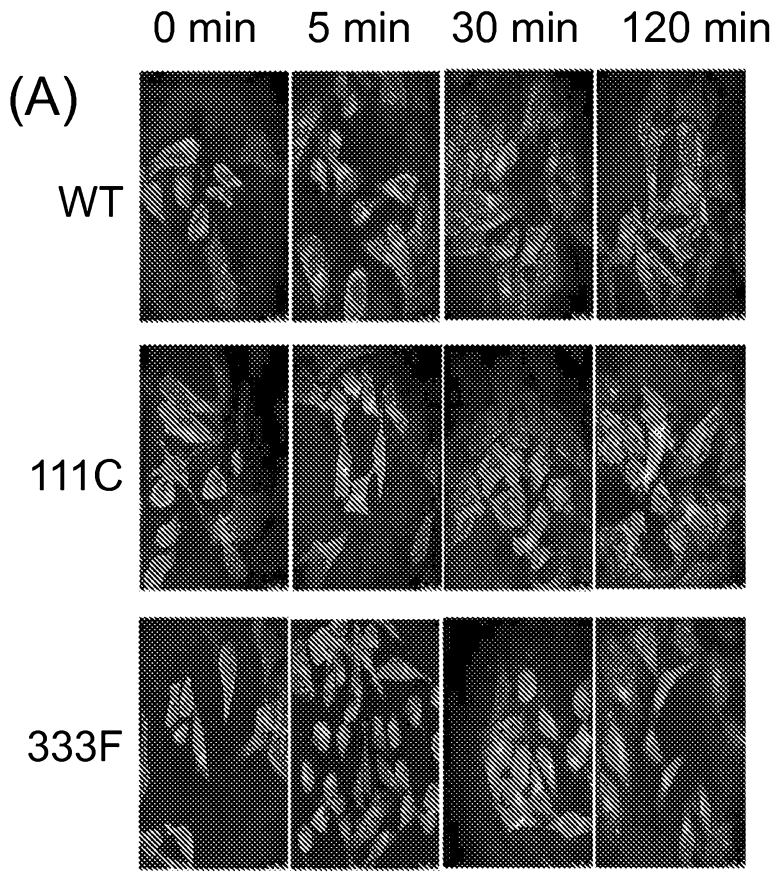

(A)
NP WT 111C 333F
Cytosolic
Membrane
Nuclear
Cytoskeletal
(B)
Percentage of the total internalized PBs
(sum of each subcellular fraction)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
WT
111
333
cytosolic
membrane
nuclear
cytoskeletal

Figure 6


0 min
5 min
30 min
120 min
(A)
WT
111C
333F
(B)
Nuclear Uptake
(Percent of total internalized protein)
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0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0 min
5 min
30 min
120 min
WT
111C
333F

Figure 7

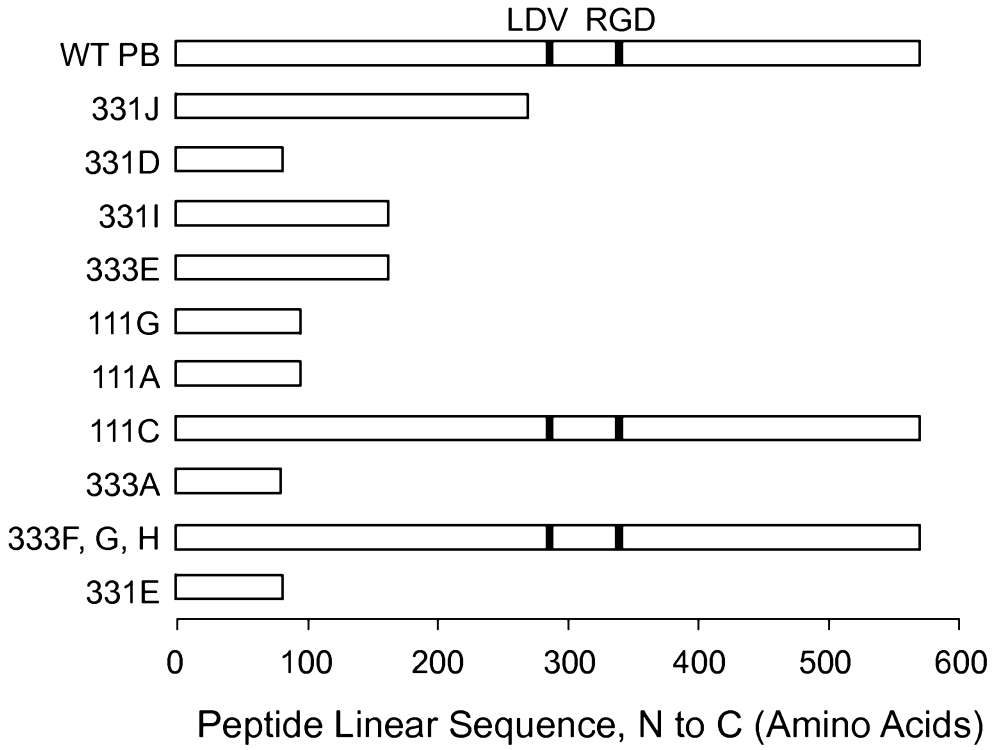
LDV
RGD
WT PB
331J
331D
331I
333E
111G
111A
111C
333A
333F, G, H
331E
0
100
200
300
400
500
600
Peptide Linear Sequence, N to C (Amino Acids)

Figure 8

Nucleic Acid Sequence:

TTTTGTTTACTTTAAGAAGGAGATATACAT
ATG CGG GGT TCT CAT CAT CAT CAT CAT CAT GGT ATG GCT AGC ATG
ACT GGT GGA CAG CAA ATG GGT CGG GAT CTG TAC GAC GAT GAC GAT
AAG GAT CGA TGG GGA AGC **ATG CGG CGC GCG GCG ATG TAT GAG GAA**
GGT CCT CCT CCC TCC TAC GAG AGT GTG GTG AGC GCG GCG CCA GTG
GCG GCG GCG CTG GGT TCT CCC TTC GAT GCT CCC CTG GAC CCG CCG
TTT GTG CCT CCG CGG TAC CTG CGG CCT ACC GGG GGG AGA AAC AGC
ATC CGT TAC TCT GAG TTG GCA CCC CTA TTC GAC ACC ACC CGT GTG
TAC CTG GTG GAC AAC AAG TCA ACG GAT GTG GCA TCC CTG AAC TAC
CAG AAC GAC CAC AGC AAC TTT CTG ACC ACG GTC ATT *T*AA AAC AAT
GAC TAC AGC CCG GGG GAG GCA AGC ACA CAG ACC ATC AAT CTT GAC
GAC CGG TCG CAC TGG GGC GG*T* GAC CTG AAA ACC ATC CTG CAT ACC
AAC ATG CCA AAT GTG AAC GAG TTC ATG TTT ACC AAT AAG TTT AAG
GCG CGG GTG ATG GTG TCG CGC TTG CCT ACT AAG GAC AAT CAG GTG
GAG CTG AAA TAC GAG TGG GTG GAG TTC ACG CTG CCC GAG GGC AAC
TAC TCC GAG ACC ATG ACC ATA GAC CTT ATG AAC AAC GCG ATC GTG
GAG CAC TAC TTG AAA GTG GGC AGA CAG AAC GGG GTT CTG GAA AGC
GAC ATC GGG GTA AAG TTT GAC ACC CGC AAC TTC AGA CTG GGG TTT
GAN CAC GTC ACT GGT CTT GTC ATG CCT GGG GTA TAT ACA AAC GAA
GCC NNC CAT CCA GAC ATC ATT TTG CTG CCA GGA TGC GGG GTG GAC
TTC ACC CAC AGC CGC CTG AGC AAC TTG TTG GGC ATC CGC AAG CGG
CAA CCC TTN CAG GAN GGC TTT AGG ATC NNC NAC GAT GAT CTG GAG
GGT GGT ANC ATT CCC GCA CTG (SEQ ID NO: 1).

