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(54) Benævnelse: **Dobbelt indgivelsessystem til heterologe antigener, der omfatter en rekombinant Listeriastamme svækket ved mutation af dal/dat og deletion af ActA, der omfatter et nukleinsyremolekyle, der koder for et listeriolysin O-prostataspecifikt antigenfusionsprotein**

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Description

FIELD OF INVENTION

[0001] Provided herein are recombinant *Listeria* strains expressing a tumor-specific antigenic polypeptide and, optionally, an angiogenic polypeptide wherein a nucleic acid molecule encoding at least one of the polypeptides is operably integrated into the *Listeria* genome in an open reading frame with a nucleic acid sequence encoding a PEST-containing polypeptide, methods of preparing same, and methods of inducing an immune response, and treating, inhibiting, or suppressing cancer or tumors comprising administering same.

BACKGROUND OF THE INVENTION

[0002] A great deal of pre-clinical evidence and early clinical trial data suggests that the anti-tumor capabilities of the immune system can be harnessed to treat patients with established cancers. The vaccine strategy takes advantage of tumor antigens associated with various types of cancers. Immunizing with live vaccines such as viral or bacterial vectors expressing a tumor-associated antigen is one strategy for eliciting strong CTL responses against tumors.

[0003] *Listeria monocytogenes* (*Lm*) is a gram positive, facultative intracellular bacterium that has direct access to the cytoplasm of antigen presenting cells, such as macrophages and dendritic cells, largely due to the pore-forming activity of listeriolysin-O (LLO). LLO is secreted by *Lm* following engulfment by the cells and perforates the phagolysosomal membrane, allowing the bacterium to escape the vacuole and enter the cytoplasm. LLO is very efficiently presented to the immune system via MHC class I molecules. Furthermore, *Lm*-derived peptides also have access to MHC class II presentation via the phagolysosome. WO 2006/017856 describes Listeria vaccine strains that express a heterologous antigen and a metabolic enzyme, and methods for their generation.

[0004] Cancer is a complex disease and combined therapeutic approaches are more likely to succeed. Not only tumor cells, but also the microenvironment that supports tumor growth, must be targeted to maximize the therapeutic efficacy. Most immunotherapies focus on single antigens to target tumor cells and therefore they have shown limited success against human cancers. A single therapeutic agent capable of targeting tumor cells and tumor microenvironment simultaneously would have an advantage over other immunotherapeutic approaches, especially if it results in a synergistic anti-tumor effect.

SUMMARY OF THE INVENTION

[0005] The invention is defined in the appended claims and any other aspects or embodiments set forth herein not falling within the scope of the claims are for information only.

[0006] In one aspect, provided herein is a recombinant *Listeria* strain comprising a first and second nucleic acid molecule, each said nucleic acid molecule encoding a heterologous antigenic polypeptide or fragment thereof, wherein said first nucleic acid molecule is operably integrated into the *Listeria* genome in an open reading frame with an endogenous PEST-containing gene. In another aspect, the present disclosure provides a vaccine comprising such a recombinant *Listeria* strain.

[0007] In another aspect, provided herein is a method of inducing an immune response to an antigen in a subject comprising administering a recombinant *Listeria* strain to said subject, wherein said recombinant *Listeria* strain comprises a first and second nucleic acid molecule, each said nucleic acid molecule encoding a heterologous antigenic polypeptide or fragment thereof, wherein said first nucleic acid molecule is operably integrated into the *Listeria* genome in an open reading frame with an endogenous PEST-containing gene.

[0008] In another aspect, provided herein is a method of treating, suppressing, or inhibiting a cancer in a subject comprising administering a recombinant *Listeria* strain comprising a first and second nucleic acid molecule, each said nucleic acid molecule encoding a heterologous antigenic polypeptide or fragment thereof, wherein said first nucleic acid molecule is operably integrated into the *Listeria* genome in an open reading frame with an endogenous PEST-containing gene.

[0009] In another aspect, provided herein is a method of treating, suppressing, or inhibiting at least one tumor in a subject comprising administering a recombinant *Listeria* strain comprising a first and second nucleic acid molecule, each said nucleic acid molecule encoding a heterologous antigenic polypeptide or fragment thereof, wherein said first nucleic acid molecule is operably integrated into the *Listeria* genome in an open reading frame with an endogenous PEST-containing gene.

[0010] In another aspect, provided herein is a recombinant *Listeria* strain comprising a nucleic acid molecule encoding a heterologous antigenic polypeptide or fragment thereof, wherein said nucleic acid molecule is operably integrated into the *Listeria* genome in an open reading frame with an endogenous PEST-containing gene.

[0011] In another aspect, provided herein is a method of producing a recombinant *Listeria* strain expressing two

antigens, the method comprising genetically fusing a first nucleic acid encoding a first antigen and a second nucleic acid encoding a second antigen into the *Listeria* genome in an open reading frame with an endogenous PEST-containing gene; and expressing said first and second antigens under conditions conducive to antigenic expression in said recombinant *Listeria* strain.

[0012] In another aspect, provided herein is a method of producing a recombinant *Listeria* strain expressing two antigens. In one aspect, the method comprises genetically fusing a first nucleic acid encoding a first antigen into the *Listeria* genome in an open reading frame with an endogenous PEST-containing gene; transforming said recombinant *Listeria* with an episomal expression vector comprising a second nucleic acid encoding a second antigen; and expressing said first and second antigens under conditions conducive to antigenic expression in said recombinant *Listeria* strain.

[0013] The present invention provides a recombinant Listeria strain comprising a nucleic acid molecule encoding an antigenic listeriolysin O (LLO) - prostate specific antigen (PSA) fusion polypeptide, wherein said recombinant Listeria is an attenuated, auxotrophic dal/dat mutant, that comprises an episomal expression vector comprising a metabolic enzyme that complements the auxotrophy of said auxotrophic mutant and wherein said recombinant Listeria further comprises a deletion of the endogenous ActA gene. The present invention further provides:

- an immunogenic composition comprising the recombinant Listeria strain of the invention, and an adjuvant;
- a recombinant Listeria strain of the invention, for use as a medicament;
- a recombinant Listeria strain of the invention, for use in inducing an immune response to PSA in a subject;
- a recombinant Listeria strain of the invention, for use in a method of preventing or delaying onset of a cancer in a subject; and
- a recombinant Listeria strain of the invention, for use in treating, suppressing, or inhibiting a cancer or tumor in a subject.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014]

Figure 1. (A) Schematic representation of the chromosomal region of the *Lmdd*-143 and *LmddA*-143 after *klk3* integration and *actA* deletion; (B) The *klk3* gene is integrated into the *Lmdd* and *LmddA* chromosome. PCR from chromosomal DNA preparation from each construct using *klk3* specific primers amplifies a band of 714 bp corresponding to the *klk3* gene, lacking the secretion signal sequence of the wild type protein.

Figure 2. (A) Map of the pADV134 plasmid. (B) Proteins from *LmddA*-134 culture supernatant were precipitated, separated in a SDS-PAGE, and the LLO-E7 protein detected by Western-blot using an anti-E7 monoclonal antibody. The antigen expression cassette consists of *hly* promoter, ORF for truncated LLO and human PSA gene (*klk3*). (C) Map of the pADV142 plasmid. (D) Western blot showed the expression of LLO-PSA fusion protein using anti-PSA and anti-LLO antibody.

Figure 3. (A) Plasmid stability *in vitro* of LmddA-LLO-PSA if cultured with and without selection pressure (D-alanine). Strain and culture conditions are listed first and plates used for CFU determination are listed after. (B) Clearance of LmddA-LLO-PSA *in vivo* and assessment of potential plasmid loss during this time. Bacteria were injected i.v. and isolated from spleen at the time point indicated. CFUs were determined on BHI and BHI + D-alanine plates.

Figure 4. (**A**) *In vivo* clearance of the strain LmddA-LLO-PSA after administration of 10^8 CFU in C57BL/6 mice. The number of CFU were determined by plating on BHI/str plates. The limit of detection of this method was 100 CFU. (B) Cell infection assay of J774 cells with 10403S, LmddA-LLO-PSA and XFL7 strains.

Figure 5. (A) PSA tetramer-specific cells in the splenocytes of naive and LmddA-LLO-PSA immunized mice on day 6 after the booster dose. (B) Intracellular cytokine staining for IFN-γ in the splenocytes of naive and LmddA-LLO-PSA immunized mice were stimulated with PSA peptide for 5 h. Specific lysis of EL4 cells pulsed with PSA peptide with in vitro stimulated effector T cells from LmddA-LLO-PSA immunized mice and naive mice at different effector/target ratio using a caspase based assay (C) and a europium based assay (D). Number of IFNγ spots in naive and immunized splenocytes obtained after stimulation for 24 h in the presence of PSA peptide or no peptide (E).

Figure 6. Immunization with *LmddA*-142 induces regression of Tramp-C1-PSA (TPSA) tumors. Mice were left untreated (n=8) (A) or immunized i.p. with *LmddA*-142 ($1x10^8$ CFU/mouse) (n=8) (B) or Lm-LLO-PSA (n=8) (C) on days 7, 14 and 21. Tumor sizes were measured for each individual tumor and the values expressed as the mean diameter in millimeters. Each line represents an individual mouse.

Figure 7. (A) Analysis of PSA-tetramer$^+$CD8$^+$ T cells in the spleens and infiltrating T-PSA-23 tumors of untreated mice and mice immunized with either an *Lm* control strain or *Lm-ddA*-LLO-PSA (*LmddA*-142). (B) Analysis of CD4$^+$ regulatory T cells, which were defined as CD25$^+$FoxP3$^+$, in the spleens and infiltrating T-PSA-23 tumors of untreated mice and mice immunized with either an *Lm* control strain or *Lm-ddA*-LLO-PSA.

Figure 8. (A) Schematic representation of the chromosomal region of the Lmdd-143 and LmddA-143 after klk3 integration and actA deletion; (B) The klk3 gene is integrated into the Lmdd and LmddA chromosome. PCR from chromosomal DNA preparation from each construct using klk3 specific primers amplifies a band of 760 bp corresponding to the klk3 gene.

Figure 9. (A) *Lmdd*-143 and *LmddA*-143 secretes the LLO-PSA protein. Proteins from bacterial culture supernatants were precipitated, separated in a SDS-PAGE and LLO and LLO-PSA proteins detected by Western-blot using an anti-LLO and anti-PSA antibodies; (B) LLO produced by *Lmdd*-143 and *LmddA*-143 retains hemolytic activity. Sheep red blood cells were incubated with serial dilutions of bacterial culture supernatants and hemolytic activity measured by absorbance at 590nm; (C) *Lmdd*-143 and *LmddA*-143 grow inside the macrophage-like J774 cells. J774 cells were incubated with bacteria for 1 hour followed by gentamicin treatment to kill extracellular bacteria. Intracellular growth was measured by plating serial dilutions of J774 lysates obtained at the indicated timepoints. *Lm* 10403S was used as a control in these experiments.

Figure 10. Immunization of mice with *Lmdd*-143 and *LmddA*-143 induces a PSA-specific immune response. C57BL/6 mice were immunized twice at 1-week interval with 1x10^8 CFU of *Lmdd-143, LmddA*-143 or *LmddA*-142 and 7 days later spleens were harvested. Splenocytes were stimulated for 5 hours in the presence of monensin with 1 μM of the PSA_{65-74} peptide. Cells were stained for CD8, CD3, CD62L and intracellular IFN-γ and analyzed in a FACS Calibur cytometer.

Figure 11. Three *Lm*-based vaccines expressing distinct HMW-MAA fragments based on the position of previously mapped and predicted HLA-A2 epitopes were designed (A). The *Lm*-tLLO-HMW-$MMA_{2160-2258}$ (also referred as *Lm*-LLO-HMW-MAA-C) strain secretes a ~62 kDa band corresponding to the tLLO-HMW-$MAA_{2160-2258}$ fusion protein (B). C57BL/6 mice (n=15) were inoculated s.c. with B16F10 cells and either immunized i.p. on days 3, 10 and 17 with *Lm*-tLLO-HMW-$MAA_{2160-2258}$ (n=8) or left untreated (n=7). BALB/c mice (n=16) were inoculated s.c. with RENCA cells and immunized i.p. on days 3, 10 and 17 with either *Lm*-HMW-MAA-C (n=8) or an equivalent dose of a control *Lm* vaccine. Mice immunized with the *Lm*-LLO-HMW-MAA-C impeded the growth of established tumors (C). FVB/N mice (n=13) were inoculated s.c. with NT-2 tumor cells and immunized i.p. on days 7, 14 and 21 with either *Lm*-HMW-MAA-C (n=5) or an equivalent dose of a control *Lm* vaccine (n=8). Immunization of mice with *Lm*-LLO-HMW-MAA-C significantly impaired the growth of tumors not engineered to express HMW-MAA, such as B16F10, RENCA and NT-2 (D). Tumor sizes were measured for each individual tumor and the values expressed as the mean diameter in millimeters ± SEM. *, $P \leq 0.05$, Mann-Whitney test.

Figure 12. Immunization with *Lm*-HMW-MAA-C promotes tumor infiltration by CD8$^+$ T cells and decreases the number of pericytes in blood vessels. (A) NT-2 tumors were removed and sectioned for immunofluorescence. Staining groups are numbered (1-3) and each stain is indicated on the right. Sequential tissues were either stained with the pan-vessel marker anti-CD31 or the anti-NG2 antibody for the HMW-MAA mouse homolog AN2, in conjunction with anti-CD8α for possible TILs. Group 3 shows isotype controls for the above antibodies and DAPI staining used as a nuclear marker. A total of 5 tumors were analyzed and a single representative image from each group is shown. CD8$^+$ cells around blood vessels are indicated by arrows. (B) Sequential sections were stained for pericytes by using the anti-NG2 and anti-alpha-smooth-muscle-cell-actin (α-SMA) antibodies. Double staining/colocalization of these two antibodies (yellow in merge image) are indicative of pericyte staining (*top*). Pericyte colocalization was quantitated using Image Pro Software and the number of colocalized objects is shown in the graph (*bottom*). A total of 3 tumors were analyzed and a single representative image from each group is shown. *, $P \leq 0.05$, Mann-Whitney test. Graph shows mean ± SEM.

DETAILED DESCRIPTION OF THE INVENTION

[0015] The invention is defined in the appended claims and any other aspects or embodiments set forth herein not falling within the scope of the claims are for information only.

[0016] One aspect described herein relates to a recombinant *Listeria* strain expressing an antigenic polypeptide in which the nucleic acid encoding the polypeptide is operably integrated into the *Listeria* genome in an open reading frame with an endogenous PEST-containing gene, which in one aspect, is LLO. In one aspect, the *Listeria* expresses two

polypeptides, one of which is a tumor-associated antigen, and one of which is an angiogenic polypeptide.

[0017] One aspect described herein provides a recombinant *Listeria* strain comprising a first and second nucleic acid molecule, each said nucleic acid molecule encoding a heterologous antigenic polypeptide or fragment thereof, wherein the first nucleic acid molecule is integrated into the *Listeria* genome in an open reading frame with an endogenous LLO gene and wherein the second nucleic acid molecule is present in an episomal expression vector within the recombinant *Listeria* strain. In one aspect, the first nucleic acid molecule encodes a KLK3 protein and the second nucleic acid molecule encodes an HMW-MAA peptide, and in one aspect, is in an open reading frame with a nucleic acid encoding a non-hemolytic LLO, truncated ActA, or PEST sequence.

[0018] One aspect described herein provides a recombinant *Listeria* strain comprising a first and second nucleic acid molecule, each said nucleic acid molecule encoding a heterologous antigenic polypeptide.

[0019] In one aspect, the first nucleic acid molecule is operably integrated into the *Listeria* genome as an open reading frame with an endogenous nucleic acid sequence encoding a polypeptide comprising a PEST sequence. In one aspect, the first nucleic acid molecule is operably integrated into the *Listeria* genome as an open reading frame with a nucleic acid sequence encoding LLO. In another aspect, the first nucleic acid molecule is operably integrated into the *Listeria* genome as an open reading frame with a nucleic acid sequence encoding ActA.

[0020] In one aspect, the first nucleic acid molecule is operably integrated into the *Listeria* genome in an open reading frame with an endogenous nucleic acid sequence encoding LLO. In one aspect, the integration does not eliminate the functionality of LLO. In another aspect, the integration does not eliminate the functionality of ActA. In one aspect, the functionality of LLO or Acta is its native functionality. In one aspect, the LLO functionality is allowing the organism to escape from the phagolysosome, while in another aspect, the LLO functionality is enhancing the immunogenicity of a polypeptide to which it is fused. In one aspect, a recombinant Listeria described herein retains LLO function, which in one aspect, is hemolytic function and in another aspect, is antigenic function. Other functions of LLO are known in the art, as are methods of and assays for evaluating LLO functionality. In one aspect, a recombinant Listeria described herein has wild-type virulence, while in another aspect, a recombinant Listeria described herein has attenuated virulence. In another aspect, a recombinant Listeria of the present invention is avirulent. In one aspect, a recombinant Listeria of the present invention is sufficiently virulent to escape the phagolysosome and enter the cytosol. The recombinant Listeria of the present invention expresses a fused antigen-LLO protein. Thus, in one aspect, the integration of the first nucleic acid molecule into the *Listeria* genome does not disrupt the structure of the endogenous PEST-containing gene, while in another aspect, it does not disrupt the function of the endogenous PEST-containing gene. In one aspect, the integration of the first nucleic acid molecule into the *Listeria* genome does not disrupt the ability of said Listeria to escape the phagolysosome.

[0021] In another aspect, the second nucleic acid molecule is operably integrated into the *Listeria* genome with said first nucleic acid molecule in an open reading frame with an endogenous polypeptide comprising a PEST sequence. Thus, in one aspect, the first and second nucleic acid molecules are integrated in frame with a nucleic acid sequence encoding LLO, while in another aspect, they are integrated in frame with a nucleic acid sequence encoding ActA. In another aspect, the second nucleic acid molecule is operably integrated into the *Listeria* genome in an open reading frame with a nucleic acid sequence encoding a polypeptide comprising a PEST sequence in a site that is distinct from the integration site of the first nucleic acid molecule. In one aspect, the first nucleic acid molecule is integrated in frame with a nucleic acid sequence encoding LLO, while the second nucleic acid molecule is integrated in frame with a nucleic acid sequence encoding ActA, while in another aspect, the first nucleic acid molecule is integrated in frame with a nucleic acid sequence encoding ActA, while the second nucleic acid molecule is integrated in frame with a nucleic acid sequence encoding LLO.

[0022] Another aspect described herein provides a recombinant *Listeria* strain comprising a first nucleic acid molecule encoding a first heterologous antigenic polypeptide or fragment thereof and a second nucleic acid molecule encoding a second heterologous antigenic polypeptide or fragment thereof, wherein said first nucleic acid molecule is integrated into the *Listeria* genome such that the first heterologous antigenic polypeptide and an endogenous PEST-containing polypeptide are expressed as a fusion protein. In one aspect, the first heterologous antigenic polypeptide and the endogenous PEST-containing polypeptide are translated in a single open reading frame, while in another aspect, the first heterologous antigenic polypeptide and the endogenous PEST-containing polypeptide are fused after being translated separately.

[0023] In one aspect, the *Listeria* genome comprises a deletion of the endogenous ActA gene, which in one aspect is a virulence factor. In one aspect, such a deletion provides a more attenuated and thus safer *Listeria* strain for human use. According to this aspect, the antigenic polypeptide is integrated in frame with LLO in the *Listeria* chromosome. In another aspect, the integrated nucleic acid molecule is integrated into the ActA locus. In another aspect, the chromosomal nucleic acid encoding ActA is replaced by a nucleic acid molecule encoding an antigen.

[0024] In another aspect, the integrated nucleic acid molecule is integrated into the *Listeria* chromosome.

[0025] In one aspect, said first nucleic acid molecule is a vector designed for site-specific homologous recombination into the *Listeria* genome. In another aspect, the construct or heterologous gene is integrated into the Listerial chromosome

using homologous recombination.

[0026] Techniques for homologous recombination are well known in the art, and are described, for example, in Frankel, FR, Hegde, S, Lieberman, J, and Y Paterson. Induction of a cell-mediated immune response to HIV gag using Listeria monocytogenes as a live vaccine vector. J. Immunol. 155: 4766 - 4774. 1995; Mata, M, Yao, Z, Zubair, A, Syres, K and Y Paterson, Evaluation of a recombinant Listeria monocytogenes expressing an HIV protein that protects mice against viral challenge. Vaccine 19:1435-45, 2001; Boyer, JD, Robinson, TM, Maciag, PC, Peng, X, Johnson, RS, Pavlakis, G, Lewis, MG, Shen, A, Siliciano, R, Brown, CR, Weiner, D, and Y Paterson. DNA prime Listeria boost induces a cellular immune response to SIV antigens in the Rhesus Macaque model that is capable of limited suppression of SIV239 viral replication. Virology. 333: 88-101, 2005. In another aspect, homologous recombination is performed as described in United States Patent No. 6,855,320. In another aspect, a temperature sensitive plasmid is used to select the recombinants. Each technique represents a separate aspect of the methods and compositions as provided herein.

[0027] In another aspect, the construct or heterologous gene is integrated into the Listerial chromosome using transposon insertion. Techniques for transposon insertion are well known in the art, and are described, *inter alia*, by Sun et al. (Infection and Immunity 1990, 58: 3770-3778) in the construction of DP-L967. Transposon mutagenesis has the advantage, in one aspect, that a stable genomic insertion mutant can be formed. In another aspect, the position in the genome where the foreign gene has been inserted by transposon mutagenesis is unknown.

[0028] In another aspect, the construct or heterologous gene is integrated into the Listerial chromosome using phage integration sites (Lauer P, Chow MY et al, Construction, characterization, and use of two LM site-specific phage integration vectors. J Bacteriol 2002;184(15): 4177-86). In another aspect, an integrase gene and attachment site of a bacteriophage (e.g. U153 or PSA listeriophage) is used to insert the heterologous gene into the corresponding attachment site, which can be any appropriate site in the genome (e.g. comK or the 3' end of the arg tRNA gene). In another aspect, endogenous prophages are cured from the attachment site utilized prior to integration of the construct or heterologous gene. In another aspect, this method results in single-copy integrants. Each possibility represents a separate aspect as provided herein.

[0029] In another aspect, the first nucleic acid sequence of methods and compositions as provided herein is operably linked to a promoter/regulatory sequence. In another aspect, the second nucleic acid sequence is operably linked to a promoter/regulatory sequence. In another aspect, each of the nucleic acid sequences is operably linked to a promoter/regulatory sequence. In one aspect, the promoter/regulatory sequence is present on an episomal plasmid comprising said nucleic acid sequence. In one aspect, endogenous Listeria promoter/regulatory sequence controls the expression of a nucleic acid sequence of the methods and compositions described herein. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0030] In another aspect, a nucleic acid sequence as provided herein is operably linked to a promoter, regulatory sequence, or combination thereof that drives expression of the encoded peptide in the *Listeria* strain. Promoter, regulatory sequences, and combinations thereof useful for driving constitutive expression of a gene are well known in the art and include, but are not limited to, for example, the P_{hlyA}, P_{ActA}, hly, ActA, and p60 promoters of *Listeria*, the *Streptococcus* bac promoter, the *Streptomyces griseus* sgiA promoter, and the *B. thuringiensis* phaZ promoter. In another aspect, inducible and tissue specific expression of the nucleic acid encoding a peptide as provided herein is accomplished by placing the nucleic acid encoding the peptide under the control of an inducible or tissue-specific promoter/regulatory sequence. Examples of tissue-specific or inducible regulatory sequences, promoters, and combinations thereof which are useful for his purpose include, but are not limited to the MMTV LTR inducible promoter, and the SV40 late enhancer/promoter. In another aspect, a promoter that is induced in response to inducing agents such as metals, glucocorticoids, and the like, is utilized. Thus, it will be appreciated that the invention includes the use of any promoter or regulatory sequence, which is either known or unknown, and which is capable of driving expression of the desired protein operably linked thereto. In one aspect, a regulatory sequence is a promoter, while in another aspect, a regulatory sequence is an enhancer, while in another aspect, a regulatory sequence is a suppressor, while in another aspect, a regulatory sequence is a repressor, while in another aspect, a regulatory sequence is a silencer.

[0031] In one aspect, the nucleic acid construct used for integration to the *Listeria* genome contains an integration site. In one aspect, the site is a PhSA (phage from Scott A) attPP' integration site. PhSA is, in another aspect, the prophage of *L. monocytogenes* strain ScottA (Loessner, M. J., I. B. Krause, T. Henle, and S. Scherer. 1994. Structural proteins and DNA characteristics of 14 Listeria typing bacteriophages. J. Gen. Virol. 75:701-710), a serotype 4b strain that was isolated during an epidemic of human listeriosis. In another aspect, the site is any another integration site known in the art. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0032] In another aspect, the nucleic acid construct contains an integrase gene. In another aspect, the integrase gene is a PhSA integrase gene. In another aspect, the integrase gene is any other integrase gene known in the art. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0033] In one aspect, the nucleic acid construct is a plasmid. In another aspect, the nucleic acid construct is a shuttle plasmid. In another aspect, the nucleic acid construct is an integration vector. In another aspect, the nucleic acid construct is a site-specific integration vector. In another aspect, the nucleic acid construct is any other type of nucleic acid construct known in the art. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0034] The integration vector of methods and compositions as provided herein is, in another aspect, a phage vector. In another aspect, the integration vector is a site-specific integration vector. In another aspect, the vector further comprises an attPP' site. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0035] In another aspect, the integration vector is a U153 vector. In another aspect, the integration vector is an A118 vector. In another aspect, the integration vector is a PhSA vector.

[0036] In another aspect, the vector is an A511 vector (e.g. GenBank Accession No: X91069). In another aspect, the vector is an A006 vector. In another aspect, the vector is a B545 vector. In another aspect, the vector is a B053 vector. In another aspect, the vector is an A020 vector. In another aspect, the vector is an A500 vector (e.g. GenBank Accession No: X85009). In another aspect, the vector is a B051 vector. In another aspect, the vector is a B052 vector. In another aspect, the vector is a B054 vector. In another aspect, the vector is a B055 vector. In another aspect, the vector is a B056 vector. In another aspect, the vector is a B101 vector. In another aspect, the vector is a B110 vector. In another aspect, the vector is a Bill vector. In another aspect, the vector is an A153 vector. In another aspect, the vector is a D441 vector. In another aspect, the vector is an A538 vector. In another aspect, the vector is a B653 vector. In another aspect, the vector is an A513 vector. In another aspect, the vector is an A507 vector. In another aspect, the vector is an A502 vector. In another aspect, the vector is an A505 vector. In another aspect, the vector is an A519 vector. In another aspect, the vector is a B604 vector. In another aspect, the vector is a C703 vector. In another aspect, the vector is a B025 vector. In another aspect, the vector is an A528 vector. In another aspect, the vector is a B024 vector. In another aspect, the vector is a B012 vector. In another aspect, the vector is a B035 vector. In another aspect, the vector is a C707 vector.