Capital and italicized bases indicate mutations from wildtype PB

Mutations

Base 397 (wt 289) C > T, Glutamine > Stop codon
Base 471 (wt 363) C > T, Glycine > Glycine

Predicted Amino Acid Sequence:

MRRAAMYEEGPPPSYESVVSAAPVAAALGSPFDAPLDPPFVPPRYLRPTGGRNSIRYS
ELAPLFDTTRVYLVDNKSTDVASLNYQNDHSNFLTTVI (SEQ ID NO: 2)

Number of amino acids: 96

Molecular weight: 10531.8

Theoretical pI: 5.15

Figure 9

Nucleic Acid Sequence:

TTTAAGAAGGAGANATACAT

ATG CGG GGT TCT CAT CAT CAT CAT CAT CAT GGT ATG GCT AGC ATG
ACT GGT GGA CAG CAA ATG GGT CGG GAT CTG TAC GAC GAT GAC GAT
AAG GAT CGA TGG GGA TCC A***C***G CGG CGC GCG GCG ATG TAT GAG GAA
GGT CCT CCT CCC TCC TAC GAG AGT GTG GTG AGC GCG GCG CCA GTG
GCG GCG GCG CTG GGT TCT CCC TTC GAT GCT CCC CTG GAC CCG CCG
TTT GTG CCT CCG CGG TAC CTG CGG CCT ACC GGG GGG AGA AAC AGC
ATC CGT TAC TCT GAG T***G***G GCA CCC CTA TTC GAC ACC ACC CGT GTG
TAC CTG GTG GAC AAC AAG TCA ACG GAT GTG GCA TCC CTG AAC TAC
CAG AAC GAC CAC AGC AAC TTT CTG ACC ACG GTC ATT CAA AAC AAT
GAC TAC AGC CCG GGG GAG GCA AGC ACA CAG ACC ATC AAT CTT GAC
GAC CGG TCG CAC TGG GGC GGC GAC CTG AAA ACC ATC CTG CAT ACC
AAC ATG CCA AAT GTG AAC GAG TTC ATG TTT ACC AAT AAG TTT AAG
GCG CGG GTG ATG GTG TCG CGC TTG CCT ACT AAG GAC AAT CAG GTG
GAG CTG AAA TAC GAG TGG GTG GAG TTC ACG CTG CCC GAG GGC AAC
TAC TCC GAG ACC ATG ACC ATA GAC CTT ATG AAC AAC GCG ATC GTG
GAG CAC TAC TTG AAA GTG GGC AGA CAG AAC GGG GTT CTG GAA AGC
GAC ATC GGG GTA AAG TTT GAC ACC CGC AAC TTC AGA CTG GGG TTT
GAC CCC GTC ACT GGT CTT GTC ATG CCT GGG GTA TAT ACA AAC GAA
GCC TTC CAT CCA GAC ATC ATT TTG CTG CCA GGA TGC GGG GTG GAC
TTC ACC CAC AGC CGC CTG ANC AAC TTG TTG GGC ATC CGC AAG CGG
CAN CCC TTC CAG GAG GNN TTT AGG ATC ANC NAC GAT GAT CNG GAG
GGN NGN ANN ATN CCC GCA CTG (SEQ ID NO: 3)

Capital and italicized bases indicate mutations from wildtype PB

Mutations
Base 110 (wt 2) T > C, Methionine > Threonine
Base 179 (wt 71) T > G, Leucine > Tryptophan
Base 1044 C > T, Serine > Serine
Base 1518 G > A, Valine > Valine

Predicted Amino Acid Sequence:

MRGSHHHHHHGMASMTGGQQMGRDLYDDDDKDRWGSTRRAAMYEEGPPPSYES
VVSAAPVAAALGSPFDAPLDPPFVPPRYLRPTGGRNSIRYSEWAPLFDTTRVYLVDN
KSTDVASLNYQNDHSNFLTTVIQNNDYSPGEASTQTINLDDRSHWGGDLKTILHTNM
PNVNEFMFTNKFKARVMVSRLPTKDNQVELKYEWVEFTLPEGNYSETMTIDLMNN
AIVEHYLKVGRQNGVLESDIGVKFDTRNFRLGFDPVTGLVMPGVYTNEAFHPDIILLP
GCGVDFTHSRLXNLLGIRKRXPFQEXFRIXXDDXEXXXXPAL (SEQ ID NO: 4)

Figure 10

Nucleic Acid Sequence:

TTTACTTTAAGAAGGAGANATACAT

ATG CGG GGT TCT CAT CAT CAT CAT CAT CAT GGT ATG GCT AGC ATG ACT GGT
GGA CAG CAA ATG GGT CGG GAT CTG TAC GAC GAT GAC GAT AAG GAT CGA TGG
GGT TCT **ATG CGG CGC GCG GCG ATG TAT GAG GAA GGT CCT CCT CCC TCC TAC**
GAG AGT GCG GTG AGC GCG GCG CCA GTG GCG GCG GCG CTG GGT TCT CCC TTC
GAT GCT CCC CTG GAC CCG CCG TTT GTG CCT CCG CGG TAC CTG CGG CCT ACC
GGG GGG AGA AAC AGC ATC CGT TAC TCT GAG TTG GCA CCC CTA TTC GAC ACC
ACC CGT GTG TAC CTG GTG GAC AAC AAG TCA ACG GAT GTG GCA TCC CTG AAC
TAC CAG AAC GAC CAC AGC AAC TTT CTG ACC ACG GTC ATT *TAA* AAC AAT GAC
TAC AGC CCG GGG GAG GCA AGC ACA CAG ACC ATC AAT CTT GAC GAC CGG TCG
CAC TGG GGC GGC GAC CTG AAA ACC ATC CTG CAT ACC AAC ATG CCA AAT GTG
AAC GAG TTC ATG TTT ACC AAT AAG TTT AAG GCG CGG GTG ATG GTG TCG CGC
TTG CCT ACT AAG GAC AAT CAG GTG GAG CTG AAA TAC GAG TGG GTG GAG TTC
ACG CTG CCC GAG GGC AAC TA***A*** TCC GAG ACC ATG ACC ATA GAC CTT ATG AAC
AAC GCG ATC GTG GAG CAC TAC TTG AAA GTG GGC AGA CAG AAC GGG GTT CTG
GAA AGC GAC ATC GGG GTA AAG TTT GAC ACC CGC AAC TTC AGA CTG GGG TTT
GAC C***A***C GTC ACT GGT CTT GTC ATG CCT GGG GTA TAT ACA AAC GAA GCC TTC
CAT CCA GAC ATC ATT TTG CTG CCA GGA TGC GGG GTG GAC TTC ACC CAC AGC
CGC CTG ANC AAC TTG NTG GGC ATC CGC AAG CGG CAA CCN TTN CAG GAG GGC
TTT AGG ATC ANC TAC GAT GAT CTG GAG GGT G (SEQ ID NO: 5)

Capital and italicized bases indicate mutations from wildtype

Mutations

Base 161 (wt 53) T > C, Valine > Alanine
Base 398 (wt 290) C > T, Glutamine > Stop
Base 633 (wt 525) C > A, Tyrosine > Stop
Base 780 (wt 672) C > A, Proline > Histidine

Amino Acid Sequence :

MRRAAMYEEGPPPSYESAVSAAPVAAALGSPFDAPLDPPFVPPRYLRPTGGRNSIRYSELAPL
FDTTRVYLVDNKSTDVASLNYQNDHSNFLTTVI (SEQ ID NO: 6)

Number of amino acids: 96

Molecular weight: 10503.7

Theoretical pI: 5.15

Figure 11

Nucleic Acid Sequence:

TTAAGAAGGAGANATACAT

ATG CGG GGT TCT CAT CAT CAT CAT CAT CAT GGT ATG GCT AGC ATG ACT GGT
GGA CAG CAA ATG GGT CGG GAT CTG TAC GAC GAT GAC GAT AAG GAT CGA TGG
GGT TCC **ATG CGG CGC GCG GCG ATG TAT GAG GAA GGT CCT CCT CCC TAC TAC**
GAG AGT GTG GTG AGC GCG GCG CCA GTG GCG GCG GCG CTG GGT TCT ACC TTC
GAT GCT CCC CTG GAC CCG CCG TTT GTG CCT CCG CGG TAC CTG CGG CCT ACC
GGG GGG AGA AAC AGC ATC CGT TAC TCT GAG TTG GCA CCC CTA TTC GAC ACC
ACC CGT GTG TAC CTG GTG GAC AAC AAG TCA ACG GAT GTG GCA TCC CTG AAC
TAC *TAG* AAC GAC CAC AGC AAC TTT CTG ACC ACG GTC ATT CAA AAC AAT GAC
TAC AGC CCG GGG GAG GCA AGC ACA CAG ACC ATC AAT CTT GAC GAC CGG TCG
CAC TGG GGC GGC GAC CTG AAA ACC ATC CTG CAT ACC AAC ATG CCA AAT GTG
AAC GAG TTC ATG T***A***T ACC AAT AAG TTT AAG GCG CGG GTG ATG GTG TCG CGC
TTG CCT ACT AAG GAC AAT CAG GTG GAG CTG AAA TAC GAG TGG GTG G***G***G TTC
ACG CTG CCC GAG GGC AAC TAC TCC GAG ACC ATG ACC ATA GAC CTT ATG AAC
AAC GCG ATC GTG GAG CAC TAC TTG AAA GTG GGC AGA CAG AAC GGG GTT CTG
GAA AGC GAC ATC GGG GTA AAG TTT GAC ACC CGC AAC TTC AGA CTG NGG TTT
GAC CCC GTC ACT GGT CTT GTC ATG CCT GGG GTA TAT ACA AAC GAA GCC TTC
CAT CCA GAC ATC ATT TTG CTG CCA GGA TGN GGG GTG GNC TTC ACC CAC AGC
CGC CNG AGC AAC TTG TTG GGC ATC CGC AAG CNG CAA CCN TTC TAG GAG GGC
TTT ANG ATC ACC TAC NAT GAT CTG GAG GGN (SEQ ID NO: 7)

Mutations

Base 149 (wt 41) C > A, Serine > Tyrosine
Base 199 (wt 91) C > A, Proline > Threonine
Base 361 (wt 253) C > T, Glutamine > Stop
Base 524 (wt 416) T > A Phenylalanine > Tyrosine
Base 608 (wt 500) A > G Glutamate > Glycine

Predicted Amino Acid Sequence:

MRRAAMYEEGPPPYYESVVSAAPVAAALGSTFDAPLDPPFVPPRYLRPTGGRNSIRYSELAPLFDTTR
VYLVDNKSTDVASLNY (SEQ ID NO: 8)

Number of amino acids: 84

Molecular weight: 9241.4

Theoretical pI: 5.17

Figure 12

Nucleic Acid Sequence:

TTTAAGAAGGAGANATACAT

ATG CGG GGT TCT CAT CAT CAT CAT CAT CAT GGT ATG GCT AGC ATG
ACT GGT GGA CAG CAA ATG GGT CGG GAT CTG TAC GAC GAT GAC GAT
AAG GAT CGA TGG CGT TCC **ATG CGG CGC GCG GCG ATG TAT GAG GAA**
GGT CCT CCT CCC TCC TAC GAG AGT GTG GTG AGC GCG GCG CCA GTG
GCG GCG GCG CTG GGT TCT CCC TTC GAT GCT CCC CTG GAC CCG CCG
TTT GTG CCT CCG CGG TAC CTG CGG CCT ACC GGG GGG AGA AAC AGC
ATC CGT TAC TCT GAG TTG GCA CCC CTA TTC GAC ACC ACC CGT GTG
TAC CTG GTG GAC AAC AAG TCA ACG GAT GTG GCA TCC CTG AAC TAC
CAG AAC GAC CAC AGC AAC TTT CTG ACC ACG GTC ATT CAA AAC AAT
GAC TAC AGC CCG GGG GAG GCA AGC ACA CAG ACC ATC AAT CTT GAC
GAC CGG TCG CAC TGG GGC GGC GAC CTG AAA ACC ATC CTG CAT ACC
AAC ATG CCA AAT GTG AAC GAG TTC ATG TTT ACC AAT AAG TTT AAG
GCG CGG GTG ATG GTG TCG CGC TTG CCT ACT AAG GAC AAT CAG GTG
GAG CTG AAA TAC GAG TAG GTG GAG TTC ACG CTG CCC GAG GGC AAC
TAC TCC GAG ACC ATG ACC ATA GAC CTT ATG AAC AAC GCG ATC GTG
GAG CAC TAC TTG AAA GTG GGC AGA CAG AAC GGG GTT CTG GAA AGC
GAC ATC GGG GTA AAG TTT GAC ACC CGC AAC TTC AGA CTG GGG TTT
GAC CCC GTC ACT GGT CTT GTC ATG CCT GGG GTA TAT ACA AAC GAA
GCC TTC CAT CCA GAC ATC ATT TTG CTG CCA GGA TGC GGG GTG GNC
TTC ACC CAC AGC CGC CTG AGC AAC TTG TTG GGC ATC CNC AAG CNG
CAA CCC NTT CCA GGA GGG CTT TAG GAT CAC CTA CGA TGA TCT GGA
GGG (SEQ ID NO: 9)

Mutations

Base 602 (wt 494) G > A, Glutamine > Stop

Predicted Amino Acid Sequence:

MRRAAMYEEGPPPSYESVVSAAPVAAALGSPFDAPLDPPFVPPRYLRPTGGRNSIRYS
ELAPLFDTTRVYLVDNKSTDVASLNYQNDHSNFLTTVIQNNDYSPGEASTQTINLDD
RSHWGGDLKTILHTNMPNVNEFMFTNKFKARVMVSRLPTKDNQVELKYE (SEQ ID
NO: 10)