[0037] In another aspect, the vector is an A005 vector. In another aspect, the vector is an A620 vector. In another aspect, the vector is an A640 vector. In another aspect, the vector is a B021 vector. In another aspect, the vector is an HSO47 vector. In another aspect, the vector is an H10G vector. In another aspect, the vector is an H8/73 vector. In another aspect, the vector is an H19 vector. In another aspect, the vector is an H21 vector. In another aspect, the vector is an H43 vector. In another aspect, the vector is an H46 vector. In another aspect, the vector is an H107 vector. In another aspect, the vector is an H108 vector. In another aspect, the vector is an H110 vector. In another aspect, the vector is an H163/84 vector. In another aspect, the vector is an H312 vector. In another aspect, the vector is an H340 vector. In another aspect, the vector is an H387 vector. In another aspect, the vector is an H391/73 vector. In another aspect, the vector is an H684/74 vector. In another aspect, the vector is an H924A vector. In another aspect, the vector is an fMLUP5 vector. In another aspect, the vector is a syn (=P35) vector. In another aspect, the vector is a 00241 vector. In another aspect, the vector is a 00611 vector. In another aspect, the vector is a 02971A vector. In another aspect, the vector is a 02971C vector. In another aspect, the vector is a 5/476 vector. In another aspect, the vector is a 5/911 vector. In another aspect, the vector is a 5/939 vector. In another aspect, the vector is a 5/11302 vector. In another aspect, the vector is a 5/11605 vector. In another aspect, the vector is a 5/11704 vector. In another aspect, the vector is a 184 vector. In another aspect, the vector is a 575 vector. In another aspect, the vector is a 633 vector. In another aspect, the vector is a 699/694 vector. In another aspect, the vector is a 744 vector. In another aspect, the vector is a 900 vector. In another aspect, the vector is a 1090 vector. In another aspect, the vector is a 1317 vector. In another aspect, the vector is a 1444 vector. In another aspect, the vector is a 1652 vector. In another aspect, the vector is a 1806 vector. In another aspect, the vector is a 1807 vector. In another aspect, the vector is a 1921/959 vector. In another aspect, the vector is a 1921/11367 vector. In another aspect, the vector is a 1921/11500 vector. In another aspect, the vector is a 1921/11566 vector. In another aspect, the vector is a 1921/12460 vector. In another aspect, the vector is a 1921/12582 vector. In another aspect, the vector is a 1967 vector. In another aspect, the vector is a 2389 vector. In another aspect, the vector is a 2425 vector. In another aspect, the vector is a 2671 vector. In another aspect, the vector is a 2685 vector. In another aspect, the vector is a 3274 vector. In another aspect, the vector is a 3550 vector. In another aspect, the vector is a 3551 vector. In another aspect, the vector is a 3552 vector. In another aspect, the vector is a 4276 vector. In another aspect, the vector is a 4277 vector. In another aspect, the vector is a 4292 vector. In another aspect, the vector is a 4477 vector. In another aspect, the vector is a 5337 vector. In another aspect, the vector is a 5348/11363 vector. In another aspect, the vector is a 5348/11646 vector. In another aspect, the vector is a 5348/12430 vector. In another aspect, the vector is a 5348/12434 vector. In another aspect, the vector is a 10072 vector. In another aspect, the vector is a 11355C vector. In another aspect, the vector is a 11711A vector. In another aspect, the vector is a 12029 vector. In another aspect, the vector is a 12981 vector. In another aspect, the vector is a 13441 vector. In another aspect, the vector is a 90666 vector. In another aspect, the vector is a 90816 vector. In another aspect, the vector is a 93253 vector. In another aspect, the vector is a 907515 vector. In another aspect, the vector is a 910716 vector. In another aspect, the vector is a NN-*Listeria* vector. In another aspect, the vector is a O1761 vector. In another aspect, the vector is a 4211 vector. In another aspect, the vector is a 4286 vector.

[0038] In another aspect, the integration vector is any other site-specific integration vector known in the art that is capable of infecting *Listeria.* Each possibility represents a separate aspect of the methods and compositions as provided herein. In another aspect, the integration vector or plasmid of methods and compositions as provided herein does not confer antibiotic resistance to the *Listeria* vaccine strain. In another aspect, the integration vector or plasmid does not contain an antibiotic resistance gene. Each possibility represents a separate aspect of the methods and compositions

as provided herein.

[0039] Another aspect described herein provides an isolated nucleic acid encoding a recombinant polypeptide. In one aspect, the isolated nucleic acid comprises a sequence sharing at least 85% homology with a nucleic acid encoding a recombinant polypeptide as provided herein. In another aspect, the isolated nucleic acid comprises a sequence sharing at least 90% homology with a nucleic acid encoding a recombinant polypeptide as provided herein. In another aspect, the isolated nucleic acid comprises a sequence sharing at least 95% homology with a nucleic acid encoding a recombinant polypeptide as provided herein. In another aspect, the isolated nucleic acid comprises a sequence sharing at least 97% homology with a nucleic acid encoding a recombinant polypeptide as provided herein. In another aspect, the isolated nucleic acid comprises a sequence sharing at least 99% homology with a nucleic acid encoding a recombinant polypeptide as provided herein.

[0040] In one aspect, provided herein is a method of producing a recombinant *Listeria* strain expressing two distinct heterologous antigens. In another aspect, the recombinant *Listeria* expresses at least 3 or more distinct heterologous antigens. In another aspect, the recombinant *Listeria* expresses 4 or more distinct heterologous antigens. In another aspect, the recombinant *Listeria* expresses 5 or more distinct heterologous antigens.

[0041] In another aspect, the method comprises genetically fusing a first nucleic acid encoding a first antigen into the *Listeria* genome in an open reading frame with an endogenous polypeptide comprising a PEST sequence. In another aspect, the method comprises genetically fusing at least 2 nucleic acids encoding two distinct heterologous antigens in the *Listeria* genome in an open reading frame with an endogenous polypeptide comprising a PEST sequence. In another aspect, the method comprises genetically fusing at least 3 nucleic acids encoding two distinct heterologous antigens in the *Listeria* genome in an open reading frame with an endogenous polypeptide comprising a PEST sequence. In another aspect, the method comprises genetically fusing at least 4 nucleic acids encoding two distinct heterologous antigens in the *Listeria* genome in an open reading frame with an endogenous polypeptide comprising a PEST sequence. In another aspect, the method comprises genetically fusing at least 5 nucleic acids encoding two distinct heterologous antigens in the *Listeria* genome in an open reading frame with an endogenous polypeptide comprising a PEST sequence.

[0042] In another aspect, the method comprises transforming said recombinant *Listeria* with an episomal expression vector comprising a second nucleic acid encoding a second antigen. In another aspect, the method comprises transforming said recombinant *Listeria* with an episomal expression vector comprising at least 2 nucleic acids encoding at least two distinct heterologous antigens. In another aspect, the method comprises transforming said recombinant *Listeria* with an episomal expression vector comprising at least 3 nucleic acids encoding at least three distinct heterologous antigens. In another aspect, the method comprises transforming said recombinant *Listeria* with an episomal expression vector comprising at least 4 nucleic acids encoding at least four distinct heterologous antigens. In another aspect, the method comprises transforming said recombinant *Listeria* with an episomal expression vector comprising at least 5 nucleic acids encoding at least five distinct heterologous antigens.

[0043] In yet another aspect, the method comprises expressing said first and second antigens under conditions conducive to antigenic expression, that are known in the art, in said recombinant *Listeria* strain.

[0044] In another aspect, the method comprises transforming said recombinant *Listeria* with at least 1 episomal expression vector comprising heterologous antigens as described hereinabove. In another aspect, the method comprises transforming said recombinant *Listeria* with at least 2 episomal expression vector comprising heterologous antigens as described hereinabove. In another aspect, the method comprises transforming said recombinant *Listeria* with at least 3 episomal expression vector comprising heterologous antigens as described hereinabove. In another aspect, the method comprises transforming said recombinant *Listeria* with at least 4 episomal expression vector comprising heterologous antigens as described hereinabove.

[0045] In another aspect, the recombinant *Listeria* strain may express more than two antigens, some of which are expressed from one or more nucleic acid molecules integrated into the *Listeria* chromosome and some of which are expressed via one or more episomal expression vectors present in the recombinant *Listeria* strain. Thus, as described hereinabove, in one aspect, a recombinant *Listeria* strain as provided herein comprises two or more episomal expression vectors, each of which expresses a separate antigenic polypeptide, in one aspect. In one aspect, one or more of the antigens are expressed as a fusion protein with LLO, which in one aspect, is non-hemolytic LLO, and, in another aspect, truncated LLO. In one aspect, a recombinant *Listeria* strain as provided herein targets tumors by eliciting immune responses to two separate antigens, which are expressed by two different cell types, which in one aspect are a cell surface antigen and an anti-angiogenic polypeptide, while in another aspect, a recombinant *Listeria* strain as provided herein targets tumors by eliciting an immune response to two different antigens expressed by the same cell type, which in one aspect are prostate specific antigen (PSA) and prostate-specific membrane antigen (PSMA), which in one aspect is FOLH1. In another aspect, a recombinant *Listeria* strain as provided herein targets tumors by eliciting an immune response to two different antigens as described hereinbelow or as are known in the art.

[0046] In one aspect, a first antigen of the compositions and methods of the described herein is directed against a specific cell surface antigen or tumor target, and a second antigen is directed against an angiogenic antigen or tumor microenvironment. In another aspect, the first and second antigens of the compositions and methods of the described

herein are polypeptides expressed by tumor cells, or in another aspect, polypeptides expressed in a tumor microenvironment. In another aspect, the first antigen of the compositions and methods of the described herein is a polypeptide expressed by a tumor and the second antigen of the compositions and methods of the described herein is a receptor target, NO Synthetase, Arg-1, or other enzyme known in the art.

[0047] In one aspect, provided herein is a method of producing a recombinant *Listeria* strain expressing two antigens, the method comprising, in one aspect, genetically fusing a first nucleic acid encoding a first antigen and a second nucleic acid encoding a second antigen into the *Listeria* genome in an open reading frame with a native polypeptide comprising a PEST sequence. In another aspect, the expressing said first and second antigens are produced under conditions conducive to antigenic expression in said recombinant *Listeria* strain.

[0048] In one aspect, the recombinant *Listeria* strain of the composition and methods as provided herein comprises an episomal expression vector comprising the second nucleic acid molecule encoding a heterologous antigen. In another aspect, the second nucleic acid molecule encoding a heterologous antigen is present in said episomal expression vector in an open reading frame with a polypeptide comprising a PEST sequence.

[0049] In another aspect, an episomal expression vector of the methods and compositions as provided herein comprises an antigen fused in frame to a nucleic acid sequence encoding a PEST-like AA sequence. In one aspect, the antigen is HMW-MAA, and in another aspect, a HMW-MAA fragment. In another aspect, the PEST-like AA sequence is KENSISSMAPPASPPASPKTPIEKKHADEIDK (SEQ ID NO: 1). In another aspect, the PEST-like sequence is KENSISSMAPPASPPASPK (SEQ ID No: 2). In another aspect, fusion of an antigen to any LLO sequence that includes one of the PEST-like AA sequences enumerated herein can enhance cell mediated immunity against HMW-MAA.

[0050] In another aspect, the PEST-like AA sequence is a PEST-like sequence from a *Listeria* ActA protein. In another aspect, the PEST-like sequence is KTEEQPSEVNTGPR (SEQ ID NO: 3), KASVTDTSEGDLDSSMQSADESTPQPLK (SEQ ID NO: 4), KNEEVNASDFPPPPTDEELR (SEQ ID NO: 5), or RGGIPTSEEFSSLNSGDFTDDENSETTEEEIDR (SEQ ID NO: 6). In another aspect, the PEST-like sequence is from *Listeria seeligeri* cytolysin, encoded by the lso gene. In another aspect, the PEST-like sequence is RSEVTISPAETPESPPATP (SEQ ID NO: 7). In another aspect, the PEST-like sequence is from Streptolysin O protein of *Streptococcus sp.* In another aspect, the PEST-like sequence is from *Streptococcus pyogenes* Streptolysin O, e.g. KQNTASTETTTTNEQPK (SEQ ID NO: 8) at AA 35-51. In another aspect, the PEST-like sequence is from *Streptococcus equisimilis* Streptolysin O, e.g. KQNTANTETTTTNEQPK (SEQ ID NO: 9) at AA 38-54. In another aspect, the PEST-like sequence has a sequence selected from SEQ ID NO: 3-9. In another aspect, the PEST-like sequence has a sequence selected from SEQ ID NO: 1-9. In another aspect, the PEST-like sequence is another PEST-like AA sequence derived from a prokaryotic organism.

[0051] Identification of PEST-like sequences is well known in the art, and is described, for example in Rogers S et al (Amino acid sequences common to rapidly degraded proteins: the PEST hypothesis. Science 1986; 234(4774):364-8) and Rechsteiner M et al (PEST sequences and regulation by proteolysis. Trends Biochem Sci 1996; 21(7):267-71). "PEST-like sequence" refers, in another aspect, to a region rich in proline (P), glutamic acid (E), serine (S), and threonine (T) residues. In another aspect, the PEST-like sequence is flanked by one or more clusters containing several positively charged amino acids. In another aspect, the PEST-like sequence mediates rapid intracellular degradation of proteins containing it. In another aspect, the PEST-like sequence fits an algorithm disclosed in Rogers et al. In another aspect, the PEST-like sequence fits an algorithm disclosed in Rechsteiner et al. In another aspect, the PEST-like sequence contains one or more internal phosphorylation sites, and phosphorylation at these sites precedes protein degradation. In one aspect, a sequence referred to herein as a PEST-like sequence is a PEST sequence.

[0052] In one aspect, PEST-like sequences of prokaryotic organisms are identified in accordance with methods such as described by, for example Rechsteiner and Rogers (1996, Trends Biochem. Sci. 21:267-271) for *LM* and in Rogers S et al (Science 1986; 234(4774):364-8). Alternatively, PEST-like AA sequences from other prokaryotic organisms can also be identified based on this method. Other prokaryotic organisms wherein PEST-like AA sequences would be expected to include, but are not limited to, other *Listeria* species. In one aspect, the PEST-like sequence fits an algorithm disclosed in Rogers et al. In another aspect, the PEST-like sequence fits an algorithm disclosed in Rechsteiner et al. In another aspect, the PEST-like sequence is identified using the PEST-find program.

[0053] In another aspect, identification of PEST motifs is achieved by an initial scan for positively charged amino acids R, H, and K within the specified protein sequence. All amino acids between the positively charged flanks are counted and only those motifs are considered further, which contain a number of amino acids equal to or higher than the window-size parameter. In another aspect, a PEST-like sequence must contain at least 1 P, 1 D or E, and at least 1 S or T.

[0054] In another aspect, the quality of a PEST motif is refined by means of a scoring parameter based on the local enrichment of critical amino acids as well as the motifs hydrophobicity. Enrichment of D, E, P, S and T is expressed in mass percent (w/w) and corrected for 1 equivalent of D or E, 1 of P and 1 of S or T. In another aspect, calculation of hydrophobicity follows in principle the method of J. Kyte and R.F. Doolittle (Kyte, J and Dootlittle, RF. J. Mol. Biol. 157, 105 (1982). For simplified calculations, Kyte-Doolittle hydropathy indices, which originally ranged from -4.5 for arginine to +4.5 for isoleucine, are converted to positive integers, using the following linear transformation, which yielded values from 0 for arginine to 90 for isoleucine.

$$\text{Hydropathy index} = 10 * \text{Kyte-Doolittle hydropathy index} + 45$$

[0055] In another aspect, a potential PEST motif's hydrophobicity is calculated as the sum over the products of mole percent and hydrophobicity index for each amino acid species. The desired PEST score is obtained as combination of local enrichment term and hydrophobicity term as expressed by the following equation:

$$\text{PEST score} = 0.55 * \text{DEPST} - 0.5 * \text{hydrophobicity index}.$$

[0056] In another aspect, "PEST sequence," "PEST-like sequence" or "PEST-like sequence peptide" refers to a peptide having a score of at least +5, using the above algorithm. In another aspect, the term refers to a peptide having a score of at least 6. In another aspect, the peptide has a score of at least 7. In another aspect, the score is at least 8. In another aspect, the score is at least 9. In another aspect, the score is at least 10. In another aspect, the score is at least 11. In another aspect, the score is at least 12. In another aspect, the score is at least 13. In another aspect, the score is at least 14. In another aspect, the score is at least 15. In another aspect, the score is at least 16. In another aspect, the score is at least 17. In another aspect, the score is at least 18. In another aspect, the score is at least 19. In another aspect, the score is at least 20. In another aspect, the score is at least 21. In another aspect, the score is at least 22. In another aspect, the score is at least 22. In another aspect, the score is at least 24. In another aspect, the score is at least 24. In another aspect, the score is at least 25. In another aspect, the score is at least 26. In another aspect, the score is at least 27. In another aspect, the score is at least 28. In another aspect, the score is at least 29. In another aspect, the score is at least 30. In another aspect, the score is at least 32. In another aspect, the score is at least 35. In another aspect, the score is at least 38. In another aspect, the score is at least 40. In another aspect, the score is at least 45. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0057] In another aspect, the PEST-like sequence is identified using any other method or algorithm known in the art, e.g the CaSPredictor (Garay-Malpartida HM, Occhiucci JM, Alves J, Belizario JE. Bioinformatics. 2005 Jun;21 Suppl 1:i169-76). In another aspect, the following method is used:

[0058] A PEST index is calculated for each stretch of appropriate length (e.g. a 30-35 amino acid stretch) by assigning a value of 1 to the amino acids Ser, Thr, Pro, Glu, Asp, Asn, or Gln. The coefficient value (CV) for each of the PEST residue is 1 and for each of the other amino acids (non-PEST) is 0.

[0059] Each method for identifying a PEST-like sequence represents a separate aspect as provided herein.

[0060] In another aspect, the PEST-like sequence is any other PEST-like sequence known in the art. Each PEST-like sequence and type thereof represents a separate aspect as provided herein.

[0061] One aspect described herein provides fusion proteins, which in one aspect, are expressed by Listeria. In one aspect, such fusion proteins are fused to a PEST-like sequence which, in one aspect, refers to fusion to a protein fragment comprising a PEST-like sequence. In another aspect, the term includes cases wherein the protein fragment comprises surrounding sequence other than the PEST-like sequence. In another aspect, the protein fragment consists of the PEST-like sequence. Thus, in another aspect, "fusion" refers to two peptides or protein fragments either linked together at their respective ends or embedded one within the other. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0062] In another aspect, a recombinant *Listeria* strain of the compositions and methods as provided herein comprises a full length LLO polypeptide, which in one aspect, is hemolytic.

[0063] In another aspect, the recombinant *Listeria* strain comprises a non-hemolytic LLO polypeptide. In another aspect, the polypeptide is an LLO fragment. In another aspect, the oligopeptide is a complete LLO protein. In another aspect, the polypeptide is any LLO protein or fragment thereof known in the art. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0064] In another aspect, an LLO protein fragment is utilized in compositions and methods as provided herein. In one aspect, a truncated LLO protein is encoded by the episomal expression vector as provided herein that expresses a polypeptide, that is, in one aspect, an antigen, in another aspect, an angiogenic factor, or, in another aspect, both an antigen and angiogenic factor. In another aspect, the LLO fragment is an N-terminal fragment.

[0065] In another aspect, the N-terminal LLO fragment has the sequence:

MKKIMLVFITLILVSLPIAQQTEAKDASAFNKENSISSVAPPASPPASPKTPIEKKH
ADEIDKYIQGLDYNKNNVLVYHGDAVTNVPPRKGYKDGNEYIVVEKKKKSINQNNADI
QVVNAISSLTYPGALVKANSELVENQPDVLPVKRDSLTLSIDLPGMTNQDNKIVVKNATK
SNVNNAVNTLVERWNEKYAQAYSNVSAKIDYDDEMAYSESQLIAKFGTAFKAVNNSLN
VNFGAISEGKMQEEVISFKQIYYNVNVNEPTRPSRFFGKAVTKEQLQALGVNAENPPAYI
SSVAYGRQVYLKLSTNSHSTKVKAAFDAAVSGKSVSGDVELTNIIKNSSFKAVIYGGSAK

DEVQIIDGNLGDLRDILKKGATFNRETPGVPIAYTTNFLKDNELAVIKNNSEYIETTSKAY TDGKINIDHSGGYVAQFNI-SWDEVNYD (SEQ ID NO: 10). In another aspect, an LLO AA sequence of methods and compositions as provided herein comprises the sequence set forth in SEQ ID No: 10. In another aspect, the LLO AA sequence is a homologue of SEQ ID No: 10. In another aspect, the LLO AA sequence is a variant of SEQ ID No: 10. In another aspect, the LLO AA sequence is a fragment of SEQ ID No: 10. In another aspect, the LLO AA sequence is an isoform of SEQ ID No: 10. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0066] In another aspect, the LLO fragment has the sequence:
mkkimlvfitlilvslpiaqqteakdasafnkensissvappasppaspktpiekkhadeidkyiqgldynknnvlvyhgda
vtnvpprkgykdgneyivvekkkksinqnnadiqvvnaissltypgalvkanselvenqpdvlpvkrdsltlsidlpgmtnqdnkivv
knatksnvnnavntlverwnekyaqaysnvsakidyddemaysesqliakfgtafkavnnslnvnfgaisegkmqeevisfkqiyyn
vnvneptrpsrffgkavtkeqlqalgvnaenppayissvaygrqvylklstnshstkvkaafdaavsgksvsgdveltniiknssfkaviy
ggsakdevqiidgnlgdlrdilkkgatfnretpgvpiayttnflkdnelaviknnseyiettskaytd (SEQ ID NO: 11). In another aspect, an LLO AA sequence of methods and compositions as provided herein comprises the sequence set forth in SEQ ID No: 11. In another aspect, the LLO AA sequence is a homologue of SEQ ID No: 11. In another aspect, the LLO AA sequence is a variant of SEQ ID No: 11. In another aspect, the LLO AA sequence is a fragment of SEQ ID No: 11. In another aspect, the LLO AA sequence is an isoform of SEQ ID No: 11. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0067] The LLO protein used in the compositions and methods as provided herein has, in another aspect, the sequence:
MKKIMLVFITLILVSLPIAQQTEAKDASAFNKENSISSMAPPASPPASPKTPIEKK
HADEIDKYIQGLDYNKNNVLVYHGDAVTNVPPRKGYKDGNEYIVVEKKKKSINQNNAD
IQVVNAISSLTYPGALVKANSELVENQPDVLPVKRDSLTLSIDLPGMTNQDNKIVVKNAT
KSNVNNAVNTLVERWNEKYAQAYPNVSAKIDYDDEMAYSESQLIAKFGTAFKAVNNSL
NVNFGAISEGKMQEEVISFKQIYYNVNVNEPTRPSRFFGKAVTKEQLQALGVNAENPPA
YISSVAYGRQVYLKLSTNSHSTKVKAAFDAAVSGKSVSGDVELTNIIKNSSFKAVIYGGS
AKDEVQIIDGNLGDLRDILKKGATFNRETPGVPIAYTTNFLKDNELAVIKNNSEYIETTSK
AYTDGKINIDHSGGYVAQFNISWDEVNYDPEGNEIVQHKNWSENNKSKLAHFTSSIYLP
GNARNINVYAKECTGLAWEWWRTVIDDRNLPLVKNRNISIWGTTLYPKYSNKVDNPIE (GenBank Accession No. P13128; SEQ ID NO: 12; nucleic acid sequence is set forth in GenBank Accession No. X15127). The first 25 AA of the proprotein corresponding to this sequence are the signal sequence and are cleaved from LLO when it is secreted by the bacterium. Thus, in this aspect, the full length active LLO protein is 504 residues long. In another aspect, the above LLO fragment is used as the source of the LLO fragment incorporated in a vaccine as provided herein. In another aspect, an LLO AA sequence of methods and compositions as provided herein comprises the sequence set forth in SEQ ID NO: 12. In another aspect, the LLO AA sequence is a homologue of SEQ ID NO: 12. In another aspect, the LLO AA sequence is a variant of SEQ ID NO: 12. In another aspect, the LLO AA sequence is a fragment of SEQ ID NO: 12. In another aspect, the LLO AA sequence is an isoform of SEQ ID NO: 12. Each possibility represents a separate aspect as provided herein.

[0068] The LLO protein used in the compositions and methods as provided herein has, in another aspect, the sequence:
MKKIMLVFITLILVSLPIAQQTEAKDASAFNKENSISSVAPPASPPASPKTPIEKKHADEIDK
YIQGLDYNKNNVLVYHGDAVTNVPPRKGYKDGNEYIVVEKKKKSINQNNADIQVVNAI
SSLTYPGALVKANSELVENQPDVLPVKRDSLTLSIDLPGMTNQDNKIVVKNATKSNVNN
AVNTLVERWNEKYAQAYSNVSAKIDYDDEMAYSESQLIAKFGTAFKAVNNSLNVNFGA
ISEGKMQEEVISFKQIYYNVNVNEPTRPSRFFGKAVTKEQLQALGVNAENPPAYISSVAY
GRQVYLKLSTNSHSTKVKAAFDAAVSGKSVSGDVELTNIIKNSSFKAVIYGGSAKDEVQII
DGNLGDLRDILKKGATFNRETPGVPIAYTTNFLKDNELAVIKNNSEYIETTSKAYTD (SEQ ID NO: 13). In another aspect, an LLO AA sequence of methods and compositions as provided herein comprises the sequence set forth in SEQ ID NO: 13. In another aspect, the LLO AA sequence is a homologue of SEQ ID NO: 13. In another aspect, the LLO AA sequence is a variant of SEQ ID NO: 13. In another aspect, the LLO AA sequence is a fragment of SEQ ID NO: 13. In another aspect, the LLO AA sequence is an isoform of SEQ ID NO: 13. Each possibility represents a separate aspect as provided herein.

[0069] In one aspect, the amino acid sequence of the LLO polypeptide of the compositions and methods as provided herein is from the *Listeria monocytogene*s 10403S strain, as set forth in Genbank Accession No.: ZP_01942330, EBA21833, or is encoded by the nucleic acid sequence as set forth in Genbank Accession No.: NZ_AARZ01000015 or AARZ01000015.1. In another aspect, the LLO sequence for use in the compositions and methods as provided herein is from *Listeria monocytogene*s, which in one aspect, is the 4b F2365 strain (in one aspect, Genbank accession number: YP_012823), the EGD-e strain (in one aspect, Genbank accession number: NP_463733), or any other strain of *Listeria monocytogene*s known in the art.

[0070] In another aspect, the LLO sequence for use in the compositions and methods as provided herein is from Flavobacteriales bacterium HTCC2170 (in one aspect, Genbank accession number: ZP_01106747 or EAR01433; in

one aspect, encoded by Genbank accession number: NZ_AAOC01000003). In one aspect, proteins that are homologous to LLO in other species, such as alveolysin, which in one aspect, is found in Paenibacillus alvei (in one aspect, Genbank accession number: P23564 or AAA22224; in one aspect, encoded by Genbank accession number: M62709) may be used in the compositions and methods as provided herein. Other such homologous proteins are known in the art.

[0071] Each LLO protein and LLO fragment represents a separate aspect of the methods and compositions as provided herein.

[0072] In another aspect, homologues of LLO from other species, including known lysins, or fragments thereof may be used to create a fusion protein of LLO with an antigen of the compositions and methods as provided herein, which in one aspect, is HMW-MAA, and in another aspect is a fragment of HMW-MAA.

[0073] In another aspect, the LLO fragment of methods and compositions as provided herein, is a PEST-like domain. In another aspect, an LLO fragment that comprises a PEST sequence is utilized as part of a composition or in the methods as provided herein.

[0074] In another aspect, the LLO fragment does not contain the activation domain at the carboxy terminus. In another aspect, the LLO fragment does not include cysteine 484. In another aspect, the LLO fragment is a non-hemolytic fragment. In another aspect, the LLO fragment is rendered non-hemolytic by deletion or mutation of the activation domain. In another aspect, the LLO fragment is rendered non-hemolytic by deletion or mutation of cysteine 484. In another aspect, an LLO sequence is rendered non-hemolytic by deletion or mutation at another location.

[0075] In another aspect, the LLO fragment consists of about the first 441 AA of the LLO protein. In another aspect, the LLO fragment comprises about the first 400-441 AA of the 529 AA full length LLO protein. In another aspect, the LLO fragment corresponds to AA 1-441 of an LLO protein disclosed herein. In another aspect, the LLO fragment consists of about the first 420 AA of LLO. In another aspect, the LLO fragment corresponds to AA 1-420 of an LLO protein disclosed herein. In another aspect, the LLO fragment consists of about AA 20-442 of LLO. In another aspect, the LLO fragment corresponds to AA 20-442 of an LLO protein disclosed herein. In another aspect, any ΔLLO without the activation domain comprising cysteine 484, and in particular without cysteine 484, are suitable for methods and compositions as provided herein.

[0076] In another aspect, the LLO fragment corresponds to the first 400 AA of an LLO protein. In another aspect, the LLO fragment corresponds to the first 300 AA of an LLO protein. In another aspect, the LLO fragment corresponds to the first 200 AA of an LLO protein. In another aspect, the LLO fragment corresponds to the first 100 AA of an LLO protein. In another aspect, the LLO fragment corresponds to the first 50 AA of an LLO protein, which in one aspect, comprises one or more PEST-like sequences.

[0077] In another aspect, the LLO fragment contains residues of a homologous LLO protein that correspond to one of the above AA ranges. The residue numbers need not, in another aspect, correspond exactly with the residue numbers enumerated above; e.g. if the homologous LLO protein has an insertion or deletion, relative to an LLO protein utilized herein.

[0078] In another aspect, a recombinant *Listeria* strain of the methods and compositions as provided herein comprise a nucleic acid molecule operably integrated into the *Listeria* genome as an open reading frame with an endogenous ActA sequence. In another aspect, an episomal expression vector as provided herein comprises a fusion protein comprising an antigen fused to an ActA or a truncated ActA. In one aspect, the antigen is HMW-MAA, while in another aspect, it's an immunogenic fragement of HMW-MAA.

[0079] In one aspect, an antigen of the methods and compositions as provided herein is fused to an ActA protein, which in one aspect, is an N-terminal fragment of an ActA protein, which in one aspect, comprises or consists of the first 390 AA of ActA, in another aspect, the first 418 AA of ActA, in another aspect, the first 50 AA of ActA, in another aspect, the first 100 AA of ActA, which in one aspect, comprise a PEST-like sequence such as that provided in SEQ ID NO: 2. In another aspect, an N-terminal fragment of an ActA protein utilized in methods and compositions as provided herein comprises or consists of the first 150 AA of ActA, in another aspect, the first approximately 200 AA of ActA, which in one aspect comprises 2 PEST-like sequences as described herein. In another aspect, an N-terminal fragment of an ActA protein utilized in methods and compositions as provided herein comprises or consists of the first 250 AA of ActA, in another aspect, the first 300 AA of ActA. In another aspect, the ActA fragment contains residues of a homologous ActA protein that correspond to one of the above AA ranges. The residue numbers need not, in another aspect, correspond exactly with the residue numbers enumerated above; e.g. if the homologous ActA protein has an insertion or deletion, relative to an ActA protein utilized herein, then the residue numbers can be adjusted accordingly, as would be routine to a skilled artisan using sequence alignment tools such as NCBI BLAST that are well-known in the art.

[0080] In another aspect, the N-terminal portion of the ActA protein comprises 1, 2, 3, or 4 PEST-like sequences, which in one aspect are the PEST-like sequences specifically mentioned herein, or their homologs, as described herein or other PEST-like sequences as can be determined using the methods and algorithms described herein or by using alternative methods known in the art.

[0081] An N-terminal fragment of an ActA protein utilized in methods and compositions as provided herein has, in another aspect, the sequence set forth in SEQ ID NO: 14:

MRAMMVVFITANCITINPDIIFAATDSEDSSLNTDEWEEEKTEEQPSEVNTGPRYETAREVSSRDIKELEKSNKVRNTN
KADLIAMLKEKAEKGPNINNNNSEQTENAAINEEASGADRPAIQVERRHPGLPSDSAAEIKKRRKAIASSDSELESLTYP
DKPTKVNKKKVAKESVADASESDLDSSMQSADESSPQPLKANQQPFFPKVFKKIKDAGKWVRDKIDENPEVKKAIVD
KSAGLIDQLLTKKKSEEVNASDFPPPPTDEELRLALPETPMLLGFNAPATSEPSSFEFPPPPTDEELR
LALPETPMLLGFNAPATSEPSSFEFPPPPTEDELEIIRETASSLDSSFTRGDLASLRNAIN-
RHSQNFSDFPPIPTEEELNGRGGRP (SEQ ID NO: 14). In another aspect, the ActA fragment comprises the sequence set forth in SEQ ID NO: 14. In another aspect, the ActA fragment is any other ActA fragment known in the art. In another aspect, the ActA protein is a homologue of SEQ ID NO: 14. In another aspect, the ActA protein is a variant of SEQ ID NO: 14. In another aspect, the ActA protein is an isoform of SEQ ID NO: 14. In another aspect, the ActA protein is a fragment of SEQ ID NO: 14. In another aspect, the ActA protein is a fragment of a homologue of SEQ ID NO: 14. In another aspect, the ActA protein is a fragment of a variant of SEQ ID NO: 14. In another aspect, the ActA protein is a fragment of an isoform of SEQ ID NO: 14. Each possibility represents a separate aspect as provided herein. Each possibility represents a separate aspect as provided herein.

[0082] In another aspect, the recombinant nucleotide encoding a fragment of an ActA protein comprises the sequence set forth in SEQ ID NO: 15:
atgcgtgcgatgatggtggttttcattactgccaattgcattacgattaaccccgacataatatttgcagcgacagatagcgaagattctagtctaaacacagatgaatgg
gaagaagaaaaaacagaagagcaaccaagcgaggtaaatacgggaccaagatacgaaactgcacgtgaagtaagttcacgtgatattaaagaactagaaaa
atcgaataaagtgagaaatacgaacaaagcagacctaatagcaatgttgaaagaaaaagcagaaaaaggtccaaatatcaataataacaacagtgaacaaact
gagaatgcggctataaatgaagaggcttcaggagccgaccgaccagctatacaagtggagcgtcgtcatccaggattgccatcggatagcgcagcggaaattaa
aaaaagaaggaaagccatagcatcatcggatagtgagcttgaaagccttacttatccggataaaccaacaaaagtaaataagaaaaaagtggcgaaagagtca
gttgcggatgcttctgaaagtgacttagattctagcatgcagtcagcagatgagtcttcaccacaacctttaaaagcaaaccaacaaccatttttccctaaagtatttaaa
aaaataaaagatgcggggaaatgggtacgtgataaaatcgacgaaaatcctgaagtaaagaaagcgattgttgataaaagtgcagggttaattgaccaattattaa
ccaaaaagaaaagtgaagaggtaaatgcttcggacttcccgccaccacctacggatgaagagttaagacttgctttgccagagacaccaatgcttcttggttttaatgc
tcctgctacatcagaaccgagctcattcgaatttccaccaccacctacggatgaagagttaagacttgctttgccagagacgccaatgcttcttggttttaatgctcctgct
acatcggaaccgagctcgttcgaatttccaccgcctccaacagaagatgaactagaaatcatccgggaaacagcatcctcgctagattctagttttacaagagggga
tttagctagtttgagaaatgctattaatcgccatagtcaaaatttctctgatttcccaccaatcccaacagaagaagagttgaacgggagaggcggtagacca (SEQ ID NO: 15). In another aspect, the recombinant nucleotide has the sequence set forth in SEQ ID NO: 15. In another aspect, the recombinant nucleotide comprises any other sequence that encodes a fragment of an ActA protein. Each possibility represents a separate aspect of the methods and compositions as provided herein .

[0083] An N-terminal fragment of an ActA protein utilized in methods and compositions as provided herein has, in another aspect, the sequence set forth in SEQ ID NO: 16: MRAMMVVFITANCITINPDIIFAATDSEDSSLNTDEWEEEK-
TEEQPSEVNTGPRYETAREVSSRDIEELEKSNKVKNTNKADLIAMLKAKAEKGPNNNNNNGEQTGNVAINEEASGVD
RPTLQVERRHPGLSSDSAAEIKKRRKAIASSDSELESLTYPDKPTKANKRKVAKESVVDASE
SDLDSSMQSADESTPQPLKANQKPFFPKVFKKIKDAGKWVRDKIDENPEVKKAIVDKSAGLIDQLLTKKKSEEVNASD
FPPPPTDEELRLALPETPMLLGFNAPTPSEPSSFEFPPPPTDEELRLALPETPMLLGFNAPATSEPSSFEFPPPPTEDE
LEIMRETAPSLDSSFTSGDLASLRSAINRHSENFSDFPLIPTEEELNGRGGRP (SEQ ID NO: 16), which in one aspect is the first 390 AA for ActA from *Listeria monocytogenes,* strain 10403S. In another aspect, the ActA fragment comprises the sequence set forth in SEQ ID NO: 16. In another aspect, the ActA fragment is any other ActA fragment known in the art. In another aspect, the ActA protein is a homologue of SEQ ID NO: 16. In another aspect, the ActA protein is a variant of SEQ ID NO: 16. In another aspect, the ActA protein is an isoform of SEQ ID NO: 16. In another aspect, the ActA protein is a fragment of SEQ ID NO: 16. In another aspect, the ActA protein is a fragment of a homologue of SEQ ID NO: 16. In another aspect, the ActA protein is a fragment of a variant of SEQ ID NO: 16. In another aspect, the ActA protein is a fragment of an isoform of SEQ ID NO: 16. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0084] In another aspect, the recombinant nucleotide encoding a fragment of an ActA protein comprises the sequence set forth in SEQ ID NO: 17:
atgcgtgcgatgatggtagttttcattactgccaactgcattacgattaaccccgacataatatttgcagcgacagatagcgaagattccagtctaaacacagatgaatg
ggaagaagaaaaaacagaagagcagccaagcgaggtaaatacgggaccaagatacgaaactgcacgtgaagtaagttcacgtgatattgaggaactagaaa
aatcgaataaagtgaaaaatacgaacaaagcagacctaatagcaatgttgaaagcaaaagcagagaaaggtccgaataacaataataacaacggtgagcaaa
caggaaatgtggctataaatgaagaggcttcaggagtcgaccgaccaactctgcaagtggagcgtcgtcatccaggtctgtcatcggatagcgcagcggaaattaa
aaaaagaagaaaagccatagcgtcgtcggatagtgagcttgaaagccttacttatccagataaaccaacaaaagcaaataagagaaaagtggcgaaagagtca
gttgtggatgcttctgaaagtgacttagattctagcatgcagtcagcagacgagtctacaccacaacctttaaaagcaaatcaaaaaccatttttccctaaagtatttaaa
aaaataaaagatgcggggaaatgggtacgtgataaaatcgacgaaaatcctgaagtaaagaaagcgattgttgataaaagtgcagggttaattgaccaattattaa
ccaaaaagaaaagtgaagaggtaaatgcttcggacttcccgccaccacctacggatgaagagttaagacttgctttgccagagacaccgatgcttctcggttttaatg
ctcctactccatcggaaccgagctcattcgaatttccgccgccacctacggatgaagagttaagacttgctttgccagagacgccaatgcttcttggttttaatgctcctgct
acatcggaaccgagctcattcgaatttccaccgcctccaacagaagatgaactagaaattatgcgggaaacagcaccttcgctagattctagttttacaagcggggat
ttagctagtttgagaagtgctattaatcgccatagcgaaaatttctctgatttcccactaatcccaacagaagaagagttgaacgggagaggcggtagacca (SEQ ID NO: 17), which in one aspect, is the first 1170 nucleotides encoding ActA in *Listeria monocytogenes* 10403S strain.

In another aspect, the recombinant nucleotide has the sequence set forth in SEQ ID NO: 17. In another aspect, the recombinant nucleotide comprises any other sequence that encodes a fragment of an ActA protein. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0085] In another aspect, the ActA fragment is another ActA fragment known in the art, which in one aspect, is any fragment comprising a PEST sequence. Thus, in one aspect, the ActA fragment is amino acids 1-100 of the ActA sequence. In another aspect, the ActA fragment is amino acids 1-200 of the ActA sequence. In another aspect, the ActA fragment is amino acids 200-300 of the ActA sequence. In another aspect, the ActA fragment is amino acids 300-400 of the ActA sequence. In another aspect, the ActA fragment is amino acids 1-300 of the ActA sequence. In another aspect, a recombinant nucleotide as provided herein comprises any other sequence that encodes a fragment of an ActA protein. In another aspect, the recombinant nucleotide comprises any other sequence that encodes an entire ActA protein. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0086] In one aspect, the ActA sequence for use in the compositions and methods as provided herein is from *Listeria monocytogenes*, which in one aspect, is the EGD strain, the 10403S strain (Genbank accession number: DQ054585) the NICPBP 54002 strain (Genbank accession number: EU394959), the S3 strain (Genbank accession number: EU394960), the NCTC 5348 strain (Genbank accession number: EU394961), the NICPBP 54006 strain (Genbank accession number: EU394962), the M7 strain (Genbank accession number: EU394963), the S19 strain (Genbank accession number: EU394964), or any other strain of *Listeria monocytogenes* which is known in the art.

[0087] In one aspect, the sequence of the deleted *actA* region in the strain, LmddΔactA is as follows:
gcgccaaatcattggttgattggtgaggatgtctgtgtgcgtgggtcgcgagatgggcgaataagaagcattaaagatcctgacaaatataatcaagcggctcatatg
aaagattacgaatcgcttccactcacagaggaaggcgactggggcggagttcattataatagtggtatcccgaataaagcagcctataatactatcactaaacttgga
aaagaaaaaacagaacagctttattttcgcgccttaaagtactatttaacgaaaaaatcccagtttaccgatgcgaaaaaagcgcttcaacaagcagcgaaagattt
atatggtgaagatgcttctaaaaaagttgctgaagcttgggaagcagttggggttaactgattaacaaatgttagagaaaaattaattctccaagtgatattcttaaaata
attcatgaatatttttttcttatattagctaattaagaagataactaactgctaatccaatttttaacggaacaaattagtgaaaatgaaggccgaattttccttgttctaaaaag
gttgtattagcgtatcacgaggagggagtataagtgggattaaacagatttatgcgtgcgatgatggtggttttcattactgccaattgcattacgattaaccccgac**gtcg**
acccatacgacgttaattcttgcaatgttagctattggcgtgttctctttaggggcgtttatcaaaattattcaattaagaaaaaaataattaaaaacacagaacgaaagaa
aaagtgaggtgaatgatatgaaattcaaaaaggtggttctaggtatgtgcttgatcgcaagtgttctagtctttccggtaacgataaaagcaaatgcctgttgtgatgaat
acttacaaacacccgcagctccgcatgatattgacagcaaattaccacataaacttagttggtccgcggataacccgacaaatactgacgtaaatacgcactattggc
ttttaaacaagcggaaaaaatactagctaaagatgtaaatcatatgcgagctaatttaatgaatgaacttaaaaaattcgataaacaaatagctcaaggaatatatga
tgcggatcataaaaatccatattatgatactagtacatttttatctcatttttataatcctgatagagataatacttatttgccgggttttgctaatgcgaaaataacaggagca
aagtatttcaatcaatcggtgactgattaccgagaagggaa (SEQ ID NO: 18). In one aspect, the underlined region contains *actA* sequence element that is present in the LmddΔactA strain. In one aspect, the bold sequence gtcgac represent the site of junction of the N-T and C-T sequence.

[0088] In one aspect, the recombinant *Listeria* strain of the compositions and methods as provided herein comprise a first or second nucleic acid molecule that encodes a High Molecular Weight-Melanoma Associated Antigen (HMW-MAA), or, in another aspect, a fragment of HMW-MAA.

[0089] In one aspect, HMW-MAA is also known as the melanoma chondroitin sulfate proteoglycan (MCSP), and in another aspect, is a membrane-bound protein of 2322 residues. In one aspect, HMW-MAA is expressed on over 90% of surgically removed benign nevi and melanoma lesions, and is also expressed in basal cell carcinoma, tumors of neural crest origin (e.g. astrocytomas, gliomas, neuroblastomas and sarcomas), childhood leukemias, and lobular breast carcinoma lesions. In another aspect, HMW-MAA is highly expressed on both activated pericytes and pericytes in tumor angiogeneic vasculature which, in another aspect is associated with neovascularization *in vivo*. In another aspect, immunization of mice with the recombinant *Listeria,* as provided herein, that expresses a fragment of HMW-MAA (residues 2160 to 2258), impairs the growth of tumors not engineered to express HMW-MAA (Figure 9D). In another aspect, immunization of mice with the recombinant *Listeria* expressing a fragment of HMW-MAA (residues 2160 to 2258) decreases the number of pericytes in the tumor vasculature. In another aspect, immunization of mice with the recombinant *Listeria* expressing a fragment of HMW-MAA (residues 2160 to 2258) causes infiltration of $CD8^+$T cells around blood vessels and into the tumor.

[0090] In one aspect, a murine homolog of HMW-MAA, known as NG2 or AN2, has 80% homology to HMW-MAA, as well as similar expression pattern and function. In another aspect, HMW-MAA is highly expressed on both activated pericytes and pericytes in tumor angiogenic vasculature. In one aspect, activated pericytes are associated with neovascularization in vivo. In one aspect, activated pericytes are involved in angiogenesis. In another aspect, angiogenesis is important for survival of tumors. In another aspect, pericytes in tumor angiogenic vasculature are associated with neovascularization in vivo. In another aspect, activated pericytes are important cells in vascular development, stabilization, maturation and remodeling. Therefore, in one aspect, besides its role as a tumor-associated antigen, HMW-MAA is also a potential universal target for anti-angiogenesis using an immunotherapeutic approach. As described herein (Example 8), results obtained using an *Lm*-based vaccine against this antigen has supported this possibility.

[0091] In another aspect, one of the antigens of the methods and compositions provided herein is expressed in activated pericytes. In another aspect, at least one of the antigens is expressed in activated pericytes.

[0092] The HMW-MAA protein from which HMW-MAA fragments as provided herein are derived is, in another aspect, a human HMW-MAA protein. In another aspect, the HMW-MAA protein is a mouse protein. In another aspect, the HMW-MAA protein is a rat protein. In another aspect, the HMW-MAA protein is a primate protein. In another aspect, the HMW-MAA protein is from any other species known in the art. In another aspect, the HMW-MAA protein is melanoma chondroitin sulfate proteoglycan (MCSP). In another aspect, an AN2 protein is used in methods and compositions as provided herein. In another aspect, an NG2 protein is used in methods and compositions as provided herein.

[0093] In another aspect, the HMW-MAA protein of methods and compositions as provided herein has the sequence:
MQSGRGPPLPAPGLALALTLTMLARLASAASFFGENHLEVPVATALTDIDLQLQFSTSQPEALLLLAAGPADHLLLQLY
SGRLQVRLVLGQEELRLQTPAETLLSDSIPHTVVLTVVEGWATLSVDGFLNASSAVPGAPLEVPYGLFVGGTGTLGLP
YLRGTSRPLRGCLHAATLNGRSLLRPLTPDVHEGCAEEFSASDDVALGFSGPHSLAAFPAWGTQDEGTLEFTLTTQS
RQAPLAFQAGGRRGDFIYVDIFEGHLRAVVEKGQGTVLLHNSVPVADGQPHEVSVHINAHRLEISVDQYPTHTSNRG
VLSYLEPRGSLLLGGLDAEASRHLQEHRLGLTPEATNASLLGCMEDLSVNGQRRGLREALLTRNMAAGCRLEEEEYE
DDAYGHYEAFSTLAPEAWPAMELPEPCVPEPGLPPVFANFTQLLTISPLVVAEGGTAWLEWRHVQPTLDLMEAELRK
SQVLFSVTRGARHGELELDIPGAQARKMFTLLDVVNRKARFIHDGSEDTSDQLVLEVSVTARVPMPSCLRRGQTYLLP
IQVNPVNDPPHIIFPHGSLMVILEHTQKPLGPEVFQAYDPDSACEGLTFQVLGTSSGLPVERRDQPGEPATEFSCREL
EAGSLVYVHRGGPAQDLTFRVSDGLQASPPATLKVVAIRPAIQIHRSTGLRLAQGSAMPILPANLSVETNAVGQDVSVL
FRVTGALQFGELQKQGAGGVEGAEWWATQAFHQRDVEQGRVRYLSTDPQHHAYDTVENLALEVQVGQEILSNLSF
PVTIQRATVWMLRLEPLHTQNTQQETLTTAHLEATLEEAGPSPPTFHYEVVQAPRKGNLQLQGTRLSDGQGFTQDDI
QAGRVTYGATARASEAVEDTFRFRVTAPPYFSPLYTFPIHIGGDPDAPVLTNVLLVVPEGGEGVLSADHLFVKSLNSA
SYLYEVMERPRHGRLAWRGTQDKTTMVTSFTNEDLLRGRLVYQHDDSETTEDDIPFVATRQGESSGDMAWEEVRG
VFRVAIQPVNDHAPVQTISRIFHVARGGRRLLTTDDVAFSDADSGFADAQLVLTRKDLLFGSIVAVDEPTRPIYRFTQE
DLRKRRVLFVHSGADRGWIQLQVSDGQHQATALLEVQASEPYLRVANGSSLVVPQGGQGTIDTAVLHLDTNLDIRSG
DEVHYHVTAGPRWGQLVRAGQPATAFSQQDLLDGAVLYSHNGSLSPRDTMAFSVEAGPVHTDATLQVTIALEGPLA
PLKLVRHKKIYVFQGEAAEIRRDQLEAAQEAVPPADIVFSVKSPPSAGYLVMVSRGALADEPPSLDPVQSFSQEAVDT
GRVLYLHSRPEAWSDAFSLDVASGLGAPLEGVLVELEVLPAAIPLEAQNFSVPEGGSLTLAPPLLRVSGPYFPTLLGLS
LQVLEPPQHGALQKEDGPQARTLSAFSWRMVEEQLIRYVHDGSETLTDSFVLMANASEMDRQSHPVAFTVTVLPVN
DQPPILTTNTGLQMWEGATAPIPAEALRSTDGDSGSEDLVYTIEQPSNGRVVLRGAPGTEVRSFTQAQLDGGLVLFS
HRGTLDGGFRFRLSDGEHTSPGHFFRVTAQKQVLLSLKGSQTLTVCPGSVQPLSSQTL
RASSSAGTDPQLLLYRVVRGPQLGRLFHAQQDSTGEALVNFTQAEVYAGNILYEHEMPPEPFWEAHDTLELQLSSPP
ARDVAATLAVAVSFEAACPQRPSHLWKNKGLWVPEGQRARITVAALDASNLLASVPSPQRSEHDVLFQVTQFPSRG
QLLVSEEPLHAGQPHFLQSQLAAGQLVYAHGGGGTQQDGFHFRAHLQGPAGASVAGPQTSEAFAITVRDVNERPPQ
PQASVPLRLTRGSRAPISRAQLSVVDPDSAPGEIEYEVQRAPHNGFLSLVGGGLGPVTRFTQADVDSGRLAFVANGS
SVAGIFQLSMSDGASPPLPMSLAVDILPSAIEVQLRAPLEVPQALGRSSLSQQQLRVVSDREEPEAAYRLIQGPQYGH
LLVGGRPTSAFSQFQIDQGEVVFAFTNFSSSHDHFRVLALARGVNASAVVNVTVRALLHVWAGGPWPQGATLRLDPT
VLDAGELANRTGSVPRFRLLEGPRHGRVVRVPRARTEPGGSQLVEQFTQQDLEDGRLGLEVGRPEGRAPGPAGDS
LTLELWAQGVPPAVASLDFATEPYNAARPYSVALLSVPEAARTEAGKPESSTPTGEPGPMASSPEPAVAKGGFLSFL
EANMFSVIIPMCLVLLLLALILPLLFYLRKRNKTGKHDVQVLTAKPRNGLAGDTETFRKVEPGQAIPLTAVPGQGPPPG
GQPDPELLQFCRTPNPALKNGQYWV (SEQ ID No: 19). In another aspect, an HMW-MAA AA sequence of methods and compositions as provided herein comprises the sequence set for thin SEQ ID No: 19. In another aspect, the HMW-MAA AA sequence is ahomologue of SEQ ID No: 19. In another aspect, the HMW-MAA AA sequence is a variant of SEQ ID No: 19. In another aspect, the HMW-MAA AA sequence is a fragment of SEQ ID No: 19. In another aspect, the HMW-MAA AA sequence is an isoform of SEQ ID No: 19. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0094] In another aspect, the HMW-MAA protein of methods and compositions as provided herein is encoded by the sequence:
atgcagtccggccgcggccccccacttccagccccggcctggccttggctttgaccctgactatgttggccagacttgcatccgcggcttccttcttcggtgagaacca
cctggaggtgcctgtggccacggctctgaccgacatagacctgcagctgcagttctccacgtcccagcccgaagccctccttctcctggcagcaggcccagctgacc
acctcctgctgcagctctactctggacgcctgcaggtcagacttgttctgggccaggaggagctgaggctgcagactccagcagagacgctgctgagtgactccatcc
cccacactgtggtgctgactgtcgtagagggctgggccacgttgtcagtcgatgggtttctgaacgcctcctcagcagtcccaggagccccctagaggtcccctatgg
gctctttgttgggggcactgggacccttggcctgccctacctgaggggaaccagccgacccctgaggggttgcctccatgcagccaccctcaatggccgcagcctcct
ccggcctctgaccccgatgtgcatgagggctgtgctgaagagttttctgccagtgatgatgtggccctgggcttctctgggccccactctctggctgccttccctgcctgg
ggcactcaggacgaaggaaccctagagtttacactcaccacacagagccggcaggcaccccttggccttccaggcaggggccggcgtggggacttcatctatgtg
gacatatttgagggccacctgcgggccgtggtggagaagggccagggtaccgtattgctccacaacagtgtgcctgtggccgatgggcagccccatgaggtcagtg
tccacatcaatgctcaccggctggaaatctccgtggaccagtaccctacgcatacttcgaaccgaggagtcctcagctacctggagccacggggcagtctccttctcg
gggggctggatgcagaggcctctcgtcacctccaggaacaccgcctgggcctgacaccagaggccaccaatgcctccctgctgggctgcatggaagacctcagtg
tcaatggccagaggcgggggctgcgggaagctttgctgacgcgcaacatggcagccggctgcaggctggaggaggaggagtatgaggacgatgcctatggaca
ttatgaagctttctccaccctggcccctgaggcttggccagccatggagctgcctgagccatgcgtgcctgagccagggctgcctcctgtctttg ccaatttcac-
ccagctgctgactatcagcccactggtggtggccgaggggggcacagcctggcttgagtggaggcatgtgcagcccacgctggacctgatggaggctgagctgcg