Number of amino acids: 164

Molecular weight: 18400.5

Theoretical pI: 5.58

Figure 13

Nucleic Acid Sequence:

TTTAAGAAGGAGANATACAT

ATG CGG GGT TCT CAT CAT CAT CAT CAT CAT GGT ATG GCT AGC ATG ACT GGT
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GAG AGT GTG GTG AGC GCG GCG CCA GTG GCG GCG GCG CTG GGT TCT CCC TTC
GAT GCT CCC CTG GAC CCG CCG TTT GTG CCT CCG CGA TAC CTG CGG CCT ACC
GGG GGG AGA AAC AGC ATC CGT TAC TCT GAG TTG GCA CCC CTA TTC GAC ACC
ACC CGT GTG TAC CTG GTG GAC *T*AC AAG TCA ACG GAT GTG GCA TCC CTG AAC
TAC CAG AAC GAC CAC AGC AAC TTT CTG ACC ACG GTC ATT CAA AAC AAT GAC
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CAC TGG GGC GGC GAC CTG AAA ACC ATC CTG CAT ACC AAC ATG CCA AAT GTG
AAC GAG TTC ATG TTT ACC AAT AAG TTT AAG GCG CGG GTG ATG GTG TCG CGC
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AAC GCG ATC GTG GAG CAC TAC TTG AAA GTG GGC AGA CAG AAC GGG GTT CTG
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GAC CCC GTC ACT GGT CTT GTC ATG CCT GGG GTA TAT ACA AAC GAA GCC NTC
CAT CCA GAC ATC ATT TTG CTG CCA GGA TGC GGG GTG NAC TTC ACC CAC AGC
CGC CTG AGC AAC TTG TTG GGC ATC CGC AAG CGG CAA CCC NTN CCA GGA GGG
CTT TAG GNT CAC CTA CGA TGA TCT GGA GGG NTG GNA NCA TTC CCG CNC TGT
CTG CCA GGA TGC GGG GTG GAC TTC ACC CAC AGC CGC CTG AGC AAC TTG TTG
GGC ATC CGC AAG CGG CAA CCC TT CCA GGA GGG CTT TAG GAT CAC CTA C
GCT TCG (SEQ ID NO: 11)

Mutations

Base 285 (wt 178) A > G, Arg > Arg
Base 328 (wt 220) A > T, Asn > Tyr
Base 616 (wt 508) C > A, Leu > Met

Predicted Amino Acid Sequence:

MRRAAMYEEGPPPSYESVVSAAPVAAALGSPFDAPLDPPFVPPRYLRPTGGRNSIRYSELAPL
FDTTRVYLVDYKSTDVASLNYQNDHSNFLTTVIQNNDYSPGEASTQTINLDDRSHWGGDLK
TILHTNMPNVNEFMFTNKFKARVMVSRLPTKDNQVELKYEWVEFTMPEGNYSETMTIDLMN
NAIVEHYLKVGRQNGVLESDIGVKFDTRNFRLGFDPVTGLVMPGVYTNEAXHPDIILLPGCG
VXFTHSRLSNLLGIRKRQPXPGGL (SEQ ID NO: 12)

Number of amino acids: 271

Molecular weight: 30339.9

Theoretical pI: 5.70

Figure 14

Nucleic Acid Sequence:

TTTAAGAAGGAGANATACAT

ATG CGG GGT TCT CAT CAT CAT CAT CAT CAT GGT ATG GCT AGC ATG ACT GGT
GGA CAG CAA ATG GGT CGG GAT CTG TAC GAC GAT GAC GAT AAG GAT CGA TGG
PCR Tag **ATG CGG CGC GCG GCG ATG TAT GAG GAA GGT CCT CCT CCC TCC TAC**
GAG AGT GTG GTG AGC GCG GCG CCA GTG GCG GCG GCG CTG GGT TCT CCC TTC
GAT GCT CCC CTG GAC CCG CCG TTT GTG CCT CCG CGG TAC CTG CGG CCT ACC
GGG GGG AGA AAC AGC ATC CGT TAC TCT GAG TTG GCA CCC CTA TTC GAC ACC
ACC CGT GTG TAC CTG GTG GAC AAC AAG TCA ACG GTG TGG CAT CCC TGA ACT
ACC AGA ACG ACC ACA GCA ACT TTC TGA CCA CGG TCA TTC AAA ACA GTG ACT
ACA GCC CGG GGG AGG CAA GCA CAC AGA CCA TCA ATC TTG ACG ACC GGT CGC
ACT GGG GCG GCG ACC TGA AAA CCA TCC TGC ATA CCA ACA TGC CAA ATG TGA
ACG AGT TCA TGT TTA CCA ATA AGT TTA AGG CGC GGG TGA TGG TGT CGC GCT
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AAT GCG ACA TCG GGG TAA AGT TTG ACA CCC GCA ACT TCA GAC TGG GGT TTG
ATC CCG TCA CTG GTC TTG TCA TGN CTG GGG TAT ATA CAA ACG AAG CCT TCC
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GCC TGA GCA ACT TGN TGG GCA TCC GCA TGC GGC AAN CNT TNC NGG AGG GCT
TTA GGA TCA CCN ACG ATG ATC TGN AGG (SEQ ID NO: 13)

Mutations

Deletion at base 340 (wt 232) Causes a frame shift.
Wt amino acid sequence is: …Thr-Asp-Val-Ala-Ser-Leu…
Mutated sequence is: …Thr-Val-Trp-His-Pro-Stop

Predicted Amino Acid Sequence:

5'3' Frame 1
G S **Met** R R A A **Met** Y E E G P P P S Y E S V V S A A P V A A A L G S P F D A P L D P P F V
P P R Y L R P T G G R N S I R Y S E L A P L F D T T R V V Y L V D N K S T V W H P **Stop** (SEQ ID NO: 14)

Encodes 81 amino acids

Figure 15

Nucleic Acid Sequence:

TTTACTTTAAGAAGGAGANATACAT

ATG CGG GGT TCT CAT CAT CAT CAT NAT CAT GGT ATG GCT AGC ATG ACT GGT
GGA CAG CAA ATG GGT CGG GAT CTG TAC GAC GAT GAC GAT AAG GAT CGA TGG
GCA TCC **ATG CGG CGC GCG GCG ATG TAT GAG GAA GGT CCT CCT CCC TCC TAC**
GAG AGT GTG GTG AGC GCG GCG CCA GTG GCG GCG GCG CTG GGT TCT CCC TTC
GAT GCT CCC CTG GAC CCG CCG TTT GTG CCT CCG CGG TAC CTG CGG CCT ACC
GGG GGG AGA AAC AGC ATC CGT TAC TCT GAG TTG GCA CCC CTA TTC GAC ACC
ACC CGT GTG TAC CTG GTG GAC AAC AAG TCA ACG GTG TGG CAT CCC TGA ACT
ACC AGA ACG ACC ACA GCA ACT TTC TGA CCA CGG TCA TTC AAA ACA GTG ACT
ACA GCC CGG GGG AGG CAA GCA CAC AGA CCA TCA ATC TTG ACG ACC GGT CGC
ACT GGG GCG GCG ACC TGA AAA CCA TCC TGC ATA CCA ACA TGC CAA ATG TGA
ACG AGT TCA TGT TTA CCA ATA AGT TTA AGG CGC GGG TGA TGG TGT CGC GCT
TGC CTA CTA AGG ACA ATC AGG TGG AGC TGA AAT ACG AGT GGG TGG AGT TCA
CGC TGC CCG AGG GCA ACT ACT CCG AGA CCA TGA CCA TAG ACC TTA TGA ACA
ACG CGA TCG TGG AGC ACT ACT TGA AAG TGG GCA GAC AGA ACG GGG TTC TGG
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ATC CAG ACA TCA TTT TGC TGC CAG GAT GCG GGG TGG ACT TCA CCC ACA GCC
GCC TGA GCA ACT TGT TGG GCA TCC GCA TGC GGC AAC CCT TNC AGG AGG GCT
TTA GGA TCA CCT ACG ATG ATC TGN AGG GNN GNA NCA TTN CCC GCA CTG NTG
GAN GT (SEQ ID NO: 15)

Deletion at base 340 (wt 232) Causes a frame shift.