caaatcccaggtgctgttcagcgtgacccgaggggcacgccatggcgagctcgagctggacatcccgggagcccaggcacgaaaaatgttcaccctcctggacgt
ggtgaaccgcaaggcccgcttcatccacgatggctctgaggacacctccgaccagctggtgctggaggtgtcggtgacggctcgggtgcccatgccctcatgccttc
ggaggggccaaacatacctcctgcccatccaggtcaaccctgtcaatgacccaccccacatcatcttcccacatggcagcctcatggtgatcctggaacacacgca
gaagccgctggggcctgaggttttccaggcctatgacccggactctgcctgtgagggcctcaccttccaggtccttggcacctcctctggcctccccgtggagcgccga
gaccagcctggggagccggcgaccgagttctcctgccgggagttggaggccggcagcctagtctatgtccaccgcggtggtcctgcacaggacttgacgttccggg
tcagcgatggactgcaggccagccccccggccacgctgaaggtggtggccatccggccggccatacagatccaccgcagcacagggttgcgactggcccaagg
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ccgcgtggtccgcgtgccccgagccaggacggagcccgggggcagccagctggtggagcagttcactcagcaggaccttgaggacgggaggctggggctgga
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ggcgagccaggccccatggcatccagccctgagcccgctgtggccaagggaggcttcctgagcttccttgaggccaacatgttcagcgtcatcatccccatgtgcct
ggtacttctgctcctggcgctcatcctgcccctgctcttctacctccgaaaacgcaacaagacgggcaagcatgacgtccaggtcctgactgccaagccccgcaacg
gcctggctggtgacaccgagaccttccgcaaggtggagccaggccaggccatcccgctcacagctgtgcctggccaggggccccctccaggaggccagcctgac
ccagagctgctgcagttctgccggacacccaaccctgcccttaagaatggccagtactgggtgtgaggcctggcctgggcccagatgctgatcgggccagggaca
ggc (SEQ ID No: 20). In another aspect, the recombinant nucleotide has the sequence set forth in SEQ ID NO: 20. In another aspect, an HMW-MAA-encoding nucleotide of methods and compositions as provided herein comprises the sequence set forth in SEQ ID No: 20. In another aspect, the HMW-MAA-encoding nucleotide is a homologue of SEQ ID No: 20. In another aspect, the HMW-MAA-encoding nucleotide is a variant of SEQ ID No: 20. In another aspect, the HMW-MAA-encoding nucleotide is a fragment of SEQ ID No: 20. In another aspect, the HMW-MAA-encoding nucleotide

is an isoform of SEQ ID No: 20. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0095] In another aspect, the HMW-MAA protein of methods and compositions as provided herein has an AA sequence set forth in a GenBank entry having an Accession Numbers selected from NM_001897 and X96753. In another aspect, the HMW-MAA protein is encoded by a nucleotide sequence set forth in one of the above GenBank entries. In another aspect, the HMW-MAA protein comprises a sequence set forth in one of the above GenBank entries. In another aspect, the HMW-MAA protein is a homologue of a sequence set forth in one of the above GenBank entries. In another aspect, the HMW-MAA protein is a variant of a sequence set forth in one of the above GenBank entries. In another aspect, the HMW-MAA protein is a fragment of a sequence set forth in one of the above GenBank entries. In another aspect, the HMW-MAA protein is an isoform of a sequence set forth in one of the above GenBank entries. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0096] The HMW-MAA fragment utilized in the present disclosure comprises, in another aspect, AA 360-554. In another aspect, the fragment consists essentially of AA 360-554. In another aspect, the fragment consists of AA 360-554. In another aspect, the fragment comprises AA 701-1130. In another aspect, the fragment consists essentially of AA 701-1130. In another aspect, the fragment consists of AA 701-1130. In another aspect, the fragment comprises AA 2160-2258. In another aspect, the fragment consists essentially of 2160-2258. In another aspect, the fragment consists of 2160-2258. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0097] In another aspect, the recombinant *Listeria* of the compositions and methods as provided herein comprise a plasmid that encodes a recombinant polypeptide that is, in one aspect, angiogenic, and in another aspect, antigenic. In one aspect, the polypeptide is HMW-MAA, and in another aspect, the polypeptide is a HMW-MAA fragment. In another aspect, the plasmid further encodes a non-HMW-MAA peptide. In one aspect, the non-HMW-MAA peptide enhances the immunogenicity of the polypeptide. In one aspect, the HMW-MAA fragment of methods and compositions as provided herein is fused to the non-HMW-MAA AA sequence. In another aspect, the HMW-MAA fragment is embedded within the non-HMW-MAA AA sequence. In another aspect, an HMW-MAA-derived peptide is incorporated into an LLO fragment, ActA protein or fragment, or PEST-like sequence. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0098] The non-HMW-MAA peptide is, in one aspect, a listeriolysin (LLO) oligopeptide. In another aspect, the non-HMW-MAA peptide is an ActA oligopeptide. In another aspect, the non-HMW-MAA peptide is a PEST-like oligopeptide. In one aspect, fusion to LLO, ActA, PEST-like sequences and fragments thereof enhances the cell-mediated immunogenicity of antigens. In one aspect, fusion to LLO, ActA, PEST-like sequences and fragments thereof enhances the cell-mediated immunogenicity of antigens in a variety of expression systems. In another aspect, the non-HMW-MAA peptide is any other immunogenic non-HMW-MAA peptide known in the art. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0099] In one aspect, the recombinant *Listeria* strain of the compositions and methods as provided herein express a heterologous antigenic polypeptide that is expressed by a tumor cell. In one aspect, the recombinant *Listeria* strain of the compositions and methods as provided herein comprise a first or second nucleic acid molecule that encodes a Prostate Specific Antigen (PSA), which in one aspect, is a marker for prostate cancer that is highly expressed by prostate tumors, which in one aspect is the most frequent type of cancer in American men and, in another aspect, is the second cause of cancer related death in American men. In one aspect, PSA is a kallikrein serine protease (KLK3) secreted by prostatic epithelial cells, which in one aspect, is widely used as a marker for prostate cancer.

[0100] In one aspect, the recombinant Listeria strain as provided herein comprises a nucleic acid molecule encoding KLK3 protein.

[0101] In another aspect, the KLK3 protein has the sequence:
MWVPVVFLTLSVTWIGAAPLILSRIVGGWECEKHSQPWQVLVASRGRAVCGGVLVHPQWVLTAAHCIRNKSVILLGR
HSLFHPEDTGQVFQVSHSFPHPLYDMSLLKNRFLRPGDDSSHDLMLLRLSEPAELTDAVKVMDLPTQEPALGTTCYA
SGWGSIEPEEFLTPKKLQCVDLHVISNDVCAQVHPQKVTKFMLCAGRWTGGKSTCSGDSGGPLVCNGVLQGITSWG
SEPCALPERPSLYTKVVHYRKWIKDTIVANP (SEQ ID No: 21; GenBank Accession No. CAA32915). In another aspect, the KLK3 protein is a homologue of SEQ ID No: 21. In another aspect, the KLK3 protein is a variant of SEQ ID No: 21. In another aspect, the KLK3 protein is an isomer of SEQ ID No: 21. In another aspect, the KLK3 protein is a fragment of SEQ ID No: 21. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0102] In another aspect, the KLK3 protein has the sequence:
IVGGWECEKHSQPWQVLVASRGRAVCGGVLVHPQWVLTAAHCIRNKSVILLGRHSLFHPEDTGQVFQVSHSFPHPL
YDMSLLKNRFLRPGDDSSHDLMLLRLSEPAELTDAVKVMDLPTQEPALGTTCYASGWGSIEPEEFLTPKKLQCVDLH
VISNDVCAQVHPQKVTKFMLCAGRWTGGKSTCSGDSGGPLVCYGVLQGITSWGSEPCALP-
ERPSLYTKVVHYRKWIKDTIVANP (SEQ ID No: 22). In another aspect, the KLK3 protein is a homologue of SEQ ID No: 22. In another aspect, the KLK3 protein is avariant of SEQ ID No: 22. In another aspect, the KLK3 protein is an isomer of SEQ ID No: 22. In another aspect, the KLK3 protein is a fragment of SEQ ID No: 22. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0103] In another aspect, the KLK3 protein has the sequence: IVGGWECEKHSQPWQVLVASRGRAVCGGVLVHPQWVLTAAHCIRNKSVILLGRHSLFHPEDTGQVFQVSHSFPHPL YDMSLLKNRFLRPGDDSSHDLMLLRLSEPAELTDAVKVMDLPTQEPALGTTCYASGWGSIEPEEFLTPKKLQCVDLH VISNDVCAQVHPQKVTKFMLCAGRWTGGKSTCSGDSGGPLVCNGVLQGITSWGSEPCALP-ERPSLYTKVVHYRKWIKDTIVANP (SEQ ID No: 23; GenBank Accession No. AAA59995.1). In another aspect, the KLK3 protein is a homologue of SEQ ID No: 23. In another aspect, the KLK3 protein is a variant of SEQ ID No: 23. In another aspect, the KLK3 protein is an isomer of SEQ ID No: 23. In another aspect, the KLK3 protein is a fragment of SEQ ID No: 23. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0104] In another aspect, the KLK3 protein is encoded by a nucleotide molecule having the sequence:

ggtgtcttaggcacactggtcttggagtgcaaaggatctaggcacgtgaggctttgtatgaagaatcggggatcgtacccacccccgtttctgtttcatcctgggcatgtc
tcctctgcctttgtcccctagatgaagtctccatgagctacaagggcctggtgcatccagggtgatctagtaattgcagaacagcaagtgctagctctccctccccttcca
cagctctgggtgtgggaggggggttgtccagcctccagcagcatggggagggccttggtcagcctctgggtgccagcagggcaggggcggagtcctggggaatgaa
ggttttatagggctcctgggggaggctccccagccccaagcttaccacctgcacccggagagctgtgtcaccatgtgggtcccggttgtcttcctcaccctgtccgtgac
gtggattggtgagaggggccatggttgggggatgcaggagagggagccagccctgactgtcaagctgaggctctttccccccaacccagcaccccagcccag
acagggagctgggctcttttctgtctctcccagccccacttcaagcccatacccccagtcccctccatattgcaacagtcctcactcccacaccaggtccccgctccctc
ccacttaccccagaactttcttcccatttgcccagccagctccctgctcccagctgctttactaaaggggaagttcctgggcatctccgtgtttctctttgtggggctcaaaac
ctccaaggacctctctcaatgccattggttccttggaccgtatcactggtccatctcctgagcccctcaatcctatcacagtctactgacttttcccattcagctgtgagtgtcc
aaccctatcccagagaccttgatgcttggcctcccaatcttgccctaggatacccagatgccaaccagacacctccttctttcctagccaggctatctggcctgagacaa
caaatgggtccctcagtctggcaatgggactctgagaactcctcattccctgactcttagccccagactcttcattcagtggcccacattttccttaggaaaaacatgagc
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aactgttttctcaacctgggacttcctaaactttccaaaaccttcctcttccagcaactgaacctcgccataaggcacttatccctggttcctagcacccctatcccctcag
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ccagctccccctgcccatgtcccaggactcccagccttggttctctgcccccgtgtcttttcaaacccacatcctaaatccatctcctatcgagtcccccagttcccctgt
caaccctgattcccctgatctagcacccccctctgcaggcgctgcgcccctcatcctgtctcggattgtgggaggctgggagtgcgagaagcattcccaaccctggcag
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gcgcggtggctcacacctgtaatcccagcactttgggaggccaaggcaggtagatcacctgaggtcaggagttcgagaccagcctggccaactggtgaaacccca
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gaagtcggctctggagacatttctctcttcttccaaagctgggaactgctatctgttatctgcctgtccaggtctgaaagataggattgcccaggcagaaactgggactga
cctatctcactctctccctgcttttaccttagggtgattctgggggcccacttgtctgtaatggtgtgcttcaaggtatcacgtcatggggcagtgaaccatgtgccctgccc
gaaaggccttccctgtacaccaaggtggtgcattaccggaagtggatcaaggacaccatcgtggccaacccctgagcacccctatcaagtccctattgtagtaaactt
ggaaccttggaaatgaccaggccaagactcaagcctccccagttctactgacctttgtccttaggtgtgaggtccagggttgctaggaaaagaaatcagcagacaca
ggtgtagaccagagtgtttcttaaatggtgtaattttgtcctctctgtgtcctggggaatactggccatgcctggagacatatcactcaatttctctgaggacacagttaggat
ggggtgtctgtgttatttgtgggatacagagatgaaagaggggtgggatcc (SEQ ID No: 24; GenBank Accession No. X14810). In another aspect, the KLK3 protein is encoded by residues 401..446, 1688..1847, 3477..3763, 3907..4043, and 5413..5568 of SEQ ID No: 24. In another aspect, the KLK3 protein is encoded by a homologue of SEQ ID No: 24. In another aspect, the KLK3 protein is encoded by a variant of SEQ ID No: 24. In another aspect, the KLK3 protein is encoded by an isomer of SEQ ID No: 24. In another aspect, the KLK3 protein is encoded by a fragment of SEQ ID No: 24. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0105] In another aspect, the KLK3 protein has the sequence:
MWVPVVFLTLSVTWIGAAPLILSRIVGGWECEKHSQPWQVLVASRGRAVCGGVLVHPQWVLTAAHCIRNKSVILLGR
HSLFHPEDTGQVFQVSHSFPHPLYDMSLLKNRFLRPGDDSSHDLMLLRLSEPAELTDAVKVMDLPTQEPALGTTCYA
SGWGSIEPEEFLTPKKLQCVDLHVISNDVCAQVHPQKVTKFMLCAGRWTGGKSTCSW-
VILITELTMPALPMVLHGSLVPWRGGV (SEQ ID No: 25; GenBank Accession No. NP_001025218) In another aspect, the KLK3 protein is a homologue of SEQ ID No: 25. In another aspect, the KLK3 protein is a variant of SEQ ID No: 25. In another aspect, the KLK3 protein is an isomer of SEQ ID No: 25. In another aspect, the KLK3 protein is a fragment of SEQ ID No: 25. Each possibility represents a separate aspect as provided herein.

[0106] In another aspect, the KLK3 protein is encoded by a nucleotide molecule having the sequence:
agccccaagcttaccacctgcacccggagagctgtgtcaccatgtgggtcccggttgtcttcctcaccctgtccgtgacgtggattggtgctgcacccctcatcctgtctc
ggattgtgggaggctgggagtgcgagaagcattcccaaccctggcaggtgcttgtggcctctcgtggcagggcagtctgcggcggtgttctggtgcacccccagtgg
gtcctcacagctgcccactgcatcaggaacaaaagcgtgatcttgctgggtcggcacagcctgtttcatcctgaagacacaggccaggtatttcaggtcagccacag
cttcccacacccgctctacgatatgagcctcctgaagaatcgattcctcaggccaggtgatgactccagccacgacctcatgctgctccgcctgtcagagcctgccga
gctcacggatgctgtgaaggtcatggacctgcccacccaggagccagcactggggaccacctgctacgcctcaggctggggcagcattgaaccagaggagttctt
gaccccaaagaaacttcagtgtgtggacctccatgttatttccaatgacgtgtgtgcgcaagttcaccctcagaaggtgaccaagttcatgctgtgtgctggacgctgga
caggggcaaaagcacctgctcgtgggtcattctgatcaccgaactgaccatgccagccctgccgatggtcctccatggctccctagtgccct ggagaggaggt-
gtctagtcagagagtagtcctggaaggtggcctctgtgaggagccacggggacagcatcctgcagatggtcctggcccttgtcccaccgacctgtctacaaggactgt
cctcgtggaccctcccctctgcacaggagctggaccctgaagtcccttccccaccggccaggactggagcccctacccctctgttggaatccctgcccaccttcttctg
gaagtcggctctggagacatttctctcttcttccaaagctgggaactgctatctgttatctgcctgtccaggtctgaaagataggattgcccaggcagaaactgggactga
cctatctcactctctccctgcttttaccttagggtgattctgggggcccacttgtctgtaatggtgtgcttcaaggtatcacgtcatggggcagtgaaccatgtgccctgccc
gaaaggccttccctgtacaccaaggtggtgcattaccggaagtggatcaaggacaccatcgtggccaacccctgagcacccctatcaacccccctattgtagtaaactt
ggaaccttggaaatgaccaggccaagactcaagcctccccagttctactgacctttgtccttaggtgtgaggtccagggttgctaggaaaagaaatcagcagacaca
ggtgtagaccagagtgtttcttaaatggtgtaattttgtcctctctgtgtcctggggaatactggccatgcctggagacatatcactcaatttctctgaggacacagatagga
tggggtgtctgtgttatttgtggggtacagagatgaaagaggggtgggatccacactgagagagtggagagtgacatgtgctggacactgtccatgaagcactgagc
agaagctggaggcacaacgcaccagacactcacagcaaggatggagctgaaaacataacccactctgtcctggaggcactgggaagcctagagaaggctgtg
agccaaggagggagggtcttcctttggcatgggatggggatgaagtaaggagagggactggacccccctggaagctgattcactatgggggggaggtgtattgaagtc
ctccagacaaccctcagatttgatgatttcctagtagaactcacagaaataaagagctgttatactgtg (SEQ ID No: 26; GenBank Accession No. NM_001030047). In another aspect, the KLK3 protein is encoded by residues 42-758 of SEQ ID No: 26. In another aspect, the KLK3 protein is encoded by a homologue of SEQ ID No: 26. In another aspect, the KLK3 protein is encoded by a variant of SEQ ID No: 26. In another aspect, the KLK3 protein is encoded by an isomer of SEQ ID No: 26. In another aspect, the KLK3 protein is encoded by a fragment of SEQ ID No: 26. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0107] In another aspect, the KLK3 protein has the sequence: MWVPVVFLTLSVTWIGAAPLILSRIVG-
GWECEKHSQPWQVLVASRGRAVCGGVLVHPQ WVLTAAHCIRK (SEQ ID No: 27; GenBank Accession No. NP_001025221). In another aspect, the KLK3 protein is a homologue of SEQ ID No: 27. In another aspect, the KLK3 protein is a variant of SEQ ID No: 27. In another aspect, the sequence of the KLK3 protein comprises SEQ ID No: 27. In another aspect, the KLK3 protein is an isomer of SEQ ID No: 27. In another aspect, the KLK3 protein is a fragment of SEQ ID No: 27. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0108] In another aspect, the KLK3 protein is encoded by a nucleotide molecule having the sequence:
agccccaagcttaccacctgcacccggagagctgtgtcaccatgtgggtcccggttgtcttcctcaccttccgtgacgtggattggtgctgcaccctcatcctgtctcg
gattgtgggaggctgggagtgcgagaagcattcccaaccctggcaggtgcttgtggcctctcgtggcagggcagtctgcggcggtgttctggtgcacccccagtgggt
cctcacagctgcccactgcatcaggaagtgagtaggggcctggggtctggggagcaggtgtctgtgtcccagaggaataacagctgggcattttccccaggataac
ctctaaggccagccttgggactgggggagagagggaaagttctggttcaggtcacatggggaggcagggttggggctggaccaccctccccatggctgcctgggtc
tccatctgtgttcctctatgtctctttgtgtcgctttcattatgtctcttggtaactggcttcggttgtgtctctccgtgtgactattttgttctctctctccctctcttctctgtcttcagt
(SEQ ID No: 28; GenBank Accession No. NM_001030050). In another aspect, the KLK3 protein is encoded by residues 42-758 of SEQID No: 28. In another aspect, the KLK3 protein is encoded by a homologue of SEQ ID No: 28. In another aspect, the KLK3 protein is encoded by a variant of SEQ ID No: 28. In another aspect, the KLK3 protein is encoded by

an isomer of SEQ ID No: 28. In another aspect, the KLK3 protein is encoded by a fragment of SEQ ID No: 28. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0109] In another aspect, the KLK3 protein that is the source of the KLK3 peptide has the sequence: MWVPVVFLTLSVTWIGAAPLILSRIVGGWECEKHSQPWQVLVASRGRAVCGGVLVHPQWVLTAAHCIRNKSVILLGR HSLFHPEDTGQVFQVSHSFPHPLYDMSLLKNRFLRPGDDSSIEPEEFLTPKKLQCVDLHVISNDVCAQVHPQKVTKFM LCAGRWTGGKSTCSGDSGGPLVCNGVLQGITSWGSEPCALPERPSLYTKVVHYRKWIKDTIVANP (SEQ ID No: 29; GenBank Accession No. NP_001025220). In another aspect, the KLK3 protein is a homologue of SEQ ID No: 29. In another aspect, the KLK3 protein is a variant of SEQ ID No: 29. In another aspect, the KLK3 protein is an isomer of SEQ ID No: 29. In another aspect, the KLK3 protein is a fragment of SEQ ID No: 29. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0110] In another aspect, the KLK3 protein is encoded by a nucleotide molecule having the sequence: agccccaagcttaccacctgcacccggagagctgtgtcaccatgtgggtcccggttgtcttcctcaccctgtccgtgacgtggattggtgctgcacccctcatcctgtctc ggattgtgggaggctgggagtgcgagaagcattcccaaccctggcaggtgcttgtggcctctcgtggcagggcagtctgcggcggtgttctggtgcacccccagtgg gtcctcacagctgcccactgcatcaggaacaaaagcgtgatcttgctgggtcggcacagcctgtttcatcctgaagacacaggccaggtatttcaggtcagccacag cttcccacacccgctctacgatatgagcctcctgaagaatcgattcctcaggccaggtgatgactccagcattgaaccagaggagttcttgaccccaaagaaacttca gtgtgtggacctccatgttatttccaatgacgtgtgtgcgcaagttcaccctcagaaggtgaccaagttcatgctgtgtgctggacgctggacaggggcaaaagcacc tgctcgggtgattctgggggcccacttgtctgtaatggtgtgcttcaaggtatcacgtcatggggcagtgaaccatgtgccctgcccgaaaggccttccctgtacaccaa ggtggtgcattaccggaagtggatcaaggacaccatcgtggccaacccctgagcacccctatcaacccctattgtagtaaacttggaaccttggaaatgaccaggc caagactcaagcctccccagttctactgacctttgtccttaggtgtgaggtccagggttgctaggaaaagaaatcagcagacacaggtgtagaccagagtgtttcttaa atggtgtaattttgtcctctctgtgtcctggggaatactggccatgcctggagacatatcactcaatttctctgaggacacagataggatggggtgtctgtgttatttgtggggt acagagatgaaagaggggtgggatccacactgagagagtggagagtgacatgtgctggacactgtccatgaagcactgagcagaagctggaggcacaacgca ccagacactcacagcaaggatggagctgaaaacataacccactctgtcctggaggcactgggaagcctagagaaggctgtgagccaaggagggagggtcttcct ttggcatgggatggggatgaagtaaggagagggactggacccccctggaagctgattcactatgggggaggtgtattgaagtcctccagacaaccctcagatttgat gatttcctagtagaactcacagaaataaagagctgttatactgtg (SEQ ID No: 30; GenBank Accession No. NM_001030049). In another aspect, the KLK3 protein is encoded by residues 42-758 of SEQ ID No: 30. In another aspect, the KLK3 protein is encoded by a homologue of SEQ ID No: 30. In another aspect, the KLK3 protein is encoded by a variant of SEQ ID No: 30. In another aspect, the KLK3 protein is encoded by an isomer of SEQ ID No: 30. In another aspect, the KLK3 protein is encoded by a fragment of SEQ ID No: 30. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0111] In another aspect, the KLK3 protein has the sequence: MWVPVVFLTLSVTWIGAAPLILSRIVG-GWECEKHSQPWQVLVASRGRAVCGGVLVHPQWVLTAAHCIRKPGDDSSHDLMLLRLSEPAELTDAVKVMDLPTQE PALGTTCYASGWGSIEPEEFLTPKKLQCVDLHVISNDVCAQVHPQKVTKFMLCAGRWTGG-KSTCSGDSGGPLVCNGVLQGITSWGSEPCALPERPSLYTKVVHYRKWIKDTIVANP (SEQ ID No: 31; GenBank Accession No. NP_001025219). In another aspect, the KLK3 protein is a homologue of SEQ ID No: 31. In another aspect, the KLK3 protein is a variant of SEQ ID No: 31. In another aspect, the KLK3 protein is an isomer of SEQ ID No: 31. In another aspect, the KLK3 protein is a fragment of SEQ ID No: 31. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0112] In another aspect, the KLK3 protein is encoded by a nucleotide molecule having the sequence: agccccaagcttaccacctgcacccggagagctgtgtcaccatgtgggtcccggttgtcttcctcaccctgtccgtgacgtggattggtgctgcacccctcatcctgtctc ggattgtgggaggctgggagtgcgagaagcattcccaaccctggcaggtgcttgtggcctctcgtggcagggcagtctgcggcggtgttctggtgcacccccagtgg gtcctcacagctgcccactgcatcaggaagccaggtgatgactccagccacgacctcatgctgctccgcctgtcagagcctgccgagctcacggatgctgtgaaggt catggacctgcccacccaggagccagcactggggaccacctgctacgcctcaggctggggcagcattgaaccagaggagttcttgaccccaaagaaacttcagtg tgtggacctccatgttatttccaatgacgtgtgtgcgcaagttcaccctcagaaggtgaccaagttcatgctgtgtgctggacgctggacaggggcaaaagcacctgc tcgggtgattctgggggcccacttgtctgtaatggtgtgcttcaaggtatcacgtcatggggcagtgaaccatgtgccctgcccgaaaggccttccctgtacaccaaggt ggtgcattacccaaggacaccatcgtggccaacccctgagcacccctatcaacccctattgtagtaaacttggaaccttggaaatgaccaggccaagactcaagc ctccccagttctactgacctttgtccttaggtgtgaggtccagggttgctaggaaaagaaatcagcagacacaggtgtagaccagagtgtttcttaaatggtgtaattttgt cctctctgtgtcctggggaatactggccatgcctggagacatatcactcaatttctctgaggacacagataggatggggtgtctgtgttatttgtggggtacagagatgaa agaggggtgggatccacactgagagagtggagagtgacatgtgctggacactgtccatgaagcactgagcagaagctggaggcacaacgcaccagacactcac agcaaggatggagctgaaaacataacccactctgtcctggaggcactgggaagcctagagaaggctgtgagccaaggagggagggtcttcctttggcatgggatg ggatgaagtaaggagagggactggacccccctggaagctgattcactatgggggaggtgtattgaagtcctccagacaaccctcagatttgatgatttcctagtaga actcacagaaataaagagctgttatactgtg (SEQ ID No: 32; GenBank Accession No. NM_001030048). In another aspect, the KLK3 protein is encoded by residues 42-758 of SEQ ID No: 32. In another aspect, the KLK3 protein is encoded by a homologue of SEQ ID No: 32. In another aspect, the KLK3 protein is encoded by a variant of SEQ ID No: 32. In another aspect, the KLK3 protein is encoded by an isomer of SEQ ID No: 32. In another aspect, the KLK3 protein is encoded by a fragment of SEQ ID No: 32. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0113] In another aspect, the KLK3 protein has the sequence: MWVPVVFLTLSVTWIGAAPLILSRIVG-GWECEKHSQPWQVLVASRGRAVCGGVLVHPQWVLTAAHCIRNKSVILLGRHSLFHPEDTGQVFQVSHSFPHPLYDM

SLLKNRFLRPGDDSSHDLMLLRLSEPAELTDAVKVMDLPTQEPALGTTCYASGWGSIEPEEFLTPKKLQCVDLHVISN DVCAQVHPQKVTKFMLCAGRWTGGKSTCSGDSGGPLVCNGVLQGITSWGSEPCALP-ERPSLYTKVVHYRKWIKDTIVANP (SEQ ID No: 33; GenBank Accession No. NP_001639). In another aspect, the KLK3 protein is a homologue of SEQ ID No: 33. In another aspect, the KLK3 protein is a variant of SEQ ID No: 33. In another aspect, the KLK3 protein is an isomer of SEQ ID No: 33. In another aspect, the KLK3 protein is a fragment of SEQ ID No: 33. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0114] In another aspect, the KLK3 protein is encoded by anucleotide molecule having the sequence: agccccaagcttaccacctgcacccggagagctgtgtcaccatgtgggtcccggttgtcttcctcaccctgtccgtgacgtggattggtgctgcacccctcatcctgtctc ggattgtgggaggctgggagtgcgagaagcattcccaaccctggcaggtgcttgtggcctctcgtggcagggcagtctgcggcggtgttctggtgcacccccagtgg gtcctcacagctgcccactgcatcaggaacaaaagcgtgatcttgctgggtcggcacagcctgtttcatcctgaagacacaggccaggtatttcaggtcagccacag cttcccacacccgctctacgatatgagcctcctgaagaatcgattcctcaggccaggtgatgactccagccacgacctcatgctgctccgcctgtcagagcctgccga gctcacggatgctgtgaaggtcatggacctgcccacccaggagccagcactggggaccacctgctacgcctcaggctggggcagcattgaaccagaggagttctt gaccccaaagaaacttcagtgtgtggacctccatgttatttccaatgacgtgtgtgcgcaagttcaccctcagaaggtgaccaagttcatgctgtgtgctggacgctgga caggggcaaaagcacctgctcgggtgattctgggggcccacttgtctgtaatggtgtgcttcaaggtatcacgtcatggggcagtgaaccatgtgccctgcccgaaa ggccttccctgtacaccaaggtggtgcattaccggaagtggatcaaggacaccatcgtggccaacccctgagcacccctatcaacccccttattgtagtaaacttggaa ccttggaaatgaccaggccaagactcaagcctccccagttctactgacctttgtccttaggtgtgaggtccagggttgctaggaaaagaaatcagcagacacaggtgt agaccagagtgtttcttaaatggtgtaattttgtcctctctgtgtcctggggaatactggccatgcctggagacatatcactcaatttctctgaggacacagataggatggg gtgtctgtgttatttgtggggtacagagatgaaagaggggtgggatccacactgagagagtggagagtgacatgtgctggacactgtccatgaagcactgagcagaa gctggaggcacaacgcaccagacactcacagcaaggatggagctgaaaacataacccactctgtcctggaggcactgggaagcctagagaaggctgtgagcc aaggagggagggtcttcctttggcatgggatggggatgaagtaaggagagggactggacccccctggaagctgattcactatgggggggaggtgtattgaagtcctcc agacaaccctcagatttgatgatttcctagtagaactcacagaaataaagagctgttatactgtg (SEQ ID No: 34; GenBank Accession No. NM_001648). In another aspect, the KLK3 protein is encoded by residues 42-827 of SEQ ID No: 34. In another aspect, the KLK3 protein is encoded by a homologue of SEQ ID No: 34. In a nother aspect, the KLK3 protein is encoded by a variant of SEQ ID No: 34. In another aspect, the KLK3 protein is encoded by an isomer of SEQ ID No: 34. In another aspect, the KLK3 protein is encoded by a fragment of SEQ ID No: 34. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0115] In another aspect, the KLK3 protein has the sequence: MWVPVVFLTLSVTWIGAAPLILSRIVGGWECEKHSQPWQVLVASRGRAVCGGVLVHPQWVLTAAHCIRNKSVILLGR HSLFHPEDTGQVFQVSHSFPHPLYDMSLLKNRFLRPGDDSSHDLMLLRLSEPAELTDAVKVMDLPTQEPALGTTCYA SGWGSIEPEEFLTPKKLQCVDLHVISNDVCAQVHPQKVTKFMLCAGRWTGGKSTCSGDSGGPLVCNGVLQGITSWG SEPCALPERPSLYTKVVHYRKWIKDTIVANP (SEQ ID No: 35 GenBank Accession No. AAX29407.1). In another aspect, the KLK3 protein is a homologue of SEQ ID No: 35. In another aspect, the KLK3 protein is a variant of SEQ ID No: 35. In another aspect, the KLK3 protein is an isomer of SEQ ID No: 35. In another aspect, the sequence of the KLK3 protein comprises SEQ ID No: 35. In another aspect, the KLK3 protein is a fragment of SEQ ID No: 35. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0116] In another aspect, the KLK3 protein is encoded by a nucleotide molecule having the sequence: gggggagccccaagcttaccacctgcacccggagagctgtgtcaccatgtgggtcccggttgtcttcctcaccctgtccgtgacgtggattggtgctgcacccctcatcc tgtctcggattgtgggaggctgggagtgcgagaagcattcccaaccctggcaggtgcttgtggcctctcgtggcagggcagtctgcggcggtgttctggtgcaccccca gtgggtcctcacagctgcccactgcatcaggaacaaaagcgtgatcttgctgggtcggcacagcctgtttcatcctgaagacacaggccaggtatttcaggtcagcca cagcttcccacacccgctctacgatatgagcctcctgaagaatcgattcctcaggccaggtgatgactccagccacgacctcatgctgctccgcctgtcagagcctgc cgagctcacggatgctgtgaaggtcatggacctgcccacccaggagccagcactggggaccacctgctacgcctcaggctggggcagcattgaaccagaggagt tcttgaccccaaagaaacttcagtgtgtggacctccatgttatttccaatgacgtgtgtgcgcaagttcaccctcagaaggtgaccaagttcatgctgtgtgctggacgct ggacaggggcaaaagcacctgctcgggtgattctgggggcccacttgtctgtaatggtgtgcttcaaggtatcacgtcatggggcagtgaaccatgtgccctgcccg aaaggccttccctgtacaccaaggtggtgcattaccggaagtggatcaaggacaccatcgtggccaacccctgagcacccctatcaactccctattgtagtaaacttg gaaccttggaaatgaccaggccaagactcaggcctccccagttctactgacctttgtccttaggtgtgaggtccagggttgctaggaaaagaaatcagcagacacag gtgtagaccagagtgtttcttaaatggtgtaattttgtcctctctgtgtcctggggaatactggccatgcctggagacatatcactcaatttctctgaggacacagataggat ggggtgtctgtgttatttgtggggtacagagatgaaagaggggtgggatccacactgagagagtggagagtgacatgtgctggacactgtccatgaagcactgagca gaagctggaggcacaacgcaccagacactcacagcaaggatggagctgaaaacataacccactctgtcctggaggcactgggaagcctagagaaggctgtga gccaaggagggagggtcttcctttggcatgggatggggatgaagtagggagagggactggacccccctggaagctgattcactatgggggggaggtgtattgaagtcc tccagacaaccctcagatttgatgatttcctagtagaactcacagaaataaagagctgttatactgcgaaaaaaaaaaaaaaaaaaaaaaaaaaa (SEQ ID No: 36; GenBank Accession No. BC056665). In another aspect, the KLK3 protein is encoded by residues 47-832 of SEQ ID No: 36. In another aspect, the KLK3 protein is encoded by a homologue of SEQ ID No: 36. In another aspect, the KLK3 protein is encoded by a variant of SEQ ID No: 36. In another aspect, the KLK3 protein is encoded by an isomer of SEQ ID No: 36. In another aspect, the KLK3 protein is encoded by a fragment of SEQ ID No: 36. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0117] In another aspect, the KLK3 protein has the sequence: MWVPVVFLTLSVTWIGAAPLILSRIVG-GWECEKHSQPWQVLVASRGRAVCGGVLVHPQWVLTAAHCIRNKSVILLGRHSLFHPEDTGQVFQVSHSFPHPLYDM SLLKNRFLRPGDDSSIEPEEFLTPKKLQCVDLHVISNDVCAQVHPQKVTKFMLCAGRWTGG-

KSTCSGDSGGPLVCNGVLQGITSWGSEPCALPERPSLYTKVVHYRKWIKDTIVA (SEQ ID No: 37; GenBank Accession No. AJ459782). In another aspect, the KLK3 protein is a homologue of SEQ ID No: 37. In another aspect, the KLK3 protein is a variant of SEQ ID No: 37. In another aspect, the KLK3 protein is an isomer of SEQ ID No: 37. In another aspect, the KLK3 protein is a fragment of SEQ ID No: 37. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0118] In another aspect, the KLK3 protein has the sequence: MWVPVVFLTLSVTWIGAAPLILSRIVGGWECEKHSQPWQVLVASRGRAVCGGVLVHPQWVLTAAHCIRNKSVILLGRHSLFHPEDTGQVFQVSHSFPHPLYDMSLLKNRFLRPGDDSSHDLMLLRLSEPAELTDAVKVMDLPTQEPALGTTCYASGWGSIEPEEFLTPKKLQCVDLHVISNDVCAQVHPQKVTKFMLCAGRWTGGKSTCSVSHPYSQDLEGKGEWGP (SEQ ID No: 38, GenBank Accession No. AJ512346). In another aspect, the KLK3 protein is a homologue of SEQ ID No: 38. In another aspect, the KLK3 protein is a variant of SEQ ID No: 38. In another aspect, the KLK3 protein is an isomer of SEQ ID No: 38. In another aspect, the sequence of the KLK3 protein comprises SEQ ID No: 38. In another aspect, the KLK3 protein is a fragment of SEQ ID No: 38. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0119] In another aspect, the KLK3 protein has the sequence: MWVPVVFLTLSVTWIGERGHGWGDAGEGASPDCQAEALSPPTQHPSPDRELGSFLSLPA PLQAHTPSPSILQQSSLPHQVPAPSHLPQNFLPIAQPAPCSQLLY (SEQ ID No: 39 GenBank Accession No. AJ459784). In another aspect, the KLK3 protein is a homologue of SEQ ID No: 39. In another aspect, the KLK3 protein is avariant of SEQ ID No: 39. In another aspect, the sequence of the KLK3 protein comprises SEQ ID No: 39. In another aspect, the KLK3 protein is an isomer of SEQ ID No: 39. In another aspect, the KLK3 protein is a fragment of SEQ ID No: 39. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0120] In another aspect, the KLK3 protein has the sequence: MWVPVVFLTLSVTWIGAAPLILSRIVGGWECEKHSQPWQVLVASRGRAVCGGVLVHPQWVLTAAHCIRNKSVILLGRHSLFHPEDTGQVFQVSHSFPHPLYDMSLLKNRFLRPGDDSS
HDLMLLRLSEPAELTDAVKVMDLPTQEPALGTTCYASGWGSIEPEEFLTPKKLQCVDLHVISNDVCAQVHPQKVTKFMLCAGRWTGGKSTCSGDSGGPLVCNGVLQGITSWGSEPCALPERPSLYTKVVHYRKWIKDTIVANP (SEQ ID No: 40 GenBank Accession No. AJ459783). In another aspect, the KLK3 protein is a homologue of SEQ ID No: 40. In another aspect, the KLK3 protein is a variant of SEQ ID No: 40. In another aspect, the KLK3 protein is an isomer of SEQ ID No: 40. In another aspect, the KLK3 protein is a fragment of SEQ ID No: 40. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0121] In another aspect, the KLK3 protein is encoded by a nucleotide molecule having the sequence:
aagtttcccttctcccagtccaagaccccaaatcaccacaaaggacccaatccccagactcaagatatggtctgggcgctgtcttgtgtctcctaccctgatccctgggtt
caactctgctcccagagcatgaagcctctccaccagcaccagccaccaacctgcaaacctagggaagattgacagaattcccagcctttcccagctccccctgccc
atgtcccaggactcccagccttggttctctgcccccgtgtcttttcaaacccacatcctaaatccatctcctatccgagtcccccagttcctcctgtcaaccctgattcccctg
atctagcacccccctctgcaggtgctgcacccctcatcctgtctcggattgtgggaggctgggagtgcgagaagcattcccaaccctggcaggtgcttgtagcctctcgtg
gcagggcagtctgcggcggtgttctggtgcacccccagtgggtcctcacagctacccactgcatcaggaacaaaagcgtgatcttgctgggtcggcacagcctgtttc
atcctgaagacacaggccaggtatttcaggtcagccacagcttcccacacccgctctacgatatgagcctcctgaagaatcgattcctcaggccaggtgatgactcca
gccacgacctcatgctgctccgcctgtcagagcctgccgagctcacggatgctatgaaggtcatggacctgcccacccaggagccagcactggggaccacctgcta
cgcctcaggctggggcagcattgaaccagaggagttcttgacccccaaagaaacttcagtgtgtggacctccatgttatttccaatgacgtgtgtgcgcaagttcaccctc
agaaggtgaccaagttcatgctgtgtgctggacgctggacaggggggcaaaagcacctgctcgggtgattctgggggcccacttgtctgtaatggtgtgcttcaaggtat
cacgtcatggggcagtgaaccatgtgccctgcccgaaaggccttccctgtacaccaaggtggtgcattaccggaagtggatcaaggacaccatcgtggccaacccc
tgagcacccctatcaactccctattgtagtaaacttggaaccttggaaatgaccaggccaagactcaggcctccccagttctactgacctttgtccttaggtgtgaggtcc
agggttgctaggaaaagaaatcagcagacacaggtgtagaccagagtgtttcttaaatggtgtaattttgtcctctctgtgtcctggggaatactggccatgcctggaga
catatcactcaatttctctgaggacacagataggatggggtgtctgtgttatttgtggggtacagagatgaaagaggggtgggatccacactgagagagtggagagtg
acatgtgctggacactgtccatgaagcactgagcagaagctggaggcacaacgcaccagacactcacagcaaggatggagctgaaaacataacccactctgtcc
tggaggcactgggaagcctagagaaggctgtgaaccaaggagggagggtcttcctttggcatgggatggggatgaagtaaggagagggactgacccccctggaa
gctgattcactatgggggaggtgtattgaagtcctccagacaaccctca gatttgatgatttcctagtagaactcacagaaataaagagctgttatactgtgaa (SEQ ID No: 41; GenBank Accession No. X07730). In another aspect, the KLK3 protein is encoded by residues 67-1088 of SEQ ID No: 41. In another aspect, the KLK3 protein is encoded by a homologue of SEQ ID No: 41. In another aspect, the KLK3 protein is encoded by a variant of SEQ ID No: 41. In another aspect, the KLK3 protein is encoded by an isomer of SEQ ID No: 41. In another aspect, the KLK3 protein is encoded by a fragment of SEQ ID No: 41. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0122] In another aspect, the KLK3 protein is encoded by a sequence set forth in one of the following GenBank Accession Numbers: BC005307, AJ310938, AJ310937, AF335478, AF335477, M27274, and M26663. In another aspect, the KLK3 protein is encoded by a sequence set forth in one of the above GenBank Accession Numbers. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0123] In another aspect, the KLK3 protein is encoded by a sequence set forth in one of the following GenBank Accession Numbers: NM_001030050, NM_001030049, NM_001030048, NM_001030047, NM_001648, AJ459782,

AJ512346, or AJ459784. Each possibility represents a separate aspect of the methods and compositions as provided herein. In one aspect, the KLK3 protein is encoded by a variation of any of the sequences described herein wherein the sequence lacks MWVPVVFLTLSVTWIGAAPLILSR (SEQ ID NO: 55).

[0124] In another aspect, the KLK3 protein has the sequence that comprises a sequence set forth in one of the following GenBank Accession Numbers: X13943, X13942, X13940, X13941, and X13944. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0125] In another aspect, the KLK3 protein is any other KLK3 protein known in the art. Each KLK3 protein represents a separate aspect of the methods and compositions as provided herein.

[0126] In another aspect, the KLK3 peptide is any other KLK3 peptide known in the art. In another aspect, the KLK3 peptide is a fragment of any other KLK3 peptide known in the art. Each type of KLK3 peptide represents a separate aspect of the methods and compositions as provided herein.

[0127] "KLK3 peptide" refers, in another aspect, to a full-length KLK3 protein. In another aspect, the term refers to a fragment of a KLK3 protein. In another aspect, the term refers to a fragment of a KLK3 protein that is lacking the KLK3 signal peptide. In another aspect, the term refers to a KLK3 protein that contains the entire KLK3 sequence except the KLK3 signal peptide. "KLK3 signal sequence" refers, in another aspect, to any signal sequence found in nature on a KLK3 protein. In another aspect, a KLK3 protein of methods and compositions as provided herein does not contain any signal sequence. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0128] In another aspect, the kallikrein-related peptidase 3 (KLK3 protein) that is the source of a KLK3 peptide for use in the methods and compositions as provided herein is a PSA protein. In another aspect, the KLK3 protein is a P-30 antigen protein. In another aspect, the KLK3 protein is a gamma-seminoprotein protein. In another aspect, the KLK3 protein is a kallikrein 3 protein. In another aspect, the KLK3 protein is a semenogelase protein. In another aspect, the KLK3 protein is a seminin protein. In another aspect, the KLK3 protein is any other type of KLK3 protein that is known in the art. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0129] In another aspect, the KLK3 protein is a splice variant 1 KLK3 protein. In another aspect, the KLK3 protein is a splice variant 2 KLK3 protein. In another aspect, the KLK3 protein is a splice variant 3 KLK3 protein. In another aspect, the KLK3 protein is a transcript variant 1 KLK3 protein. In another aspect, the KLK3 protein is a transcript variant 2 KLK3 protein. In another aspect, the KLK3 protein is a transcript variant 3 KLK3 protein. In another aspect, the KLK3 protein is a transcript variant 4 KLK3 protein. In another aspect, the KLK3 protein is a transcript variant 5 KLK3 protein. In another aspect, the KLK3 protein is a transcript variant 6 KLK3 protein. In another aspect, the KLK3 protein is a splice variant RP5 KLK3 protein. In another aspect, the KLK3 protein is any other splice variant KLK3 protein known in the art. In another aspect, the KLK3 protein is any other transcript variant KLK3 protein known in the art. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0130] In another aspect, the KLK3 protein is a mature KLK3 protein. In another aspect, the KLK3 protein is a pro-KLK3 protein. In another aspect, the leader sequence has been removed from a mature KLK3 protein of methods and compositions as provided herein. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0131] In another aspect, the KLK3 protein that is the source of a KLK3 peptide of methods and compositions as provided herein is a human KLK3 protein. In another aspect, the KLK3 protein is a primate KLK3 protein. In another aspect, the KLK3 protein is a KLK3 protein of any other species known in the art. In another aspect, one of the above KLK3 proteins is referred to in the art as a "KLK3 protein." Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0132] In another aspect, the antigen of interest is a KLK9 polypeptide.

[0133] In another aspect, the antigen of interest is HPV-E7. In another aspect, the antigen is HPV-E6. In another aspect, the antigen is Her-2/neu. In another aspect, the antigen is NY-ESO-1. In another aspect, the antigen is telomerase (TERT). In another aspect, the antigen is SCCE. In another aspect, the antigen is CEA. In another aspect, the antigen is LMP-1. In another aspect, the antigen is p53. In another aspect, the antigen is carboxic anhydrase IX (CAIX). In another aspect, the antigen is PSMA. In another aspect, the antigen is prostate stem cell antigen (PSCA). In another aspect, the antigen is HMW-MAA. In another aspect, the antigen is WT-1. In another aspect, the antigen is HIV-1 Gag. In another aspect, the antigen is Proteinase 3. In another aspect, the antigen is Tyrosinase related protein 2. In another aspect, the antigen is PSA (prostate-specific antigen). In another aspect, the antigen is selected from HPV-E7, HPV-E6, Her-2, NY-ESO-1, telomerase (TERT), SCCE, HMW-MAA, WT-1, HIV-1 Gag, CEA, LMP-1, p53, PSMA, PSCA, Proteinase 3, Tyrosinase related protein 2, Mucl, PSA (prostate-specific antigen), or a combination thereof.

[0134] In another aspect, the antigen is a tumor-associated antigen, which in one aspect, is one of the following tumor antigens: a MAGE (Melanoma-Associated Antigen E) protein, e.g. MAGE 1, MAGE 2, MAGE 3, MAGE 4, a tyrosinase; a mutant ras protein; a mutant p53 protein; p97 melanoma antigen, a ras peptide or p53 peptide associated with advanced cancers; the HPV 16/18 antigens associated with cervical cancers, KLH antigen associated with breast carcinoma, CEA (carcinoembryonic antigen) associated with colorectal cancer, gp100, a MART1 antigen associated with melanoma, or the PSA antigen associated with prostate cancer. In another aspect, the antigen for the compositions and methods as

provided herein are melanoma-associated antigens, which in one aspect are TRP-2, MAGE-1, MAGE-3, gp-100, tyrosinase, HSP-70, beta-HCG, or a combination thereof.

[0135] In one aspect, the first and second nucleic acids may encode two separate antigens that serve as tumor targets, which in one aspect are Prostate Specific Antigen (PSA) and Prostate Cancer Stem Cell (PSCA) antigen. In one aspect, the polypeptide encoded by the second nucleic acid may complement or synergize the immune response to the first nucleic acid encoding an antigenic polypeptide. In another aspect, the polypeptide encoded by the second nucleic acid affects vascular growth. In one aspect, the first and second nucleic acid may encode two polypeptides that affect vascular growth, which in one aspect, work via distinct mechanisms to affect vascular growth. In one aspect, such polypeptides are EGFR-III, HMW-MAA, or a combination thereof. In one aspect, a polypeptide may serve as both a tumor antigen an angiogenic factor. In one aspect, the first nucleic acid may encode a tumor antigen, and the second nucleic acid may encode a polypeptide that is an inhibitor of the function or expression of ARG-1 or NOS or combination. In one aspect, an inhibitor of NOS is N^G-mono-methyl-L-arginine (L-NMMA), N^G-nitro-L-arginine methyl ester (L-NAME), 7-NI, L-NIL, or L-NIO. In one aspect, N-omega-nitro-L-arginine a nitric oxide synthase inhibitor and L-arginine competitive inhibitor may be encoded by the nucleic acid. In one aspect, the second nucleic acid may encode an mRNA that inhibits function or expression of ARG-1 or NOS.

[0136] In one aspect, a polypeptide expressed by the Listeria described herein may be a neuropeptide growth factor antagonist, which in one aspect is [D-Arg1, D-Phe5, D-Trp7,9, Leu11]substance P, [Arg6, D-Trp7,9, NmePhe8]substance P(6-11). These and related aspects aspects are understood by one of skill in the art.

[0137] In another aspect, the antigen is an infectious disease antigen. In one aspect, the antigen is an auto antigen or a self-antigen.

[0138] In other aspects, the antigen is derived from a fungal pathogen, bacteria, parasite, helminth, or viruses. In other aspects, the antigen is selected from tetanus toxoid, hemagglutinin molecules from influenza virus, diphtheria toxoid, HIV gp120, HIV gag protein, IgA protease, insulin peptide B, Spongospora subterranea antigen, vibriose antigens, Salmonella antigens, pneumococcus antigens, respiratory syncytial virus antigens, Haemophilus influenza outer membrane proteins, Helicobacter pylori urease, Neisseria meningitidis pilins, N. gonorrhoeae pilins, human papilloma virus antigens E1 and E2 from type HPV-16, -18, -31, -33, -35 or -45 human papilloma viruses, or a combination thereof.

[0139] In other aspects, the antigen is associated with one of the following diseases; cholera, diphtheria, Haemophilus, hepatitis A, hepatitis B, influenza, measles, meningitis, mumps, pertussis, small pox, pneumococcal pneumonia, polio, rabies, rubella, tetanus, tuberculosis, typhoid, Varicella-zoster, whooping cough3 yellow fever, the immunogens and antigens from Addison's disease, allergies, anaphylaxis, Bruton's syndrome, cancer, including solid and blood borne tumors, eczema, Hashimoto's thyroiditis, polymyositis, dermatomyositis, type 1 diabetes mellitus, acquired immune deficiency syndrome, transplant rejection, such as kidney, heart, pancreas, lung, bone, and liver transplants, Graves' disease, polyendocrine autoimmune disease, hepatitis, microscopic polyarteritis, polyarteritis nodosa, pemphigus, primary biliary cirrhosis, pernicious anemia, coeliac disease, antibody-mediated nephritis, glomerulonephritis, rheumatic diseases, systemic lupus erthematosus, rheumatoid arthritis, seronegative spondylarthritides, rhinitis, sjogren's syndrome, systemic sclerosis, sclerosing cholangitis, Wegener's granulomatosis, dermatitis herpetiformis, psoriasis, vitiligo, multiple sclerosis, encephalomyelitis, Guillain-Barre syndrome, myasthenia gravis, Lambert-Eaton syndrome, sclera, episclera, uveitis, chronic mucocutaneous candidiasis, urticaria, transient hypogammaglobulinemia of infancy, myeloma, X-linked hyper IgM syndrome, Wiskott-Aldrich syndrome, ataxia telangiectasia, autoimmune hemolytic anemia, autoimmune thrombocytopenia, autoimmune neutropenia, Waldenstrom's macroglobulinemia, amyloidosis, chronic lymphocytic leukemia, non-Hodgkin's lymphoma, malarial circumsporozite protein, microbial antigens, viral antigens, autoantigens, and lesteriosis. Each antigen represents a separate aspect of the methods and compositions as provided herein.

[0140] The immune response induced by methods and compositions as provided herein is, in another aspect, a T cell response. In another aspect, the immune response comprises a T cell response. In another aspect, the response is a CD8+ T cell response. In another aspect, the response comprises a $CD8^+$ T cell response. Each possibility represents a separate aspect as provided herein.

[0141] In one aspect, a recombinant *Listeria* of the compositions and methods as provided herein comprise an angiogenic polypeptide. In another aspect, anti-angiogenic approaches to cancer therapy are very promising, and in one aspect, one type of such anti-angiogenic therapy targets pericytes. In another aspect, molecular targets on vascular endothelial cells and pericytes are important targets for antitumor therapies. In another aspect, the platelet-derived growth factor receptor (PDGF-B/PDGFR-β) signaling is important to recruit pericytes to newly formed blood vessels. Thus, in one aspect, angiogenic polypeptides as provided herein inhibit molecules involved in pericyte signaling, which in one aspect, is PDGFR-β.