Predicted Amino Acid Sequence:

MRRAAMYEEG PPPSYESVVS AAPVAAALGS PFDAPLDPPFVPPRYLRPTG
GRNSIRYSELAPLFDTTRVY LVDNKSTVWHP (SEQ ID NO: 16)

Number of amino acids: 81

Molecular weight: 8918.1

Theoretical pI: 6.54

Figure 16

Nucleic Acid Sequence:

ATTTTGTTTACTTTAAGAAGGAGATATACAT

ATG CGG GGT TCT CAT CAT CAT CAT CAT CAT GGT ATG GCT AGC ATG ACT GGT
GGA CAG CAA ATG GGT CGG GAT CTG TAC GAC GAT GAC GAT AAG GAT CGA TGG
GGA TCC **ATG CGG CGC GCG GCG ATG TAT GAG GAA GGT CCT CCT CCC TCC TAC**
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GAT GCT CCC CTG GAC CCG CCG TTT GTG CCT CCG CGG TAC CTG CGG CCT ACC
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CAC TGG GGC GGC GAC CTG AAA ACC ATC CTG CAT ACC AAC ATG CCA AAT GTG
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GAC CCC GTC ACT GGT CTT GTC ATG CCT GGG GTA TAT ACA AAC GAA GCC TNN
CAT CCA GAC ATC ATT TTG CTG CCA GGA TGC GGG GTG GAC TTC ACC CAC AGC
CGC CTG AGC AAC TTG TTG GGC ATC CNC AAG CGG CAA CCC TTN CAG G (SEQ
ID NO: 17)

Mutations

Base 602 (wt 494) G > A Glutamine > Stop. Same mutation as 331I

Predicted Amino Acid Sequence:

MRRAAMYEEGPPPSYESVVSAAPVAAALGSPFDAPLDPPFVPPRYLRPTGGRNSIRYSELAPL
FDTTRVYLVDNKSTDVASLNYQNDHSNFLTTVIQNNDYSPGEASTQTINLDDRSHWGGDLK
TILHTNMPNVNEFMFTNKFKARVMVSRLPTKDNQVELKYE (SEQ ID NO: 18)

Number of amino acids: 164

Molecular weight: 18400.5

Theoretical pI: 5.58

Figure 17

Nucleic Acid Sequence:

Pieced Together Sequence from the 5'and 3'Data by Bradley Reinfeld

ATG CGG GGT TCT CAT CAT CAT CAT CAT CAT GGT ATG GCT AGC ATG ACT GGT
GGA CAG CAA ATG GGT CGG GAT CTG TAC GAC GAT GAC GAT AAG GAT CGA TGG
CCA TGC **ATG CGG CGC GCG GCG ATG TAT GAG GAA GGT CCT CCT CCC TCC TAC**
GAG AGT GTG GTG AGC GCG GCG CCA GTG GCG GCG GCG CTG GGT TCT CCC TTC
GAT GCT CCC CTG GAC CCA CCG TTT GTG CCT CCG CGG TAC CTG CGG CCT ACC
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ACC CGT GTG TAC CTG GTG GAC AAC AAG TCA ACG GAT GTG GCA TCC CTG AAC
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AGC AAC TTT CCG GTG GTT GGC GCC GAG CTG TTG TCC GTG CAC TCC AAG AGC
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C (SEQ ID NO: 19)

Figure 17 (continued)

Predicted Amino Acid Sequence:

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HVFNRFPENQILARPPAPTITTVSENVPALTDHGTLPLRNSIGGVQRVTITDARRRTCPYVYKAL
GIVSPRVLSSRTF (SEQ ID NO: 20)

Number of amino acids: 571

Molecular weight: 63320.2

Theoretical pI: 5.25

SEQUENCE LISTING

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65                  70                  75                  80

Tyr Leu Arg Pro Thr Gly Gly Arg Asn Ser Ile Arg Tyr Ser Glu Trp
                85                  90                  95

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            100                 105                 110

Thr Asp Val Ala Ser Leu Asn Tyr Gln Asn Asp His Ser Asn Phe Leu
        115                 120                 125

Thr Thr Val Ile Gln Asn Asn Asp Tyr Ser Pro Gly Glu Ala Ser Thr
    130                 135                 140

Gln Thr Ile Asn Leu Asp Asp Arg Ser His Trp Gly Gly Asp Leu Lys
145                 150                 155                 160

Thr Ile Leu His Thr Asn Met Pro Asn Val Asn Glu Phe Met Phe Thr
                165                 170                 175

Asn Lys Phe Lys Ala Arg Val Met Val Ser Arg Leu Pro Thr Lys Asp
            180                 185                 190

Asn Gln Val Glu Leu Lys Tyr Glu Trp Val Glu Phe Thr Leu Pro Glu
        195                 200                 205

Gly Asn Tyr Ser Glu Thr Met Thr Ile Asp Leu Met Asn Asn Ala Ile
    210                 215                 220

Val Glu His Tyr Leu Lys Val Gly Arg Gln Asn Gly Val Leu Glu Ser
225                 230                 235                 240

Asp Ile Gly Val Lys Phe Asp Thr Arg Asn Phe Arg Leu Gly Phe Asp
                245                 250                 255

Pro Val Thr Gly Leu Val Met Pro Gly Val Tyr Thr Asn Glu Ala Phe
            260                 265                 270

His Pro Asp Ile Ile Leu Leu Pro Gly Cys Gly Val Asp Phe Thr His
        275                 280                 285

Ser Arg Leu Xaa Asn Leu Leu Gly Ile Arg Lys Arg Xaa Pro Phe Gln
    290                 295                 300
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Glu Xaa Phe Arg Ile Xaa Xaa Asp Asp Xaa Glu Xaa Xaa Xaa Xaa Pro
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Ala Leu