[0142] In one aspect, the compositions of the present invention comprise an angiogenic factor, or an immunogenic fragment thereof, where in one aspect, the immunogenic fragment comprises one or more epitopes recognized by the host immune system. In one aspect, an angiogenic factor is a molecule involved in the formation of new blood vessels. In one aspect, the angiogenic factor is VEGFR2. In another aspect, an angiogenic factor described herein is Angiogenin;

Angiopoietin-1; Del-1; Fibroblast growth factors: acidic (aFGF) and basic (bFGF); Follistatin; Granulocyte colony-stimulating factor (G-CSF); Hepatocyte growth factor (HGF) /scatter factor (SF); Interleukin-8 (IL-8); Leptin; Midkine; Placental growth factor; Platelet-derived endothelial cell growth factor (PD-ECGF); Platelet-derived growth factor-BB (PDGF-BB); Pleiotrophin (PTN); Progranulin; Proliferin; Transforming growth factor-alpha (TGF-alpha); Transforming growth factor-beta (TGF-beta); Tumor necrosis factor-alpha (TNF-alpha); Vascular endothelial growth factor (VEGF)/vascular permeability factor (VPF). In another aspect, an angiogenic factor is an angiogenic protein. In one aspect, a growth factor is an angiogenic protein. In one aspect, an angiogenic protein for use in the compositions and methods described herein is Fibroblast growth factors (FGF); VEGF; VEGFR and Neuropilin 1 (NRP-1); Angiopoietin 1 (Ang1) and Tie2; Platelet-derived growth factor (PDGF; BB-homodimer) and PDGFR; Transforming growth factor-beta (TGF-β), endoglin and TGF-β receptors; monocyte chemotactic protein-1 (MCP-1); Integrins αVβ3, αVβ5 and α5β1; VE-cadherin and CD31; ephrin; plasminogen activators; plasminogen activator inhibitor-1; Nitric oxide synthase (NOS) and COX-2; AC133; or Id1/Id3. In one aspect, an angiogenic protein for use in the compositions and methods described herein n is an angiopoietin, which in one aspect, is Angiopoietin 1, Angiopoietin 3, Angiopoietin 4 or Angiopoietin 6. In one aspect, endoglin is also known as CD105; EDG; HHT1; ORW; or ORW1. In one aspect, endoglin is a TGFbeta co-receptor.

[0143] In one aspect, cancer vaccines as provided herein generate effector T cells that are able to infiltrate the tumor, destroy tumor cells and eradicate the disease. In one aspect, naturally occurring tumor infiltrating lymphocytes (TILs) are associated with better prognosis in several tumors, such as colon, ovarian and melanoma. In colon cancer, tumors without signs of micrometastasis have an increased infiltration of immune cells and a Th1 expression profile, which correlate with an improved survival of patients. Moreover, the infiltration of the tumor by T cells has been associated with success of immunotherapeutic approaches in both pre-clinical and human trials. In one aspect, the infiltration of lymphocytes into the tumor site is dependent on the up-regulation of adhesion molecules in the endothelial cells of the tumor vasculature, generally by proinflammatory cytokines, such as IFN-γ, TNF-α and IL-1. Several adhesion molecules have been implicated in the process of lymphocyte infiltration into tumors, including intercellular adhesion molecule 1 (ICAM-1), vascular endothelial cell adhesion molecule 1 (V-CAM-1), vascular adhesion protein 1 (VAP-1) and E-selectin. However, these cell-adhesion molecules are commonly down-regulated in the tumor vasculature. Thus, in one aspect, cancer vaccines as provided herein increase TILs, up-regulate adhesion molecules (in one aspect, ICAM-1, V-CAM-1, VAP-1, E-selectin, or a combination thereof), up-regulate proinflammatory cytokines (in one aspect, IFN-γ, TNF-α, IL-1, or a combination thereof), or a combination thereof.

[0144] In one aspect, the compositions and methods as provided herein provide anti-angiogenesis therapy, which in one aspect, may improve immunotherapy strategies. In one aspect, the compositions and methods as provided herein circumvent endothelial cell anergy *in vivo* by up-regulating adhesion molecules in tumor vessels and enhancing leukocyte-vessel interactions, which increases the number of tumor infiltrating leukocytes, such as $CD8^+$ T cells. Interestingly, enhanced anti-tumor protection correlates with an increased number of activated $CD4^+$ and $CD8^+$ tumor-infiltrating T cells and a pronounced decrease in the number of regulatory T cells in the tumor upon VEGF blockade.

[0145] In one aspect, delivery of anti-angiogenic antigen simultaneously with a tumor-associated antigen to a host afflicted by a tumor as described herein, will have a synergistic effect in impacting tumor growth and a more potent therapeutic efficacy.

[0146] In another aspect, targeting pericytes through vaccination will lead to cytotoxic T lymphocyte (CTL) infiltration, destruction of pericytes, blood vessel destabilization and vascular inflammation, which in another aspect is associated with up-regulation of adhesion molecules in the endothelial cells that are important for lymphocyte adherence and transmigration, ultimately improving the ability of lymphocytes to infiltrate the tumor tissue. In another aspect, concomitant delivery of a tumor-specific antigen generate lymphocytes able to invade the tumor site and kill tumor cells.

[0147] In one aspect, the platelet-derived growth factor receptor (PDGF-B/PDGFR-β) signaling is important to recruit pericytes to newly formed blood vessels. In another aspect, inhibition of VEGFR-2 and PDGFR-β concomitantly induces endothelial cell apoptosis and regression of tumor blood vessels, in one emboidment, approximately 40% of tumor blood vessels.

[0148] The recombinant *Listeria* strain of the invention is an auxotrophic *Listeria* strain that is a dal/dat mutant. In another aspect, the nucleic acid molecule is stably maintained in the recombinant bacterial strain in the absence of antibiotic selection.

[0149] Auxotrophic mutants useful as vaccine vectors may be generated in a number of ways. D-alanine auxotrophic mutants can be generated, in one aspect, via the disruption of both the dal gene and the dat gene to generate an attenuated auxotrophic strain of Listeria which requires exogenously added D-alanine for growth.

[0150] The generation of AA strains of *Listeria* deficient in D-alanine, for example, may be accomplished in a number of ways that are well known to those of skill in the art, including deletion mutagenesis, insertion mutagenesis, and mutagenesis which results in the generation of frameshift mutations, mutations which cause premature termination of a protein, or mutation of regulatory sequences which affect gene expression. In another aspect, mutagenesis can be accomplished using recombinant DNA techniques or using traditional mutagenesis technology using mutagenic chemicals or radiation and subsequent selection of mutants. In another aspect, deletion mutants are preferred because of

the accompanying low probability of reversion of the auxotrophic phenotype. In another aspect, mutants of D-alanine which are generated according to the protocols presented herein may be tested for the ability to grow in the absence of D-alanine in a simple laboratory culture assay. In another aspect, those mutants which are unable to grow in the absence of this compound are selected for further study.

[0151] In another aspect, in addition to the aforementioned D-alanine associated genes, other genes involved in synthesis of a metabolic enzyme , as provided herein, may be used as targets for mutagenesis of Listeria.

[0152] The auxotrophic *Listeria* strain of the inventioncomprises an episomal expression vector comprising a metabolic enzyme that complements the auxotrophy of said auxotrophic *Listeria* strain. In another aspect, the construct is contained in the *Listeria* strain in an episomal fashion. In another aspect, the foreign antigen is expressed from a vector harbored by the recombinant *Listeria* strain. In another aspect, said episomal expression vector lacks an antibiotic resistance marker. In one aspect, an antigen of the methods and compositions as provided herein is genetically fused to an oligopeptide comprising a PEST sequence. In another aspect, said endogenous polypeptide comprising a PEST sequence is LLO. In another aspect, said endogenous polypeptide comprising a PEST sequence is ActA. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0153] In another aspect, the metabolic enzyme complements an endogenous metabolic gene that is lacking in the remainder of the chromosome of the recombinant bacterial strain. In one aspect, the endogenous metabolic gene is mutated in the chromosome. In another aspect, the endogenous metabolic gene is deleted from the chromosome. In another aspect, said metabolic enzyme is an amino acid metabolism enzyme. In another aspect, said metabolic enzyme catalyzes a formation of an amino acid used for a cell wall synthesis in said recombinant *Listeria* strain. In another embodiment, said metabolic enzyme is an alanine racemase enzyme. In another embodiment, said metabolic enzyme is a D-amino acid transferase enzyme. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0154] In another aspect, the metabolic enzyme catalyzes the formation of an amino acid (AA) used in cell wall synthesis. In another aspect, the metabolic enzyme catalyzes synthesis of an AA used in cell wall synthesis. In another aspect, the metabolic enzyme is involved in synthesis of an AA used in cell wall synthesis. In another aspect, the AA is used in cell wall biogenesis. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0155] In another aspect, the metabolic enzyme is a synthetic enzyme for D-glutamic acid, a cell wall component.

[0156] In another aspect, the metabolic enzyme is encoded by an alanine racemase gene (dal) gene. In another aspect, the dal gene encodes alanine racemase, which catalyzes the reaction L-alanine ↔ D-alanine.

[0157] The dal gene of methods and compositions of the methods and compositions as provided herein is encoded, in another aspect, by the sequence:

atggtgacaggctggcatcgtccaacatggattgaaatagaccgcgcagcaattcgcgaaaatataaaaaatgaacaaaataaactcccggaaagtgtcgacttat
gggcagtagtcaaagctaatgcatatggtcacggaattatcgaagttgctaggacggcgaaagaagctggagcaaaaggtttctgcgtagccattttagatgaggca
ctggctcttagagaagctggatttcaagatgactttattcttgtgcttggtgcaaccagaaaagaagatgctaatctggcagccaaaaaccacatttcacttactgtttttag
agaagattggctagagaatctaacgctagaagcaacacttcgaattcatttaaaagtagatagcggtatggggcgtctcggtattcgtacgactgaagaagcacggc
gaattgaagcaaccagtactaatgatcaccaattacaactggaaggtatttacacgcattttgcaacagccgaccagctagaaactagttatttgaacaacaattagc
taagttccaaacgattttaacgagtttaaaaaaaacgaccaacttatgttcatacagccaattcagctgcttcattgttacagccacaaatcgggtttgatgcgattcgctttg
gtatttcgatgtatggattaactccctccacagaaatcaaaactagcttgccgtttgagcttaaacctgcacttgcactctataccgagatggttcatgtgaaagaacttgc
accaggcgatagcgttagctacggagcaacttatacagcaacagagcgagaatgggttgcgacattaccaattggctatgcggatggattgattcgtcattacagtgg
tttccatgttttagtagacggtgaaccagctccaatcattggtcgagtttgtatggatcaaaccatcataaaactaccacgtgaatttcaaactggttcaaaagtaacgata
attggcaaagatcatggtaacacggtaacagcagatgatgccgct caatatttagatacaattaattatgaggtaacttgtttgttaaatgagcgcataccta-
gaaaatacatccattag (SEQ ID No: 42; GenBank Accession No: AF038438). In another aspect, the nucleotide encoding dal is homologous to SEQ ID No: 42. In another aspect, the nucleotide encoding dal is a variant of SEQ ID No: 42. In another aspect, the nucleotide encoding dal is a fragment of SEQ ID No: 42. In another aspect, the dal protein is encoded by any other dal gene known in the art. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0158] In another aspect, the dal protein has the sequence:

MVTGWHRPTWIEIDRAAIRENIKNEQNKLPESVDLWAVVKANAYGHGIIEVARTAKEAGAKGFCVAILDEALALREAGF
QDDFILVLGATRKEDANLAAKNHISLTVFREDWLENLTLEATLRIHLKVDSGMGRLGIRTTEEARRIEATSTNDHQLQLE
GIYTHFATADQLETSYFEQQLAKFQTILTSLKKRPTYVHTANSAASLLQPQIGFDAIRFGISMYGLTPSTEIKTSLPFELK
PALALYTEMVHVKELAPGDSVSYGATYTATEREWVATLPIGYADGLIRHYSGFHVLVDGEPAPIIGRVCMDQTIIKLPRE
FQTGSKVTIIGKDHGNTVTADDAAQYLDTINYEVTCLLNERIPRKYIH (SEQ ID No: 43; GenBank Accession No: AF038428). In another aspect, the dal protein is homologous to SEQ ID No: 43. In another aspect, the dal protein is a variant of SEQ ID No: 43. In another aspect, the dal protein is an isomer of SEQ ID No: 43. In another aspect, the dal protein is a fragment of SEQ ID No: 43. In another aspect, the dal protein is a fragment of a homologue of SEQ ID No: 43. In another aspect, the dal protein is a fragment of a variant of SEQ ID No: 43. In another aspect, the dal protein is a fragment of an isomer of SEQ ID No: 43.

[0159] In another aspect, the dal protein is any other *Listeria* dal protein known in the art. In another aspect, the dal protein is any other gram-positive dal protein known in the art. In another aspect, the dal protein is any other dal protein known in the art. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0160] In another aspect, the dal protein of methods and compositions as provided herein retains its enzymatic activity. In another aspect, the dal protein retains 90% of wild-type activity. In another aspect, the dal protein retains 80% of wild-type activity. In another aspect, the dal protein retains 70% of wild-type activity. In another aspect, the dal protein retains 60% of wild-type activity. In another aspect, the dal protein retains 50% of wild-type activity. In another aspect, the dal protein retains 40% of wild-type activity. In another aspect, the dal protein retains 30% of wild-type activity. In another aspect, the dal protein retains 20% of wild-type activity. In another aspect, the dal protein retains 10% of wild-type activity. In another aspect, the dal protein retains 5% of wild-type activity. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0161] In another aspect, the metabolic enzyme is encoded by a D-amino acid aminotransferase gene (dat). D-glutamic acid synthesis is controlled in part by the dat gene, which is involved in the conversion of D-glu + pyr to alpha-ketoglutarate + D-ala, and the reverse reaction.

[0162] In another aspect, a dat gene utilized in the present invention has the sequence set forth in GenBank Accession Number AF038439. In another aspect, the dat gene is any another dat gene known in the art. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0163] The dat gene of methods and compositions of the methods and compositions as provided herein is encoded, in another aspect, by the sequence:

atgaaagtattagtaaataaccatttagttgaaagagaagatgccacagttgacattgaagaccgcggatatcagtttggtgatggtgtatatgaagtagttcgtctatat
aatggaaaattctttacttataatgaacacattgatcgcttatatgctagtgcagcaaaaattgacttagttattccttattccaaagaagagctacgtgaattacttgaaaa
attagttgccgaaaataatatcaatacagggaatgtctatttacaagtgactcgtggtgttcaaaacccacgtaatcatgtaatccctgatgatttccctctagaaggcgttt
taacagcagcagctcgtgaagtacctagaaacgagcgtcaattcgttgaaggtggaacggcgattacagaagaagatgtgcgctggttacgctgtgatattaagagc
ttaaacctttaggaaatattctagcaaaaaataaagcacatcaacaaaatgctttggaagctattttacatcgcggggaacaagtaacagaatgttctgcttcaaacgtt
tctattattaaagatggtgtattatggacgcatgcggcagataacttaatcttaaatggtatcactcgtcaagttatcattgatgttgcgaaaaagaatggcattcctgttaaa
gaagcggatttcactttaacagaccttcgtgaagcggatgaagtgttcatttcaagtacaactattgaaattacacctattacgcatattgacggagttcaagtagctgac
ggaaaacgtggaccaattacagcgcaacttcatcaatattttgtagaagaaatcactcgtgcatgtggcgaattagagtttgcaaaataa (SEQ ID No: 44; GenBank Accession No: AF038439). In another aspect, the nucleotide encoding dat is homologous to SEQ ID No: 44. In another aspect, the nucleotide encoding dat is a variant of SEQ ID No: 44. In another aspect, the nucleotide encoding dat is a fragment of SEQ ID No: 44. In another aspect, the dat protein is encoded by any other dat gene known in the art. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0164] In another aspect, the dat protein has the sequence:

MKVLVNNHLVEREDATVDIEDRGYQFGDGVYEVVRLYNGKFFTYNEHIDRLYASAAKIDLVIPYSKEELRELLEKLVAE
NNINTGNVYLQVTRGVQNPRNHVIPDDFPLEGVLTAAAREVPRNERQFVEGGTAITEEDVRWLRCDIKSLNLLGNILAK
NKAHQQNALEAILHRGEQVTECSASNVSIIKDGVLWTHAADNLILNGITRQVIIDVAKKNGIPVKEADFTLTDLREADEVF
ISSTTIEITPITHIDGVQVADGKRGPITAQLHQYFVEEITRACGELEFAK (SEQ ID No: 45; GenBank Accession No: AF038439). In another aspect, the dat protein is homologous to SEQ ID No: 45. In another aspect, the dat protein is a variant of SEQ ID No: 45. In another aspect, the dat protein is an isomer of SEQ ID No: 45. In another aspect, the dat protein is a fragment of SEQ ID No: 45. In another aspect, the dat protein is a fragment of a homologue of SEQ ID No: 45. In another aspect, the dat protein is a fragment of a variant of SEQ ID No: 45. In another aspect, the dat protein is a fragment of an isomer of SEQ ID No: 45.

[0165] In another aspect, the dat protein is any other *Listeria* dat protein known in the art. In another aspect, the dat protein is any other gram-positive dat protein known in the art. In another aspect, the dat protein is any other dat protein known in the art. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0166] In another aspect, the dat protein of methods and compositions of the methods and compositions as provided herein retains its enzymatic activity. In another aspect, the dat protein retains 90% of wild-type activity. In another aspect, the dat protein retains 80% of wild-type activity. In another aspect, the dat protein retains 70% of wild-type activity. In another aspect, the dat protein retains 60% of wild-type activity. In another aspect, the dat protein retains 50% of wild-type activity. In another aspect, the dat protein retains 40% of wild-type activity. In another aspect, the dat protein retains 30% of wild-type activity. In another aspect, the dat protein retains 20% of wild-type activity. In another aspect, the dat protein retains 10% of wild-type activity. In another aspect, the dat protein retains 5% of wild-type activity. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0167] In another aspect, the metabolic enzyme is encoded by dga. D-glutamic acid synthesis is also controlled in part by the dga gene, and an auxotrophic mutant for D-glutamic acid synthesis will not grow in the absence of D-glutamic acid (Pucci et al, 1995, J Bacteriol. 177: 336-342). In another raspect, the recombinant *Listeria* is auxotrophic for D-glutamic acid. A further example includes a gene involved in the synthesis of diaminopimelic acid. Such synthesis genes encode beta-semialdehyde dehydrogenase, and when inactivated, renders a mutant auxotrophic for this synthesis pathway (Sizemore et al, 1995, Science 270: 299-302). In another aspect, the dga protein is any other *Listeria* dga

protein known in the art. In another aspect, the dga protein is any other gram-positive dga protein known in the art. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0168] In another aspect, the metabolic enzyme is encoded by an air (alanine racemase) gene. In another aspect, the metabolic enzyme is any other enzyme known in the art that is involved in alanine synthesis. In another aspect, the metabolic enzyme is any other enzyme known in the art that is involved in L-alanine synthesis. In another aspect, the metabolic enzyme is any other enzyme known in the art that is involved in D-alanine synthesis. In another raspect, the recombinant *Listeria* is auxotrophic for D-alanine. Bacteria auxotrophic for alanine synthesis are well known in the art, and are described in, for example, *E. coli* (Strych et al, 2002, J. Bacteriol. 184:4321-4325), Corynebacterium glutamicum (Tauch et al, 2002, J. Biotechnol 99:79-91), and *Listeria monocytogene*s (Frankel et al, U.S. Patent 6,099,848)), *Lactococcu*s species, and *Lactobacillu*s species, (Bron et al, 2002, Appl Environ Microbiol, 68: 5663-70). In another aspect, any D-alanine synthesis gene known in the art is inactivated. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0169] In another aspect, the metabolic enzyme is an amino acid aminotransferase.

[0170] In another aspect, the metabolic enzyme is encoded by serC, a phosphoserine aminotransferase. In another aspect, the metabolic enzyme is encoded by asd (aspartate beta-semialdehyde dehydrogenase), involved in synthesis of the cell wall constituent diaminopimelic acid. In another aspect, the metabolic enzyme is encoded by gsaB- glutamate-1-semialdehyde aminotransferase, which catalyzes the formation of 5-aminolevulinate from (S)-4-amino-5-oxopentanoate. In another aspect, the metabolic enzyme is encoded by HemL, which catalyzes the formation of 5-aminolevulinate from (S)-4-amino-5-oxopentanoate. In another aspect, the metabolic enzyme is encoded by aspB, an aspartate aminotransferase that catalyzes the formation of oxalozcetate and L-glutamate from L-aspartate and 2-oxoglutarate. In another aspect, the metabolic enzyme is encoded by argF-1, involved in arginine biosynthesis. In another aspect, the metabolic enzyme is encoded by aroE, involved in amino acid biosynthesis. In another aspect, the metabolic enzyme is encoded by aroB, involved in 3-dehydroquinate biosynthesis. In another aspect, the metabolic enzyme is encoded by aroD, involved in amino acid biosynthesis. In another aspect, the metabolic enzyme is encoded by aroC, involved in amino acid biosynthesis. In another aspect, the metabolic enzyme is encoded by hisB, involved in histidine biosynthesis. In another aspect, the metabolic enzyme is encoded by hisD, involved in histidine biosynthesis. In another aspect, the metabolic enzyme is encoded by hisG, involved in histidine biosynthesis. In another aspect, the metabolic enzyme is encoded by metX, involved in methionine biosynthesis. In another aspect, the metabolic enzyme is encoded by proB, involved in proline biosynthesis. In another aspect, the metabolic enzyme is encoded by argR, involved in arginine biosynthesis. In another aspect, the metabolic enzyme is encoded by argJ, involved in arginine biosynthesis. In another aspect, the metabolic enzyme is encoded by thiI, involved in thiamine biosynthesis. In another aspect, the metabolic enzyme is encoded by LMOf2365_1652, involved in tryptophan biosynthesis. In another aspect, the metabolic enzyme is encoded by aroA, involved in tryptophan biosynthesis. In another aspect, the metabolic enzyme is encoded by ilvD, involved in valine and isoleucine biosynthesis. In another aspect, the metabolic enzyme is encoded by ilvC, involved in valine and isoleucine biosynthesis. In another aspect, the metabolic enzyme is encoded by leuA, involved in leucine biosynthesis. In another aspect, the metabolic enzyme is encoded by dapF, involved in lysine biosynthesis. In another aspect, the metabolic enzyme is encoded by thrB, involved in threonine biosynthesis (all GenBank Accession No. NC_002973).

[0171] In another aspect, the metabolic enzyme is a tRNA synthetase. In another aspect, the metabolic enzyme is encoded by the trpS gene, encoding tryptophanyltRNA synthetase. In another aspect, the metabolic enzyme is any other tRNA synthetase known in the art. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0172] In another aspect, a recombinant *Listeria* strain as provided herein has been passaged through an animal host. In another aspect, the passaging maximizes efficacy of the strain as a vaccine vector. In another aspect, the passaging stabilizes the immunogenicity of the *Listeria* strain. In another aspect, the passaging stabilizes the virulence of the *Listeria* strain. In another aspect, the passaging increases the immunogenicity of the *Listeria* strain. In another aspect, the passaging increases the virulence of the *Listeria* strain. In another aspect, the passaging removes unstable sub-strains of the *Listeria* strain. In another aspect, the passaging reduces the prevalence of unstable sub-strains of the *Listeria* strain. In another aspect, the passaging attenuates the strain, or in another aspect, makes the strain less virulent. Methods for passaging a recombinant *Listeria* strain through an animal host are well known in the art, and are described, for example, in United States Patent Application Serial No. 10/541,614. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0173] The recombinant *Listeria* strain of the invention is, in another embodiment, a recombinant *Listeria monocytogene*s strain. In another aspect, the *Listeria* strain is a recombinant *Listeria seeligeri* strain. In another aspect, the *Listeria* strain is a recombinant *Listeria grayi* strain. In another aspect, the *Listeria* strain is a recombinant *Listeria ivanovii* strain. In another aspect, the *Listeria* strain is a recombinant *Listeria murrayi* strain. In another aspect, the *Listeria* strain is a recombinant *Listeria welshimeri* strain. In another aspect, the Listeria strain is a recombinant strain of any other *Listeria* species known in the art. Each possibility represents a separate aspect as provided herein. In another aspect,

the sequences of *Listeria* proteins for use in the methods and compositions as provided herein are from any of the above-described strains.

[0174] In one aspect, a *Listeria monocytogene*s strain as provided herein is the EGD strain, the 10403S strain, the NICPBP 54002 strain, the S3 strain, the NCTC 5348 strain, the NICPBP 54006 strain, the M7 strain, the S19 strain, or another strain of *Listeria monocytogene*s which is known in the art.

[0175] In another aspect, the recombinant *Listeria* strain is a vaccine strain, which in one aspect, is a bacterial vaccine strain.

[0176] In one aspect, a vaccine is a composition which elicits an immune response to an antigen or polypeptide in the composition as a result of exposure to the composition. In another aspect, the vaccine additionally comprises an adjuvant, cytokine, chemokine, or combination thereof. In another aspect, the vaccine or composition additionally comprises antigen presenting cells (APCs), which in one aspect are autologous, while in another aspect, they are allogeneic to the subject.

[0177] In one aspect, a "vaccine" is a composition which elicits an immune response in a host to an antigen or polypeptide in the composition as a result of exposure to the composition. In one aspect, the immune response is to a particular antigen or to a particular epitope on the antigen. In one aspect, the vaccine may be a peptide vaccine, in another aspect, a DNA vaccine. In another aspect, the vaccine may be contained within and, in another aspect, delivered by, a cell, which in one aspect is a bacterial cell, which in one aspect, is a *Listeria.* In one aspect, a vaccine may prevent a subject from contracting or developing a disease or condition, wherein in another aspect, a vaccine may be therapeutic to a subject having a disease or condition. In one aspect, a vaccine described herein comprises a composition described herein and an adjuvant, cytokine, chemokine, or combination thereof.

[0178] The present invention provides an immunogenic composition comprising a recombinant Listeria of the present invention. In another aspect, the immunogenic composition of methods and compositions of the present invention comprises a recombinant vaccine vector described herein. In another aspect, the immunogenic composition comprises a plasmid described herein. The immunogenic composition of the rpesent invention comprises an adjuvant. In one aspect, a vector described herein may be administered as part of a vaccine composition. Each possibility represents a separate aspect of the present disclosure.

[0179] In another aspect, a vaccine described herein is delivered with an adjuvant. In one aspect, the adjuvant favors a predominantly Th1-mediated immune response. In another aspect, the adjuvant favors a Th1-type immune response. In another aspect, the adjuvant favors a Th1-mediated immune response. In another aspect, the adjuvant favors a cell-mediated immune response over an antibody-mediated response. In another aspect, the adjuvant is any other type of adjuvant known in the art. In another aspect, the immunogenic composition induces the formation of a T cell immune response against the target protein.