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tcgatgggga tccatgcggc gcgcggcgat gtatgaggaa ggtcctcctc cctcctacga 180

gagtgcggtg agcgcggcgc cagtggcggc ggcgctgggt tctcccttcg atgctcccct 240

ggacccgccg tttgtgcctc cgcggtacct gcggcctacc ggggggagaa acagcatccg 300

ttactctgag ttggcacccc tattcgacac cacccgtgtg tacctggtgg acaacaagtc 360

aacggatgtg gcatccctga actaccagaa cgaccacagc aactttctga ccacggtcat 420

ttaaaacaat gactacagcc cgggggaggc aagcacacag accatcaatc ttgacgaccg 480

gtcgcactgg ggcggcgacc tgaaaaccat cctgcatacc aacatgccaa atgtgaacga 540

gttcatgttt accaataagt ttaaggcgcg ggtgatggtg tcgcgcttgc ctactaagga 600

caatcaggtg gagctgaaat acgagtgggt ggagttcacg ctgcccgagg gcaactaatc 660

cgagaccatg accatagacc ttatgaacaa cgcgatcgtg gagcactact tgaaagtggg 720

cagacagaac ggggttctgg aaagcgacat cggggtaaag tttgacaccc gcaacttcag 780

actggggttt gaccacgtca ctggtcttgt catgcctggg gtatatacaa acgaagcctt 840

ccatccagac atcattttgc tgccaggatg cggggtggac ttcacccaca gccgcctgan 900

caacttgntg ggcatccgca agcggcaacc nttncaggag ggctttagga tcanctacga 960

tgatctggag ggtg 974

<210> 6
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<212> PRT
<213> Homo sapiens

<400> 6

Met Arg Arg Ala Ala Met Tyr Glu Glu Gly Pro Pro Pro Ser Tyr Glu
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Ser Ala Val Ser Ala Ala Pro Val Ala Ala Ala Leu Gly Ser Pro Phe
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Asp Ala Pro Leu Asp Pro Pro Phe Val Pro Pro Arg Tyr Leu Arg Pro
35 40 45

Thr Gly Gly Arg Asn Ser Ile Arg Tyr Ser Glu Leu Ala Pro Leu Phe
50 55 60

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

Ser Leu Asn Tyr Gln Asn Asp His Ser Asn Phe Leu Thr Thr Val Ile
85 90 95

<210> 7
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catgactggt ggacagcaaa tgggtcggga tctgtacgac gatgacgata aggatcgatg 120

gggatccatg cggcgcgcgg cgatgtatga ggaaggtcct cctccctact acgagagtgt 180

ggtgagcgcg gcgccagtgg cggcggcgct gggttctacc ttcgatgctc ccctggaccc 240

gccgtttgtg cctccgcggt acctgcggcc taccgggggg agaaacagca tccgttactc 300

tgagttggca cccctattcg acaccacccg tgtgtacctg gtggacaaca agtcaacgga 360

tgtggcatcc ctgaactact agaacgacca cagcaacttt ctgaccacgg tcattcaaaa 420

caatgactac agcccggggg aggcaagcac acagaccatc aatcttgacg accggtcgca 480

ctggggcggc gacctgaaaa ccatcctgca taccaacatg ccaaatgtga acgagttcat 540

gtataccaat aagtttaagg cgcgggtgat ggtgtcgcgc ttgcctacta aggacaatca 600

ggtggagctg aaatacgagt gggtggggtt cacgctgccc gagggcaact actccgagac 660

catgaccata gaccttatga acaacgcgat cgtggagcac tacttgaaag tgggcagaca 720

gaacggggtt ctggaaagcg acatcggggt aaagtttgac acccgcaact tcagactgng 780

gtttgacccc gtcactggtc ttgtcatgcc tggggtatat acaaacgaag ccttccatcc 840

agacatcatt ttgctgccag gatgnggggt ggncttcacc cacagccgcc ngagcaactt 900

gttgggcatc cgcaagcngc aaccnttcta ggagggcttt angatcacct acnatgatct 960

ggagggn 967

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<211> 84
<212> PRT
<213> Homo sapiens

<400> 8

Met Arg Arg Ala Ala Met Tyr Glu Glu Gly Pro Pro Pro Tyr Tyr Glu
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Ser Val Val Ser Ala Ala Pro Val Ala Ala Ala Leu Gly Ser Thr Phe
20 25 30

Asp Ala Pro Leu Asp Pro Pro Phe Val Pro Pro Arg Tyr Leu Arg Pro
35 40 45

Thr Gly Gly Arg Asn Ser Ile Arg Tyr Ser Glu Leu Ala Pro Leu Phe
50 55 60

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

Ser Leu Asn Tyr

<210> 9
<211> 968
<212> DNA
<213> Homo sapiens

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ggggatccat gcggcgcgcg gcgatgtatg aggaaggtcc tcctccctcc tacgagagtg 180

tggtgagcgc ggcgccagtg gcggcggcgc tgggttctcc cttcgatgct cccctggacc 240

cgccgtttgt gcctccgcgg tacctgcggc ctaccggggg gagaaacagc atccgttact 300

ctgagttggc acccctattc gacaccaccc gtgtgtacct ggtggacaac aagtcaacgg 360

atgtggcatc cctgaactac cagaacgacc acagcaactt tctgaccacg gtcattcaaa 420

acaatgacta cagcccgggg gaggcaagca cacagaccat caatcttgac gaccggtcgc 480

actggggcgg cgacctgaaa accatcctgc ataccaacat gccaaatgtg aacgagttca 540

tgtttaccaa taagtttaag gcgcgggtga tggtgtcgcg cttgcctact aaggacaatc 600

aggtggagct gaaatacgag taggtggagt tcacgctgcc cgagggcaac tactccgaga 660

ccatgaccat agaccttatg aacaacgcga tcgtggagca ctacttgaaa gtgggcagac 720

agaacggggt tctggaaagc gacatcgggg taaagtttga cacccgcaac ttcagactgg 780

ggtttgaccc cgtcactggt cttgtcatgc ctggggtata tacaaacgaa gccttccatc 840

cagacatcat tttgctgcca ggatgcgggg tggncttcac ccacagccgc ctgagcaact 900

tgttgggcat ccncaagcng caacccnttc caggagggct ttaggatcac ctacgatgat 960

ctggaggg 968

<210> 10
<211> 164
<212> PRT
<213> Homo sapiens

<400> 10

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Ser Val Val Ser Ala Ala Pro Val Ala Ala Ala Leu Gly Ser Pro Phe
20 25 30

Asp Ala Pro Leu Asp Pro Pro Phe Val Pro Pro Arg Tyr Leu Arg Pro
35 40 45

Thr Gly Gly Arg Asn Ser Ile Arg Tyr Ser Glu Leu Ala Pro Leu Phe
50 55 60

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

Ser Leu Asn Tyr Gln Asn Asp His Ser Asn Phe Leu Thr Thr Val Ile
85 90 95

Gln Asn Asn Asp Tyr Ser Pro Gly Glu Ala Ser Thr Gln Thr Ile Asn
100 105 110

Leu Asp Asp Arg Ser His Trp Gly Gly Asp Leu Lys Thr Ile Leu His
115 120 125

Thr Asn Met Pro Asn Val Asn Glu Phe Met Phe Thr Asn Lys Phe Lys
130 135 140

Ala Arg Val Met Val Ser Arg Leu Pro Thr Lys Asp Asn Gln Val Glu
145 150 155 160

Leu Lys Tyr Glu

<210> 11
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ggggatccat gcggcgcgcg gcgatgtatg aggaaggtcc tcctccctcc tacgagagtg 180