[0180] In another aspect, the adjuvant is MPL. In another embodiment, the adjuvant is QS21. In another aspect, the adjuvant is a TLR agonist. In another aspect, the adjuvant is a TLR4 agonist. In another aspect, the adjuvant is a TLR9 agonist. In another aspect, the adjuvant is Resiquimod®. In another aspect, the adjuvant is imiquimod. In another aspect, the adjuvant is a CpG oligonucleotide. In another aspect, the adjuvant is a cytokine or a nucleic acid encoding same. In another aspect, the adjuvant is a chemokine or a nucleic acid encoding same. In another aspect, the adjuvant is IL-12 or a nucleic acid encoding same. In another aspect, the adjuvant is IL-6 or a nucleic acid encoding same. In another aspect, the adjuvant is a lipopolysaccharide. In another aspect, the adjuvant is as described in Fundamental Immunology, 5th ed (August 2003): William E. Paul (Editor); Lippincott Williams & Wilkins Publishers; Chapter 43: Vaccines, GJV Nossal. In another aspect, the adjuvant is any other adjuvant known in the art. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0181] In one embodiment, provided herein is a *Listeria* strain of the invention for use in inducing an immune response to PSA in a subjectt. In one aspect, provided herein is a method of inducing an anti-angiogenic immune response to an antigen in a subject comprising administering a recombinant *Listeria* strain to said subject. In another aspect, said recombinant *Listeria* strain comprises a first and second nucleic acid molecule. In another aspect, each said nucleic acid molecule encodes a heterologous antigen. In yet another aspect, said first nucleic acid molecule is operably integrated into the *Listeria* genome as an open reading frame with an endogenous polypeptide comprising a PEST sequence.

[0182] In one embodiment, provided herein is a *Listeria* strain of the invention for use in treating, suppressing, or inhibiting a cancer or tumour in a subject. In another aspect, said recombinant *Listeria* strain comprises a first and second nucleic acid molecule. In another aspect, each said nucleic acid molecule encoding a heterologous antigen. In yet another aspect, said first nucleic acid molecule is operably integrated into the *Listeria* genome as an open reading frame with a nucleic acid sequence encoding an endogenous polypeptide comprising a PEST sequence. In another aspect, at least one of said antigens is expressed by at least one cell of said cancer cells.

[0183] In one embodiment, provided herein is a *Listeria* strain of the invention for use in preventing or delaying the onset to a cancer in a subjectt. In another aspect, provided herein is a method of delaying the progression to a cancer in a subject comprising administering a recombinant *Listeria* strain to said subject. In another aspect, provided herein is a method of extending the remission to a cancer in a subject comprising administering a recombinant *Listeria* strain

to said subject. In another aspect, provided herein is a method of decreasing the size of an existing tumor in a subject comprising administering a recombinant *Listeria* strain to said subject. In another aspect, provided herein is a method of preventing the growth of an existing tumor in a subject comprising administering a recombinant *Listeria* strain to said subject. In another aspect, provided herein is a method of preventing the growth of new or additional tumors in a subject comprising administering a recombinant *Listeria* strain to said subject.

[0184] In one aspect, cancer or tumors may be prevented in specific populations known to be susceptible to a particular cancer or tumor. In one aspect, such susceptibilty may be due to environmental factors, such as smoking, which in one aspect, may cause a population to be subject to lung cancer, while in another aspect, such susceptbility may be due to genetic factors, for example a population with BRCA1/2 mutations may be susceptible, in one aspect, to breast cancer, and in another aspect, to ovarian cancer. In another aspect, one or more mutations on chromosome 8q24, chromosome 17q12, and chromosome 17q24.3 may increase susceptibility to prostate cancer, as is known in the art. Other genetic and environmental factors contributing to cancer susceptibility are known in the art.

[0185] In another aspect, a method described herein further comprises the step of boosting the human subject with a recombinant Listeria strain as provided herein. In another aspect, the recombinant Listeria strain used in the booster inoculation is the same as the strain used in the initial "priming" inoculation. In another aspect, the booster strain is different from the priming strain. In another aspect, the same doses are used in the priming and boosting inoculations. In another aspect, a larger dose is used in the booster. In another aspect, a smaller dose is used in the booster. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0186] In one aspect, the first or second nucleic acid molecule encodes a prostate specific antigen (PSA) and the method is for treating, inhibiting or suppressing prostate cancer. In another aspect, the first or second nucleic acid molecule encodes PSA and the method is for treating, inhibiting or suppressing ovarian cancer. In another aspect, the first or second nucleic acid molecule encodes PSA and the method is treating, inhibiting, or suppressing metastasis of prostate cancer, which in one aspect, comprises metastasis to bone, and in another aspect, comprises metastasis to other organs. In another aspect, the first or second nucleic acid molecule encodes PSA and the method is for treating, inhibiting or suppressing metastasis of prostate cancer to bones. In yet another aspect the method is for treating, inhibiting, or suppressing metastatis of prostate cancer to other organs. In another aspect, the first or second nucleic acid molecule encodes PSA and the method is for treating, inhibiting or suppressing breast cancer. In another aspect, the first or second nucleic acid molecule encodes PSA and the method is for treating, inhibiting or suppressing both ovarian and breast cancer.

[0187] In one aspect, the first or second nucleic acid molecule encodes a High Molecular Weight-Melanoma Associated Antigen (HMW-MAA) and the method is for treating, inhibiting or suppressing melanoma. In another aspect, the first or second nucleic acid molecule encodes HMW-MAA and the method is for treating, inhibiting or suppressing breast cancer. In another aspect, the first or second nucleic acid molecule encodes HMW-MAA and the method is for treating, inhibiting or suppressing ovarian cancer. In another aspect, the first or second nucleic acid molecule encodes HMW-MAA and the method is for treating, inhibiting or suppressing benign nevi lesions. In another aspect, the first or second nucleic acid molecule encodes HMW-MAA and the method is for treating, inhibiting or suppressing basal cell carcinoma. In another aspect, the first or second nucleic acid molecule encodes HMW-MAA and the method is for treating, inhibiting or suppressing a tumor of neural crest origin, which in one aspect, is an astrocytoma, glioma, neuroblastoma, sarcoma, or combination thereof. In another aspect, the first or second nucleic acid molecule encodes HMW-MAA and the method is for treating, inhibiting or suppressing a childhood leukemia, which in one aspect, is Childhood Acute Lymphoblastic Leukemia, and in another aspect, is Childhood Acute Myeloid Leukemia (which in one aspect, is acute myelogenous leukemia, acute myeloid leukemia, acute myelocytic leukemia, or acute non-lymphocytic leukemia) and in another aspect, is acute lymphocytic leukemia (which in one aspect, is called acute lymphoblastic leukemia, and in another aspect, is acute myelogenous leukemia (also called acute myeloid leukemia, acute myelocytic leukemia, or acute non-lymphocytic leukemia) and in another aspect, is Hybrid or mixed lineage leukemia. In another aspect, the first or second nucleic acid molecule encodes HMW-MAA and the method is for treating, inhibiting or suppressing Chronic myelogenous leukemia or Juvenile Myelomonocytic Leukemia (JMML). In another aspect, the first or second nucleic acid molecule encodes HMW-MAA and the method is for treating, inhibiting or suppressing lobular breast carcinoma lesions.

[0188] The cancer that is the target of methods and compositions as provided herein is, in another aspect, a melanoma. In another aspect, the cancer is a sarcoma. In another aspect, the cancer is a carcinoma. In another aspect, the cancer is a mesothelioma (e.g. malignant mesothelioma). In another aspect, the cancer is a glioma. In another aspect, the cancer is a germ cell tumor. In another aspect, the cancer is a choriocarcinoma.

[0189] In another aspect, the cancer is pancreatic cancer. In another aspect, the cancer is ovarian cancer. In another aspect, the cancer is gastric cancer. In another aspect, the cancer is a carcinomatous lesion of the pancreas. In another aspect, the cancer is pulmonary adenocarcinoma. In another aspect, the cancer is colorectal adenocarcinoma. In another aspect, the cancer is pulmonary squamous adenocarcinoma. In another aspect, the cancer is gastric adenocarcinoma. In another aspect, the cancer is an ovarian surface epithelial neoplasm (e.g. a benign, proliferative or malignant variety thereof). In another aspect, the cancer is an oral squamous cell carcinoma. In another aspect, the cancer is non small-

cell lung carcinoma. In another aspect, the cancer is an endometrial carcinoma. In another aspect, the cancer is a bladder cancer. In another aspect, the cancer is a head and neck cancer. In another aspect, the cancer is a prostate carcinoma.

[0190] In another aspect, the cancer is a non-small cell lung cancer (NSCLC). In another aspect, the cancer is a colon cancer. In another aspect, the cancer is a lung cancer. In another aspect, the cancer is an ovarian cancer. In another aspect, the cancer is a uterine cancer. In another aspect, the cancer is a thyroid cancer. In another aspect, the cancer is a hepatocellular carcinoma. In another aspect, the cancer is a thyroid cancer. In another aspect, the cancer is a liver cancer. In another aspect, the cancer is a renal cancer. In another aspect, the cancer is a kaposis. In another aspect, the cancer is a sarcoma. In another aspect, the cancer is another carcinoma or sarcoma. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0191] In one aspect, the compositions and methods as provided herein can be used to treat solid tumors related to or resulting from any of the cancers as described hereinabove. In another aspect, the tumor is a Wilms' tumor. In another aspect, the tumor is a desmoplastic small round cell tumor.

[0192] Another aspect described herein provides a method of impeding angiogenesis of a solid tumor in a subject, comprising administering to the subject a composition comprising a recombinant *Listeria* encoding a heterologous antigen. In another aspect, the antigen is HMW-MAA. In another aspect, the antigen is fibroblast growth factor (FGF). In another aspect, the antigen is vascular endothelial growth factor (VEGF). In another aspect, the antigen is any other antigen known in the art to be involved in angiogenesis. In another aspect, the methods and compositions of impeding angiogenesis of a solid tumor in a subject, as provided herein, comprise administering to the subject a composition comprising a recombinant *Listeria* encoding two heterologous antigens. In another aspect, one of the two heterologous antigens is HMW-MAA. In another aspect, the antigen is any other antigen known in the art to be involved in angiogenesis. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0193] Methods for assessing efficacy of prostate cancer vaccines are well known in the art, and are described, for example, in Dzojic H et al (Adenovirus-mediated CD40 ligand therapy induces tumor cell apoptosis and systemic immunity in the TRAMP-C2 mouse prostate cancer model. Prostate. 2006 Jun 1;66(8):831-8), Naruishi K et al (Adenoviral vector-mediated RTVP-1 gene-modified tumor cell-based vaccine suppresses the development of experimental prostate cancer. Cancer Gene Ther. 2006 Jul;13(7):658-63), Sehgal I et al (Cancer Cell Int. 2006 Aug 23;6:21), and Heinrich JE et al (Vaccination against prostate cancer using a live tissue factor deficient cell line in Lobund-Wistar rats. Cancer Immunol Immunother 2007;56(5):725-30). Each possibility represents a separate aspect as provided herein.

[0194] In another aspect, the prostate cancer model used to test methods and compositions as provided herein is the TPSA23 (derived from TRAMP-C1 cell line stably expressing PSA) mouse model. In another aspect, the prostate cancer model is a 178-2 BMA cell model. In another aspect, the prostate cancer model is a PAIII adenocarcinoma cells model. In another aspect, the prostate cancer model is a PC-3M model. In another aspect, the prostate cancer model is any other prostate cancer model known in the art. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0195] In another aspect, the vaccine is tested in human subjects, and efficacy is monitored using methods well known in the art, e.g. directly measuring $CD4^+$ and $CD8^+$ T cell responses, or measuring disease progression, e.g. by determining the number or size of tumor metastases, or monitoring disease symptoms (cough, chest pain, weight loss, etc). Methods for assessing the efficacy of a prostate cancer vaccine in human subjects are well known in the art, and are described, for example, in Uenaka A et al (T cell immunomonitoring and tumor responses in patients immunized with a complex of cholesterol-bearing hydrophobized pullulan (CHP) and NY-ESO-1 protein. Cancer Immun. 2007 Apr 19;7:9) and Thomas-Kaskel AK et al (Vaccination of advanced prostate cancer patients with PSCA and PSA peptide-loaded dendritic cells induces DTH responses that correlate with superior overall survival. Int J Cancer. 2006 Nov 15;119(10):2428-34). Each method represents a separate aspect of the methods and compositions as provided herein.

[0196] Another aspect described herein provides a method of treating benign prostate hyperplasia (BPH) in a subject. Another aspect described herein provides a method of treating Prostatic Intraepithelial Neoplasia (PIN) in a subject

[0197] In one aspect, provided herein is a recombinant *Listeria* strain comprising a nucleic acid molecule operably integrated into the *Listeria* genome. In another aspect said nucleic acid molecule encodes (a) an endogenous polypeptide comprising a PEST sequence and (b) a polypeptide comprising an antigen in an open reading frame.

[0198] In one aspect, provided herein is a method of treating, suppressing, or inhibiting at least one tumor in a subject, comprising administering a recombinant *Listeria* strain to said subject. In another aspect, said recombinant *Listeria* strain comprises a first and second nucleic acid molecule. In another aspect, each said nucleic acid molecule encodes a heterologous antigen. In another aspect, said first nucleic acid molecule is operably integrated into the *Listeria* genome as an open reading frame with a native polypeptide comprising a PEST sequence and wherein said antigen is expressed by at least one cell of said tumor.

[0199] In one aspect, "antigen" is used herein to refer to a substance that when placed in contact with an organism, results in a detectable immune response from the organism. An antigen may be a lipid, peptide, protein, carbohydrate, nucleic acid, or combinations and variations thereof.

[0200] In one aspect, "variant" refers to an amino acid or nucleic acid sequence (or in other aspects, an organism or

tissue) that is different from the majority of the population but is still sufficiently similar to the common mode to be considered to be one of them, for example splice variants.

[0201] In one aspect, "isoform" refers to a version of a molecule, for example, a protein, with only slight differences compared to another isoform, or version, of the same protein. In one aspect, isoforms may be produced from different but related genes, or in another aspect, may arise from the same gene by alternative splicing. In another aspect, isoforms are caused by single nucleotide polymorphisms.

[0202] In one aspect, "fragment" refers to a protein or polypeptide that is shorter or comprises fewer amino acids than the full length protein or polypeptide. In another aspect, fragment refers to a nucleic acid that is shorter or comprises fewer nucleotides than the full length nucleic acid. In another aspect, the fragment is an N-terminal fragment. In another aspect, the fragment is a C-terminal fragment. In one aspect, the fragment is an intrasequential section of the protein, peptide, or nucleic acid. In one aspect, the fragment is a functional fragment. In another aspect, the fragment is an immunogenic fragment. In one aspect, a fragment has 10-20 nucleic or amino acids, while in another aspect, a fragment has more than 5 nucleic or amino acids, while in another aspect, a fragment has 100-200 nucleic or amino acids, while in another aspect, a fragment has 100-500 nucleic or amino acids, while in another aspect, a fragment has 50-200 nucleic or amino acids, while in another aspect, a fragment has 10-250 nucleic or amino acids.

[0203] In one aspect, "immunogenicity" or "immunogenic" is used herein to refer to the innate ability of a protein, peptide, nucleic acid, antigen or organism to elicit an immune response in an animal when the protein, peptide, nucleic acid, antigen or organism is administered to the animal. Thus, "enhancing the immunogenicity" in one aspect, refers to increasing the ability of a protein, peptide, nucleic acid, antigen or organism to elicit an immune response in an animal when the protein, peptide, nucleic acid, antigen or organism is administered to an animal. The increased ability of a protein, peptide, nucleic acid, antigen or organism to elicit an immune response can be measured by, in one aspect, a greater number of antibodies to a protein, peptide, nucleic acid, antigen or organism, a greater diversity of antibodies to an antigen or organism, a greater number of T-cells specific for a protein, peptide, nucleic acid, antigen or organism, a greater cytotoxic or helper T-cell response to a protein, peptide, nucleic acid, antigen or organism, and the like.

[0204] In one aspect, a "homologue" refers to a nucleic acid or amino acid sequence which shares a certain percentage of sequence identity with a particular nucleic acid or amino acid sequence. In one aspect, a sequence useful in the composition and methods as provided herein may be a homologue of a particular LLO sequence or N-terminal fragment thereof, ActA sequence or N-terminal fragment thereof, or PEST-like sequence described herein or known in the art. In one aspect, such a homolog maintains In another aspect, a sequence useful in the composition and methods as provided herein may be a homologue of an antigenic polypeptide, which in one aspect, is KLK3 or HMW-MAA or a functional fragment thereof. In one aspect, a homolog of a polypeptide and, in one aspect, the nucleic acid encoding such a homolog, described herein maintains the functional characteristics of the parent polypeptide. For example, in one aspect, a homolog of an antigenic polypeptide described herein maintains the antigenic characteristic of the parent polypeptide. In another aspect, a sequence useful in the composition and methods as provided herein may be a homologue of any sequence described herein. In one aspect, a homologue shares at least 70% identity with a particular sequence. In another aspect, a homologue shares at least 72% identity with a particular sequence. In another aspect, a homologue shares at least 75% identity with a particular sequence. In another aspect, a homologue shares at least 78% identity with a particular sequence. In another aspect, a homologue shares at least 80% identity with a particular sequence. In another aspect, a homologue shares at least 82% identity with a particular sequence. In another aspect, a homologue shares at least 83% identity with a particular sequence. In another aspect, a homologue shares at least 85% identity with a particular sequence. In another aspect, a homologue shares at least 87% identity with a particular sequence. In another aspect, a homologue shares at least 88% identity with a particular sequence. In another aspect, a homologue shares at least 90% identity with a particular sequence. In another aspect, a homologue shares at least 92% identity with a particular sequence. In another aspect, a homologue shares at least 93% identity with a particular sequence. In another aspect, a homologue shares at least 95% identity with a particular sequence. In another aspect, a homologue shares at least 96% identity with a particular sequence. In another aspect, a homologue shares at least 97% identity with a particular sequence. In another aspect, a homologue shares at least 98% identity with a particular sequence. In another aspect, a homologue shares at least 99% identity with a particular sequence. In another aspect, a homologue shares 100% identity with a particular sequence. Each possibility represents a separate aspect as provided herein.

[0205] In one aspect, it is to be understood that a homolog of any of the sequences as provided herein and/or as described herein is considered to be a part of the disclosure.

[0206] In one aspect, "functional" within the meaning of the disclosure, is used herein to refer to the innate ability of a protein, peptide, nucleic acid, fragment or a variant thereof to exhibit a biological activity or function. In one aspect, such a biological function is its binding property to an interaction partner, e.g., a membrane-associated receptor, and in another aspect, its trimerization property. In the case of functional fragments and the functional variants described herein, these biological functions may in fact be changed, e.g., with respect to their specificity or selectivity, but with retention of the basic biological function.

[0207] In one aspect, "treating" refers to both therapeutic treatment and prophylactic or preventative measures, wherein

the object is to prevent or lessen the targeted pathologic condition or disorder as described herein. Thus, in one aspect, treating may include directly affecting or curing, suppressing, inhibiting, preventing, reducing the severity of, delaying the onset of, reducing symptoms associated with the disease, disorder or condition, or a combination thereof. Thus, in one aspect, "treating" refers inter alia to delaying progression, expediting remission, inducing remission, augmenting remission, speeding recovery, increasing efficacy of or decreasing resistance to alternative therapeutics, or a combination thereof. In one aspect, "preventing" or "impeding" refers, inter alia, to delaying the onset of symptoms, preventing relapse to a disease, decreasing the number or frequency of relapse episodes, increasing latency between symptomatic episodes, or a combination thereof. In one aspect, "suppressing" or "inhibiting", refers inter alia to reducing the severity of symptoms, reducing the severity of an acute episode, reducing the number of symptoms, reducing the incidence of disease-related symptoms, reducing the latency of symptoms, ameliorating symptoms, reducing secondary symptoms, reducing secondary infections, prolonging patient survival, or a combination thereof.

[0208] In one aspect, symptoms are primary, while in another aspect, symptoms are secondary. In one aspect, "primary" refers to a symptom that is a direct result of a particular disease or disorder, while in one aspect, "secondary" refers to a symptom that is derived from or consequent to a primary cause. In one aspect, the compounds for use as described herein treat primary or secondary symptoms or secondary complications. In another aspect, "symptoms" may be any manifestation of a disease or pathological condition.

[0209] In some aspects, the term "comprising" refers to the inclusion of other recombinant polypeptides, amino acid sequences, or nucleic acid sequences, as well as inclusion of other polypeptides, amino acid sequences, or nucleic acid sequences, that may be known in the art, which in one aspect may comprise antigens or *Listeria* polypeptides, amino acid sequences, or nucleic acid sequences. In some aspects, the term "consisting essentially of' refers to a composition for use in the methods as provided herein, which has the specific recombinant polypeptide, amino acid sequence, or nucleic acid sequence, or fragment thereof. However, other polypeptides, amino acid sequences, or nucleic acid sequences may be included that are not involved directly in the utility of the recombinant polypeptide(s). In some aspects, the term "consisting" refers to a composition for use in the methods as provided herein having a particular recombinant polypeptide, amino acid sequence, or nucleic acid sequence, or fragment or combination of recombinant polypeptides, amino acid sequences, or nucleic acid sequences or fragments as provided herein, in any form or aspect as described herein.

[0210] In one aspect, the compositions for use in the methods as provided herein are administered intravenously. In another aspect, the vaccine is administered orally, whereas in another aspect, the vaccine is administered parenterally (e.g., subcutaneously, intramuscularly, and the like).

[0211] Further, in another aspect, the compositions or vaccines are administered as a suppository, for example a rectal suppository or a urethral suppository. Further, in another aspect, the pharmaceutical compositions are administered by subcutaneous implantation of a pellet. In a further aspect, the pellet provides for controlled release of an agent over a period of time. In yet another aspect, the pharmaceutical compositions are administered in the form of a capsule.

[0212] In one aspect, the route of administration may be parenteral. In another aspect, the route may be intra-ocular, conjunctival, topical, transdermal, intradermal, subcutaneous, intraperitoneal, intravenous, intra-arterial, vaginal, rectal, intratumoral, parcanceral, transmucosal, intramuscular, intravascular, intraventricular, intracranial, inhalation (aerosol), nasal aspiration (spray), intranasal (drops), sublingual, oral, aerosol or suppository or a combination thereof. For intranasal administration or application by inhalation, solutions or suspensions of the compounds mixed and aerosolized or nebulized in the presence of the appropriate carrier suitable. Such an aerosol may comprise any agent described herein. In one aspect, the compositions as set forth herein may be in a form suitable for intracranial administration, which in one aspect, is intrathecal and intracerebroventricular administration. In one aspect, the regimen of administration will be determined by skilled clinicians, based on factors such as exact nature of the condition being treated, the severity of the condition, the age and general physical condition of the patient, body weight, and response of the individual patient, etc.

[0213] In one aspect, parenteral application, particularly suitable are injectable, sterile solutions, preferably oily or aqueous solutions, as well as suspensions, emulsions, or implants, including suppositories and enemas. Ampoules are convenient unit dosages. Such a suppository may comprise any agent described herein.

[0214] In one aspect, sustained or directed release compositions can be formulated, e.g., liposomes or those wherein the active compound is protected with differentially degradable coatings, e.g., by microencapsulation, multiple coatings, etc. Such compositions may be formulated for immediate or slow release. It is also possible to freeze-dry the new compounds and use the lyophilisates obtained, for example, for the preparation of products for injection.

[0215] In one aspect, for liquid formulations, pharmaceutically acceptable carriers may be aqueous or non-aqueous solutions, suspensions, emulsions or oils. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Examples of oils are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, mineral oil, olive oil, sunflower oil, and fish-liver oil.

[0216] In one aspect, compositions of this invention are pharmaceutically acceptable. In one aspect, the term "pharmaceutically acceptable" refers to any formulation which is safe, and provides the appropriate delivery for the desired

route of administration of an effective amount of at least one compound for use in the present invention. This term refers to the use of buffered formulations as well, wherein the pH is maintained at a particular desired value, ranging from pH 4.0 to pH 9.0, in accordance with the stability of the compounds and route of administration.

[0217] In one aspect, a composition of the invention or used in the methods described herein may be administered alone or within a composition. In another aspect, compositions of this invention admixture with conventional excipients, i.e., pharmaceutically acceptable organic or inorganic carrier substances suitable for parenteral, enteral (e.g., oral) or topical application which do not deleteriously react with the active compounds may be used. In one aspect, suitable pharmaceutically acceptable carriers include but are not limited to water, salt solutions, alcohols, gum arabic, vegetable oils, benzyl alcohols, polyethylene glycols, gelatine, carbohydrates such as lactose, amylose or starch, magnesium stearate, talc, silicic acid, viscous paraffin, white paraffin, glycerol, alginates, hyaluronic acid, collagen, perfume oil, fatty acid monoglycerides and diglycerides, pentaerythritol fatty acid esters, hydroxy methylcellulose, polyvinyl pyrrolidone, etc. In another aspect, the pharmaceutical preparations can be sterilized and if desired mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or aromatic substances and the like which do not deleteriously react with the active compounds. In another aspect, they can also be combined where desired with other active agents, e.g., vitamins.

[0218] In one aspect, the compositions for use of the methods and compositions as provided herein may be administered with a carrier/diluent. Solid carriers/diluents include, but are not limited to, a gum, a starch (e.g., corn starch, pregeletanized starch), a sugar (e.g., lactose, mannitol, sucrose, dextrose), a cellulosic material (e.g., microcrystalline cellulose), an acrylate (e.g., polymethylacrylate), calcium carbonate, magnesium oxide, talc, or mixtures thereof.

[0219] In one aspect, the compositions of the methods and compositions as provided herein may comprise the composition of this invention and one or more additional compounds effective in preventing or treating cancer. In some aspects, the additional compound may comprise a compound useful in chemotherapy, which in one aspect, is Cisplatin. In another aspect, Ifosfamide, Fluorouracilor5-FU, Irinotecan, Paclitaxel (Taxol), Docetaxel, Gemcitabine, Topotecan or a combination thereof, may be administered with a composition as provided herein for use in the methods as provided herein. In another aspect, Amsacrine, Bleomycin, Busulfan, Capecitabine, Carboplatin, Carmustine, Chlorambucil, Cisplatin, Cladribine, Clofarabine, Crisantaspase, Cyclophosphamide, Cytarabine, Dacarbazine, Dactinomycin, Daunorubicin, Docetaxel, Doxorubicin, Epirubicin, Etoposide, Fludarabine, Fluorouracil, Gemcitabine, Gliadelimplants, Hydroxycarbamide, Idarubicin, Ifosfamide, Irinotecan, Leucovorin, Liposomaldoxorubicin, Liposomaldaunorubicin, Lomustine, Melphalan, Mercaptopurine, Mesna, Methotrexate, Mitomycin, Mitoxantrone, Oxaliplatin, Paclitaxel, Pemetrexed, Pentostatin, Procarbazine, Raltitrexed, Satraplatin, Streptozocin, Tegafur-uracil, Temozolomide, Teniposide, Thiotepa, Tioguanine, Topotecan, Treosulfan, Vinblastine, Vincristine, Vindesine, Vinorelbine, or a combination thereof, may be administered with a composition as provided herein for use in the methods as provided herein.