tggtgagcgc ggcgccagtg gcggcggcgc tgggttctcc cttcgatgct cccctggacc 240

cgccgtttgt gcctccgcga tacctgcggc ctaccggggg gagaaacagc atccgttact 300

ctgagttggc acccctattc gacaccaccc gtgtgtacct ggtggactac aagtcaacgg 360

atgtggcatc cctgaactac cagaacgacc acagcaactt tctgaccacg gtcattcaaa 420

acaatgacta cagcccgggg gaggcaagca cacagaccat caatcttgac gaccggtcgc 480

actggggcgg cgacctgaaa accatcctgc ataccaacat gccaaatgtg aacgagttca 540

tgtttaccaa taagtttaag gcgcgggtga tggtgtcgcg cttgcctact aaggacaatc 600

aggtggagct gaaatacgag tgggtggagt tcacgatgcc cgagggcaac tactccgaga 660

ccatgaccat agaccttatg aacaacgcga tcgtggagca ctacttgaaa gtgggcagac 720

agaacggggt tctggaaagc gacatcgggg taaagtttga cacccgcaac ttcagactgg 780

ggtttgaccc cgtcactggt cttgtcatgc ctggggtata tacaaacgaa gccntccatc 840

cagacatcat tttgctgcca ggatgcgggg tgnacttcac ccacagccgc ctgagcaact 900

tgttgggcat ccgcaagcgg caacccntnc caggagggct ttaggntcac ctacgatgat 960

ctggagggnt ggnancattc ccgcnctgtc tgccaggatg cggggtggac ttcacccaca 1020

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tcacctacga tgatctg 1097

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<400> 12

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Ser Val Val Ser Ala Ala Pro Val Ala Ala Ala Leu Gly Ser Pro Phe

20 25 30

Asp Ala Pro Leu Asp Pro Pro Phe Val Pro Pro Arg Tyr Leu Arg Pro
35 40 45

Thr Gly Gly Arg Asn Ser Ile Arg Tyr Ser Glu Leu Ala Pro Leu Phe
50 55 60

Asp Thr Thr Arg Val Tyr Leu Val Asp Tyr Lys Ser Thr Asp Val Ala
65 70 75 80

Ser Leu Asn Tyr Gln Asn Asp His Ser Asn Phe Leu Thr Thr Val Ile
85 90 95

Gln Asn Asn Asp Tyr Ser Pro Gly Glu Ala Ser Thr Gln Thr Ile Asn
100 105 110

Leu Asp Asp Arg Ser His Trp Gly Gly Asp Leu Lys Thr Ile Leu His
115 120 125

Thr Asn Met Pro Asn Val Asn Glu Phe Met Phe Thr Asn Lys Phe Lys
130 135 140

Ala Arg Val Met Val Ser Arg Leu Pro Thr Lys Asp Asn Gln Val Glu
145 150 155 160

Leu Lys Tyr Glu Trp Val Glu Phe Thr Met Pro Glu Gly Asn Tyr Ser
165 170 175

Glu Thr Met Thr Ile Asp Leu Met Asn Asn Ala Ile Val Glu His Tyr
180 185 190

Leu Lys Val Gly Arg Gln Asn Gly Val Leu Glu Ser Asp Ile Gly Val
195 200 205

Lys Phe Asp Thr Arg Asn Phe Arg Leu Gly Phe Asp Pro Val Thr Gly
210 215 220

Leu Val Met Pro Gly Val Tyr Thr Asn Glu Ala Xaa His Pro Asp Ile
225 230 235 240

Ile Leu Leu Pro Gly Cys Gly Val Xaa Phe Thr His Ser Arg Leu Ser
245 250 255

Asn Leu Leu Gly Ile Arg Lys Arg Gln Pro Xaa Pro Gly Gly Leu
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<210> 13
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<212> DNA
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ggggatccat gcggcgcgcg gcgatgtatg aggaaggtcc tcctccctcc tacgagagtg 180

tggtgagcgc ggcgccagtg gcggcggcgc tgggttctcc cttcgatgct cccctggacc 240

cgccgtttgt gcctccgcgg tacctgcggc ctaccggggg gagaaacagc atccgttact 300

ctgagttggc acccctattc gacaccaccc gtgtgtacct ggtggacaac aagtcaacgg 360

tgtggcatcc ctgaactacc agaacgacca cagcaacttt ctgaccacgg tcattcaaaa 420

cagtgactac agcccggggg aggcaagcac acagaccatc aatcttgacg accggtcgca 480

ctggggcggc gacctgaaaa ccatcctgca taccaacatg ccaaatgtga acgagttcat 540

gtttaccaat aagtttaagg cgcgggtgat ggtgtcgcgc ttgcctacta aggacaatca 600

ggtggagctg aaatacgagt gggtggagtt cacgctgccc gagggcaact actccgagac 660

catgaccata gaccttatga acaacgcgat cgtggagcac tacttgaaag tgggcagaca 720

gaacggggtt ctggaatgcg acatcggggt aaagtttgac acccgcaact tcagactggg 780

gtttgatccc gtcactggtc ttgtcatgnc tggggtatat acaaacgaag ccttccatcc 840

agacatcatt ttgctgccag gatgcggggt ggacttcacc cacagccgcc tgagcaactt 900

gntgggcatc cgcatgcggc aancnttncn ggagggcttt aggatcaccn acgatgatct 960

gnagg 965

<210> 14
<211> 83
<212> PRT
<213> Homo sapiens

<400> 14
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Tyr Glu Ser Val Val Ser Ala Ala Pro Val Ala Ala Ala Leu Gly Ser
20 25 30
Pro Phe Asp Ala Pro Leu Asp Pro Pro Phe Val Pro Pro Arg Tyr Leu
35 40 45
Arg Pro Thr Gly Gly Arg Asn Ser Ile Arg Tyr Ser Glu Leu Ala Pro
50 55 60
Leu Phe Asp Thr Thr Arg Val Tyr Leu Val Asp Asn Lys Ser Thr Val
65 70 75 80
Trp His Pro

<210> 15
<211> 999
<212> DNA
<213> Homo sapiens

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<223> n is a, c, g, or t

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<223> n is a, c, g, or t

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<223> n is a, c, g, or t

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tcgatgggga tccatgcggc gcgcggcgat gtatgaggaa ggtcctcctc cctcctacga 180

gagtgtggtg agcgcggcgc cagtggcggc ggcgctgggt tctcccttcg atgctcccct 240

ggacccgccg tttgtgcctc cgcggtacct gcggcctacc ggggggagaa acagcatccg 300

ttactctgag ttggcacccc tattcgacac cacccgtgtg tacctggtgg acaacaagtc 360

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caaaacagtg actacagccc gggggaggca agcacacaga ccatcaatct tgacgaccgg 480

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gatctgnagg gnngnancat tncccgcact gntggangt 999

<210> 16
<211> 81
<212> PRT
<213> Homo sapiens

<400> 16

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Ser Val Val Ser Ala Ala Pro Val Ala Ala Ala Leu Gly Ser Pro Phe
20 25 30

Asp Ala Pro Leu Asp Pro Pro Phe Val Pro Pro Arg Tyr Leu Arg Pro
35 40 45

Thr Gly Gly Arg Asn Ser Ile Arg Tyr Ser Glu Leu Ala Pro Leu Phe
50 55 60

Asp Thr Thr Arg Val Tyr Leu Val Asp Asn Lys Ser Thr Val Trp His
65 70 75 80

Pro

<210> 17
<211> 944
<212> DNA
<213> Homo sapiens

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<223> n is a, c, g, or t

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<223> n is a, c, g, or t

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tggtatggct agcatgactg gtggacagca aatgggtcgg gatctgtacg acgatgacga 120

taaggatcga tggggatcca tgcggcgcgc ggcgatgtat gaggaaggtc ctcctccctc 180

ctacgagagt gtggtgagcg cggcgccagt ggcggcggcg ctgggttctc ccttcgatgc 240

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catccgttac tctgagttgg cacccctatt cgacaccacc cgtgtgtacc tggtggacaa 360

caagtcaacg gatgtggcat ccctgaacta ccagaacgac cacagcaact ttctgaccac 420

ggtcattcaa aacaatgact acagcccggg ggaggcaagc acacagacca tcaatcttga 480

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gaacgagttc atgtttacca ataagtttaa ggcgcgggtg atggtgtcgc gcttgcctac 600

taaggacaat caggtggagc tgaaatacga gtaggtggag ttcacgctgc ccgagggcaa 660

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cttcagactg gggtttgacc ccgtcactgg tcttgtcatg cctggggtat atacaaacga 840

agcctnncat ccagacatca ttttgctgcc aggatgcggg gtggacttca cccacagccg 900

cctgagcaac ttgttgggca tccncaagcg gcaacccttn cagg 944

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<212> PRT
<213> Homo sapiens

<400> 18

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Ser Val Val Ser Ala Ala Pro Val Ala Ala Ala Leu Gly Ser Pro Phe
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Asp Ala Pro Leu Asp Pro Pro Phe Val Pro Pro Arg Tyr Leu Arg Pro
35 40 45

Thr Gly Gly Arg Asn Ser Ile Arg Tyr Ser Glu Leu Ala Pro Leu Phe
50 55 60

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

Ser Leu Asn Tyr Gln Asn Asp His Ser Asn Phe Leu Thr Thr Val Ile
85 90 95

Gln Asn Asn Asp Tyr Ser Pro Gly Glu Ala Ser Thr Gln Thr Ile Asn
100 105 110

Leu Asp Asp Arg Ser His Trp Gly Gly Asp Leu Lys Thr Ile Leu His
115 120 125

Thr Asn Met Pro Asn Val Asn Glu Phe Met Phe Thr Asn Lys Phe Lys
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Ala Arg Val Met Val Ser Arg Leu Pro Thr Lys Asp Asn Gln Val Glu
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Leu Lys Tyr Glu

<210> 19
<211> 1888
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<223> n is a, c, g, or t

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gacaccaccc gtgtgtacct ggtggacaac aagtcaacgg atgtggcatc cctgaactac 360

cagaacgacc acagcaactt tctgaccacg gtcattcaaa acaatgacta cagcccgggg 420

gaggcaagca cacagaccat caatcttgac gaccggtcgc actggggcgg cgacctgaaa 480

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gcgcgggtga tggtgtcgcg cttgcctact aaggacaatc aggtggagct gaaatacgag 600

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aacaacgcga tcgtggagca ctacttgaaa gtgggcagac agaacggggt tctggaaagc 720

gacatcgggg taaagtttga cacccgcaac ttcagactgg ggtttgaccc cgtcactggt 780

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Asp Ala Pro Leu Asp Pro Pro Phe Val Pro Pro Arg Tyr Leu Arg Pro
35 40 45

Thr Gly Gly Arg Asn Ser Ile Arg Tyr Ser Glu Leu Ala Pro Leu Phe
50 55 60

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

Ser Leu Asn Tyr Gln Asn Asp His Ser Asn Phe Leu Thr Thr Val Ile
85 90 95

Gln Asn Asn Asp Tyr Ser Pro Gly Glu Ala Ser Thr Gln Thr Ile Asn
100 105 110

Leu Asp Asp Arg Ser His Trp Gly Gly Asp Leu Lys Thr Ile Leu His
115 120 125

Thr Asn Met Pro Asn Val Asn Glu Phe Met Phe Thr Asn Lys Phe Lys
130 135 140

Ala Arg Val Met Val Ser Arg Leu Pro Thr Lys Asp Asn Gln Val Glu
145 150 155 160

Leu Lys Tyr Glu Trp Val Glu Xaa Thr Leu Pro Glu Gly Asn Tyr Ser
165 170 175

Glu Thr Met Thr Ile Asp Leu Met Asn Asn Ala Ile Val Glu His Tyr
180 185 190

Leu Lys Val Gly Arg Gln Asn Gly Val Leu Glu Ser Asp Ile Gly Val
195 200 205

Lys Phe Asp Thr Arg Asn Phe Arg Leu Gly Phe Asp Pro Val Thr Gly
210 215 220

Leu Val Met Pro Gly Val Tyr Thr Asn Glu Ala Phe His Pro Asp Ile
225 230 235 240

Ile Leu Leu Pro Gly Cys Gly Val Asp Phe Thr His Ser Arg Leu Ser
245 250 255

Asn Leu Leu Gly Ile Arg Lys Arg Gln Pro Phe Gln Glu Gly Phe Arg
260 265 270

Ile Thr Tyr Asp Asp Leu Glu Gly Gly Asn Ile Pro Ala Leu Leu Asp
275 280 285

Val Asp Xaa Tyr Gln Ala Ser Leu Lys Asp Asp Thr Glu Gln Gly Gly
290 295 300

Gly Gly Ala Gly Gly Ser Asn Ser Ser Gly Ser Gly Ala Glu Glu Asn
305 310 315 320

Ser Asn Ala Ala Ala Ala Ala Met Gln Pro Val Glu Asp Met Asn Asp
325 330 335

His Ala Ile Arg Gly Asp Thr Phe Ala Thr Arg Ala Glu Glu Lys Arg
340 345 350

Ala Glu Ala Glu Ala Ala Ala Glu Ala Ala Ala Pro Ala Ala Gln Pro
355 360 365

Glu Val Glu Lys Pro Gln Glu Lys Pro Val Ile Lys Pro Leu Thr Glu
370 375 380

Asp Ser Lys Lys Arg Ser Tyr Asn Leu Ile Ser Asn Asp Ser Thr Phe
385 390 395 400

Thr Gln Tyr Arg Ser Trp Tyr Leu Ala Tyr Asn Tyr Gly Asp Pro Gln
405 410 415

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Gly Ser Glu Gln Val Tyr Trp Ser Leu Pro Asp Met Met Gln Asp Pro
        435                 440                 445

Met Thr Phe Arg Ser Thr Arg Gln Ile Ser Asn Phe Pro Val Val Gly
    450                 455                 460

Ala Glu Leu Leu Ser Val His Ser Lys Ser Phe Tyr Asn Asp Gln Ala
465                 470                 475                 480

Val Tyr Ser Gln Leu Ile Arg Gln Phe Thr Ser Leu Thr His Val Phe
                485                 490                 495

Asn Arg Phe Pro Glu Asn Gln Ile Leu Ala Arg Pro Pro Ala Pro Thr
            500                 505                 510

Ile Thr Thr Val Ser Glu Asn Val Pro Ala Leu Thr Asp His Gly Thr
        515                 520                 525

Leu Pro Leu Arg Asn Ser Ile Gly Gly Val Gln Arg Val Thr Ile Thr
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Asp Ala Arg Arg Arg Thr Cys Pro Tyr Val Tyr Lys Ala Leu Gly Ile
545                 550                 555                 560

Val Ser Pro Arg Val Leu Ser Ser Arg Thr Phe
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