[0220] In another aspect, fusion proteins as provided herein are prepared by a process comprising subcloning of appropriate sequences, followed by expression of the resulting nucleotide. In another aspect, subsequences are cloned and the appropriate subsequences cleaved using appropriate restriction enzymes. The fragments are then ligated, in another aspect, to produce the desired DNA sequence. In another aspect, DNA encoding the fusion protein is produced using DNA amplification methods, for example polymerase chain reaction (PCR). First, the segments of the native DNA on either side of the new terminus are amplified separately. The 5' end of the one amplified sequence encodes the peptide linker, while the 3' end of the other amplified sequence also encodes the peptide linker. Since the 5' end of the first fragment is complementary to the 3' end of the second fragment, the two fragments (after partial purification, e.g. on LMP agarose) can be used as an overlapping template in a third PCR reaction. The amplified sequence will contain codons, the segment on the carboxy side of the opening site (now forming the amino sequence), the linker, and the sequence on the amino side of the opening site (now forming the carboxyl sequence). The insert is then ligated into a plasmid. In another aspect, a similar strategy is used to produce a protein wherein an HMW-MAA fragment is embedded within a heterologous peptide.

[0221] One aspect described herein provides a recombinant *Listeria* comprising a nucleic acid molecule encoding a heterologous antigenic polypeptide or fragment thereof, wherein said nucleic acid molecule is operably integrated into the *Listeria* genome as an open reading frame with an endogenous polypeptide comprising a PEST sequence.

[0222] In one aspect, provided herein is a recombinant *Listeria* capable of expressing and secreting two distinct heterologous antigens comprising a first antigen that is operably integrated in the genome as an open reading frame with a first polypeptide or fragment thereof comprising a PEST sequence and a second antigen that is operably integrated in the genome as an open reading frame with a second polypeptide or fragment thereof comprising a PEST sequence. In another aspect, said first or second polypeptide or fragment thereof is ActA, or LLO. In another aspect, said first or second antigen is prostate tumor-associated antigen (PSA), or High Molecular Weight-Melanoma Associated Antigen (HMWMAA). In another aspect, said fragment is an immunogenic fragment. In yet another aspect, said episomal expression vector lacks an antibiotic resistance marker.

[0223] In another aspect, the first and second antigen are distinct. In another aspect, said first and second antigens are concomitantly expressed. In another aspect, said first or second antigen are expressed at the same level. In another

aspect, said first or second antigen are differentially expressed. In another aspect, gene or protein expression is determined by methods that are well known in the art which in another aspect comprise real-time PCR, northern blotting, immunoblotting, etc. In another aspect, said first or second antigen's expression is controlled by an inducible system, while in another aspect, said first or second antigen's expression is controlled by a constitutive promoter. In another aspect, inducible expression systems are well known in the art.

[0224] In one aspect, provided herein is a method of preparing a recombinant *Listeria* capable of expressing and secreting two distinct heterologous antigens that target tumor cells and angiogenesis concomitantly. In another aspect, said method of preparing said recombinant *Listeria* comprises the steps of genetically fusing a first antigen into the genome that is operably linked to an open reading frame encoding a first polypeptide or fragment thereof comprising a PEST sequence and transforming said recombinant *Listeria* with an episomal expression vector encoding a second antigen that is operably linked to an open reading frame encoding a second polypeptide or fragment thereof comprising a PEST sequence. In another aspect, said method of preparing said recombinant *Listeria* comprises the steps of genetically fusing a first antigen into the genome that is operably linked to an open reading frame encoding a first polypeptide or fragment thereof comprising a PEST sequence and genetically fusing a second antigen that is operably linked to an open reading frame encoding a second polypeptide or fragment thereof comprising a PEST sequence.

[0225] Methods for transforming bacteria are well known in the art, and include calcium-chloride competent cell-based methods, electroporation methods, bacteriophage-mediated transduction, chemical, and physical transformation techniques (de Boer et al, 1989, Cell 56:641-649; Miller et al, 1995, FASEB J., 9:190-199; Sambrook et al. 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York; Ausubel et al., 1997, Current Protocols in Molecular Biology, John Wiley & Sons, New York; Gerhardt et al., eds., 1994, Methods for General and Molecular Bacteriology, American Society for Microbiology, Washington, DC; Miller, 1992, A Short Course in Bacterial Genetics, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.) In another aspect, the *Listeria* vaccine strain as provided herein is transformed by electroporation. Each method represents a separate aspect of the methods and compositions as provided herein.

[0226] In one aspect, provided herein is a method of inducing an immune response to an antigen in a subject comprising administering a recombinant *Listeria* strain to said subject, wherein said recombinant *Listeria* strain comprises a first and second nucleic acid molecule, each said nucleic acid molecule encoding a heterologous antigenic polypeptide or fragment thereof, wherein said first nucleic acid molecule is operably integrated into the *Listeria* genome as an open reading frame with a nucleic acid encoding an endogenous polypeptide comprising a PEST sequence.

[0227] In another aspect, provided herein is a method of inhibiting the onset of cancer, said method comprising the step of administering a recombinant *Listeria* composition that expresses two distinct heterologous antigens specifically expressed in said cancer.

[0228] In one aspect, provided herein is a method of treating a first and a second tumor in a subject, said method comprising the step of administering a recombinant *Listeria* composition that expresses two distinct heterologous antigens specifically expressed on said first and second tumor.

[0229] In another aspect, provided herein is a method of ameliorating symptoms that are associated with a cancer in a subject, said method comprising the step of administering a recombinant *Listeria* composition that expresses two distinct heterologous antigens specifically expressed in said cancer.

[0230] In one aspect, provided herein is a method of protecting a subject from cancer, said method comprising the step of administering a recombinant *Listeria* composition that expresses two distinct heterologous antigens specifically expressed in said cancer

[0231] In another aspect, provided herein is a method of delaying onset of cancer, said method comprising the step of administering a recombinant *Listeria* composition that expresses two distinct heterologous antigens specifically expressed in said cancer. In another aspect, provided herein is a method of treating metastatic cancer, said method comprising the step of administering a recombinant *Listeria* composition that expresses two distinct heterologous antigens specifically expressed in said cancer. In another aspect, provided herein is a method of preventing metastatic canceror micrometastatis, said method comprising the step of administering a recombinant *Listeria* composition that expresses two distinct heterologous antigens specifically expressed in said cancer. In another aspect, the recombinant *Listeria* composition is administered orally or parenterally.

[0232] One aspect described herein provides a method of producing a recombinant *Listeria* strain expressing two antigens, the method comprising: (a) genetically fusing a first nucleic acid encoding a first antigen into the *Listeria* genome in an open reading frame with an endogenous PEST-containing gene; (b) transforming said recombinant *Listeria* with an episomal expression vector comprising a second nucleic acid encoding a second antigen; and (c) expressing said first and second antigens under conditions conducive to antigenic expression in said recombinant *Listeria* strain. Another aspect described herein provides a method of producing a recombinant *Listeria* strain expressing two antigens, the method comprising: (a) genetically fusing a first nucleic acid encoding a first antigen and a second nucleic acid encoding a second antigen into the *Listeria* genome in an open reading frame with an endogenous PEST-containing gene; and (b) expressing said first and second antigens under conditions conducive to antigenic expression in said recombinant

Listeria strain. In one aspect, genetic fusion is via homologous recombination, as described herein. In one aspect, conditions conducive to antigenic expression are known in the art.

[0233] In another aspect of the methods and compositions as provided herein, "nucleic acids" or "nucleotide" refers to a string of at least two base-sugar-phosphate combinations. The term includes, in one aspect, DNA and RNA. "Nucleotides" refers, in one aspect, to the monomeric units of nucleic acid polymers. RNA may be, in one aspect, in the form of a tRNA (transfer RNA), snRNA (small nuclear RNA), rRNA (ribosomal RNA), mRNA (messenger RNA), antisense RNA, small inhibitory RNA (siRNA), micro RNA (miRNA) and ribozymes. The use of siRNA and miRNA has been described (Caudy AA et al, Genes & Devel 16: 2491-96 and references cited therein). DNA may be in form of plasmid DNA, viral DNA, linear DNA, or chromosomal DNA or derivatives of these groups. In addition, these forms of DNA and RNA may be single, double, triple, or quadruple stranded. The term also includes, in another aspect, artificial nucleic acids that may contain other types of backbones but the same bases. In one aspect, the artificial nucleic acid is a PNA (peptide nucleic acid). PNA contain peptide backbones and nucleotide bases and are able to bind, in one aspect, to both DNA and RNA molecules. In another aspect, the nucleotide is oxetane modified. In another aspect, the nucleotide is modified by replacement of one or more phosphodiester bonds with a phosphorothioate bond. In another aspect, the artificial nucleic acid contains any other variant of the phosphate backbone of native nucleic acids known in the art. The use of phosphothiorate nucleic acids and PNA are known to those skilled in the art, and are described in, for example, Neilsen PE, Curr Opin Struct Biol 9:353-57; and Raz NK et al Biochem Biophys Res Commun. 297:1075-84. The production and use of nucleic acids is known to those skilled in art and is described, for example, in Molecular Cloning, (2001), Sambrook and Russell, eds. and Methods in Enzymology: Methods for molecular cloning in eukaryotic cells (2003) Purchio and G. C. Fareed. Each nucleic acid derivative represents a separate aspect as provided herein.

[0234] The terms "polypeptide," "peptide" and "recombinant peptide" refer, in another aspect, to a peptide or polypeptide of any length. In another aspect, a peptide or recombinant peptide as provided herein has one of the lengths enumerated above for an HMW-MAA fragment. Each possibility represents a separate aspect of the methods and compositions as provided herein. In one aspect, the term "peptide" refers to native peptides (either degradation products, synthetically synthesized peptides or recombinant peptides) and/or peptidomimetics (typically, synthetically synthesized peptides), such as peptoids and semipeptoids which are peptide analogs, which may have, for example, modifications rendering the peptides more stable while in a body or more capable of penetrating into cells. Such modifications include, but are not limited to N terminus modification, C terminus modification, peptide bond modification, including, but not limited to, CH2-NH, CH2-S, CH2-S=O, O=C-NH, CH2-O, CH2-CH2, S=C-NH, CH=CH or CF=CH, backbone modifications, and residue modification. Methods for preparing peptidomimetic compounds are well known in the art and are specified, for example, in Quantitative Drug Design, C.A. Ramsden Gd., Chapter 17.2, F. Choplin Pergamon Press (1992). Further details in this respect are provided hereinunder.

[0235] In one aspect, "antigenic polypeptide" is used herein to refer to a polypeptide, peptide or recombinant peptide as described hereinabove that is foreign to a host and leads to the mounting of an immune response when present in, or, in another aspect, detected by, the host.

[0236] Peptide bonds (-CO-NH-) within the peptide may be substituted, for example, by N-methylated bonds (-N(CH3)-CO-), ester bonds (-C(R)H-C-O-O-C(R)-N-), ketomethylen bonds (-CO-CH2-), *-aza bonds (-NH-N(R)-CO-), wherein R is any alkyl, e.g., methyl, carba bonds (-CH2-NH-), hydroxyethylene bonds (-CH(OH)-CH2-), thioamide bonds (-CS-NH-), olefinic double bonds (-CH=CH-), retro amide bonds (-NH-CO-), peptide derivatives (-N(R)-CH2-CO-), wherein R is the "normal" side chain, naturally presented on the carbon atom.

[0237] These modifications can occur at any of the bonds along the peptide chain and even at several (2-3) at the same time. Natural aromatic amino acids, Trp, Tyr and Phe, may be substituted for synthetic non-natural acid such as TIC, naphthylelanine (Nol), ring-methylated derivatives of Phe, halogenated derivatives of Phe or o-methyl-Tyr.

[0238] In addition to the above, the peptides as provided herein may also include one or more modified amino acids or one or more non-amino acid monomers (e.g. fatty acids, complex carbohydrates etc).

[0239] In one aspect, the term "oligonucleotide" is interchangeable with the term "nucleic acid", and may refer to a molecule, which may include, but is not limited to, prokaryotic sequences, eukaryotic mRNA, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. The term also refers to sequences that include any of the known base analogs of DNA and RNA.

[0240] "Stably maintained" refers, in another aspect, to maintenance of a nucleic acid molecule or plasmid in the absence of selection (e.g. antibiotic selection) for 10 generations, without detectable loss. In another aspect, the period is 15 generations. In another aspect, the period is 20 generations. In another aspect, the period is 25 generations. In another aspect, the period is 30 generations. In another aspect, the period is 40 generations. In another aspect, the period is 50 generations. In another aspect, the period is 60 generations. In another aspect, the period is 80 generations. In another aspect, the period is 100 generations. In another aspect, the period is 150 generations. In another aspect, the period is 200 generations. In another aspect, the period is 300 generations. In another aspect, the period is 500 generations. In another aspect, the period is more than 500 generations. In another aspect, the nucleic acid molecule or plasmid is maintained stably in vitro (e.g. in culture). In another aspect, the nucleic acid molecule or plasmid is

maintained stably in vivo. In another aspect, the nucleic acid molecule or plasmid is maintained stably both in vitro and in vitro. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0241] In one aspect, the term "amino acid" or "amino acids" is understood to include the 20 naturally occurring amino acids; those amino acids often modified post-translationally in vivo, including, for example, hydroxyproline, phosphoserine and phosphothreonine; and other unusual amino acids including, but not limited to, 2-aminoadipic acid, hydroxylysine, isodesmosine, nor-valine, nor-leucine and ornithine. Furthermore, the term "amino acid" may include both D- and L-amino acids.

[0242] The term "nucleic acid" or "nucleic acid sequence" refers to a deoxyribonucleotide or ribonucleotide oligonucleotide in either single- or double-stranded form. The term encompasses nucleic acids, i.e., oligonucleotides, containing known analogues of natural nucleotides which have similar or improved binding properties, for the purposes desired, as the reference nucleic acid. The term also includes nucleic acids which are metabolized in a manner similar to naturally occurring nucleotides or at rates that are improved thereover for the purposes desired. The term also encompasses nucleic-acid-like structures with synthetic backbones. DNA backbone analogues described herein include phosphodiester, phosphorothioate, phosphorodithioate, methylphosphonate, phosphoramidate, alkyl phosphotriester, sulfamate, 3'-thioacetal, methylene(methylimino), 3'- N-carbamate, morpholino carbamate, and peptide nucleic acids (PNAs); see, e.g., Oligonucleotides and Analogues, a Practical Approach, edited by F. Eckstein, IRL Press at Oxford University Press (1991); Antisense Strategies, Annals of the New York Academy of Sciences, Volume 600, Eds. Baserga and Denhardt (NYAS 1992); Mulligan (1993) J. Med. Chem. 36:1923-1937; Antisense Research and Applications (1993, CRC Press). PNAs contain non-ionic backbones, such as N-(2-aminoethyl) glycine units. Phosphorothioate linkages are described, e.g., in WO 97/03211; WO 96/39154; Mata (1997) Toxicol. Appi. Pharmacol. 144:189-197. Other synthetic backbones encompasses by the term include methyl-phosphonate linkages or alternating methyiphosphonate and phosphodiester linkages (Strauss-Soukup (1997) Biochemistry 36:8692-8698), and benzylphosphonate linkages (Samstag (1996) Antisense Nucleic Acid Drug Dev. 6:153-156). The term nucleic acid is used interchangeably with gene, cDNA, mRNA, oligonucleotide primer, probe and amplification product.

[0243] In one aspect of the methods and compositions as provided herein, the term "recombination site" or "site-specific recombination site" refers to a sequence of bases in a nucleic acid molecule that is recognized by a recombinase (along with associated proteins, in some cases) that mediates exchange or excision of the nucleic acid segments flanking the recombination sites. The recombinases and associated proteins are collectively referred to as "recombination proteins" see, e.g., Landy, A., (Current Opinion in Genetics & Development) 3:699-707; 1993).

[0244] A "phage expression vector" or "phagemid" refers to any phage-based recombinant expression system for the purpose of expressing a nucleic acid sequence of the methods and compositions as provided herein in vitro or in vivo, constitutively or inducibly, in any cell, including prokaryotic, yeast, fungal, plant, insect or mammalian cell. A phage expression vector typically can both reproduce in a bacterial cell and, under proper conditions, produce phage particles. The term includes linear or circular expression systems and encompasses both phage-based expression vectors that remain episomal or integrate into the host cell genome.

[0245] In one aspect, the term "operably linked" as used herein means that the transcriptional and translational regulatory nucleic acid, is positioned relative to any coding sequences in such a manner that transcription is initiated. Generally, this will mean that the promoter and transcriptional initiation or start sequences are positioned 5' to the coding region.

[0246] In one aspect, an "open reading frame" or "ORF" is a portion of an organism's genome which contains a sequence of bases that could potentially encode a protein. In another aspect, the start and stop ends of the ORF are not equivalent to the ends of the mRNA, but they are usually contained within the mRNA. In one aspect, ORFs are located between the start-code sequence (initiation codon) and the stop-codon sequence (termination codon) of a gene. Thus, in one aspect, a nucleic acid molecule operably integrated into a genome as an open reading frame with an endogenous polypeptide is a nucleic acid molecule that has integrated into a genome in the same open reading frame as an endogenous polypeptide.

[0247] One aspect described herein provides a fusion polypeptide comprising a linker sequence. In one aspect, a "linker sequence" refers to an amino acid sequence that joins two heterologous polypeptides, or fragments or domains thereof. In general, as used herein, a linker is an amino acid sequence that covalently links the polypeptides to form a fusion polypeptide. A linker typically includes the amino acids translated from the remaining recombination signal after removal of a reporter gene from a display vector to create a fusion protein comprising an amino acid sequence encoded by an open reading frame and the display protein. As appreciated by one of skill in the art, the linker can comprise additional amino acids, such as glycine and other small neutral amino acids.

[0248] In one aspect, "endogenous" as used herein describes an item that has developed or originated within the reference organism or arisen from causes within the reference organism. In another aspect, endogenous refers to native.

[0249] In one aspect, "heterologous" as used herein describes a nucleic acid, amino acid, peptide, polypeptide, or protein derived from a different species than the reference species. Thus, for example, a *Listeria* strain expressing a heterologous polypeptide, in one aspect, would express a polypeptide that is not native or endogenous to the *Listeria*

strain, or in another aspect, a polypeptide that is not normally expressed by the *Listeria* strain, or in another aspect, a polypeptide from a source other than the *Listeria* strain. In another aspect, heterologous may be used to describe something derived from a different organism within the same species. In another aspect, the heterologous antigen is expressed by a recombinant strain of *Listeria,* and is processed and presented to cytotoxic T-cells upon infection of mammalian cells by the recombinant strain. In another aspect, the heterologous antigen expressed by *Listeria* species need not precisely match the corresponding unmodified antigen or protein in the tumor cell or infectious agent so long as it results in a T-cell response that recognizes the unmodified antigen or protein which is naturally expressed in the mammal.

[0250] In one aspect, an "episomal expression vector" as described herein refers to a nucleic acid vector which may be linear or circular, and which is usually double-stranded in form. In one embodiemnt, an episomal expression vector comprises a gene of interest. In another aspect, the inserted gene of interest is not interrupted or subjected to regulatory constraints which often occur from integration into cellular DNA. In another aspect, the presence of the inserted heterologous gene does not lead to rearrangement or interruption of the cell's own important regions. In another aspect, episomal vectors persist in multiple copies in the bacterial cytoplasm, resulting in amplification of the gene of interest, and, in another aspect, viral trans-acting factors are supplied when necessary. In another aspect, in stable transfection procedures, the use of episomal vectors often results in higher transfection efficiency than the use of chromosome-integrating plasmids (Belt, P.B.G.M., et al (1991) Efficient cDNA cloning by direct phenotypic correction of a mutant human cell line (HPRT2) using an Epstein-Barr virus-derived cDNA expression vector. Nucleic Acids Res. 19, 4861-4866; Mazda, O., et al. (1997) Extremely efficient gene transfection into lympho-hematopoietic cell lines by Epstein-Barr virus-based vectors. J. Immunol. Methods 204, 143-151). In one aspect, the episomal expression vectors of the methods and compositions as provided herein may be delivered to cells in vivo, ex vivo, or in vitro by any of a variety of the methods employed to deliver DNA molecules to cells. The vectors may also be delivered alone or in the form of a pharmaceutical composition that enhances delivery to cells of a subject.

[0251] In one aspect, "fused" refers to linkage by covalent bonding.

[0252] "Transforming," in one aspect, refers to engineering a bacterial cell to take up a plasmid or other heterologous DNA molecule. In another aspect, "transforming" refers to engineering a bacterial cell to express a gene of a plasmid or other heterologous DNA molecule. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0253] In another aspect, conjugation is used to introduce genetic material and/or plasmids into bacteria. Methods for conjugation are well known in the art, and are described, for example, in Nikodinovic J et al (A second generation snp-derived Escherichia coli-Streptomyces shuttle expression vector that is generally transferable by conjugation. Plasmid. 2006 Nov;56(3):223-7) and Auchtung JM et al (Regulation of a Bacillus subtilis mobile genetic element by intercellular signaling and the global DNA damage response. Proc Natl Acad Sci U S A. 2005 Aug 30;102(35):12554-9). Each method represents a separate aspect of the methods and compositions as provided herein.

[0254] "Metabolic enzyme" refers, in another aspect, to an enzyme involved in synthesis of a nutrient required by the host bacteria. In another aspect, the term refers to an enzyme required for synthesis of a nutrient required by the host bacteria. In another aspect, the term refers to an enzyme involved in synthesis of a nutrient utilized by the host bacteria. In another aspect, the term refers to an enzyme involved in synthesis of a nutrient required for sustained growth of the host bacteria. In another aspect, the enzyme is required for synthesis of the nutrient. Each possibility represents a separate aspect of the methods and compositions as provided herein.

[0255] In one aspect, the term "attenuation," as used herein, is meant a diminution in the ability of the bacterium to cause disease in an animal. In other words, the pathogenic characteristics of the attenuated Listeria strain have been lessened compared with wild-type Listeria, although the attenuated Listeria is capable of growth and maintenance in culture. Using as an example the intravenous inoculation of Balb/c mice with an attenuated Listeria, the lethal dose at which 50% of inoculated animals survive (LD.sub.50) is preferably increased above the LD.sub.50 of wild-type Listeria by at least about 10-fold, more preferably by at least about 100-fold, more preferably at least about 1,000 fold, even more preferably at least about 10,000 fold, and most preferably at least about 100,000-fold. An attenuated strain of Listeria is thus one which does not kill an animal to which it is administered, or is one which kills the animal only when the number of bacteria administered is vastly greater than the number of wild type non-attenuated bacteria which would be required to kill the same animal. An attenuated bacterium should also be construed to mean one which is incapable of replication in the general environment because the nutrient required for its growth is not present therein. Thus, the bacterium is limited to replication in a controlled environment wherein the required nutrient is provided. The attenuated strains of the present invention are therefore environmentally safe in that they are incapable of uncontrolled replication.

[0256] The term "about" as used herein means in quantitative terms plus or minus 5%, or in another aspect plus or minus 10%, or in another aspect plus or minus 15%, or in another aspect plus or minus 20%.

[0257] The term "subject" refers in one aspect to a mammal including a human in need of therapy for, or susceptible to, a condition or its sequelae. The subject may include dogs, cats, pigs, cows, sheep, goats, horses, rats, and mice and humans. In one aspect, the term "subject" does not exclude an individual that is healthy in all respects and does

not have or show signs of disease or disorder.

[0258] In one aspect, the *Listeria* as provided herein expresses a heterologous polypeptide, as described herein, in another aspect, the *Listeria* as provided herein secretes a heterologous polypeptide, as described herein, and in another aspect, the *Listeria* as provided herein expresses and secretes a heterologous polypeptide, as described herein. In another aspect, the *Listeria* as provided herein comprises a heterologous polypeptide, and in another aspect, comprises a nucleic acid that encodes a heterologous polypeptide.

[0259] In one aspect, Listeria strains as provided herein may be used in the preparation of vaccines. In one aspect, Listeria strains as provided herein may be used in the preparation of peptide vaccines. Methods for preparing peptide vaccines are well known in the art and are described, for example, in EP1408048, United States Patent Application Number 20070154953, and OGASAWARA et al (Proc. Nati. Acad. Sci. USA Vol. 89, pp. 8995-8999, October 1992). In one aspect, peptide evolution techniques are used to create an antigen with higher immunogenicity. Techniques for peptide evolution are well known in the art and are described, for example in United States Patent 6773900.

[0260] In one aspect, the vaccines of the methods and compositions as provided herein may be administered to a host vertebrate animal, preferably a mammal, and more preferably a human, either alone or in combination with a pharmaceutically acceptable carrier. In another aspect, the vaccine is administered in an amount effective to induce an immune response to the Listeria strain itself or to a heterologous antigen which the Listeria species has been modified to express. In another aspect, the amount of vaccine to be administered may be routinely determined by one of skill in the art when in possession of the present disclosure. In another aspect, a pharmaceutically acceptable carrier may include, but is not limited to, sterile distilled water, saline, phosphate buffered solutions or bicarbonate buffered solutions. In another aspect, the pharmaceutically acceptable carrier selected and the amount of carrier to be used will depend upon several factors including the mode of administration, the strain of *Listeria* and the age and disease state of the vaccinee. In another aspect, administration of the vaccine may be by an oral route, or it may be parenteral, intranasal, intramuscular, intravascular, intrarectal, intraperitoneal, or any one of a variety of well-known routes of administration. In another aspect, the route of administration may be selected in accordance with the type of infectious agent or tumor to be treated.

[0261] One aspect described herein provides a recombinant *Listeria* strain comprising a nucleic acid molecule encoding a heterologous antigenic polypeptide or fragment thereof, wherein said nucleic acid molecule is operably integrated into the *Listeria* genome in an open reading frame with an endogenous PEST-containing gene.

[0262] Another aspect described herein provides a method of inducing an immune response to an antigen in a subject comprising administering a recombinant *Listeria* strain comprising a nucleic acid molecule encoding a heterologous antigenic polypeptide or fragment thereof, wherein said nucleic acid molecule is operably integrated into the *Listeria* genome in an open reading frame with an endogenous PEST-containing gene.

[0263] Another aspect described herein provides a method of treating, suppressing, or inhibiting a cancer in a subject comprising administering a recombinant *Listeria* strain comprising a nucleic acid molecule encoding a heterologous antigenic polypeptide or fragment thereof, wherein said nucleic acid molecule is operably integrated into the *Listeria* genome in an open reading frame with an endogenous PEST-containing gene.

[0264] Another aspect described herein provides a method of treating, suppressing, or inhibiting at least one tumor in a subject comprising administering a recombinant *Listeria* strain comprising a nucleic acid molecule encoding a heterologous antigenic polypeptide or fragment thereof, wherein said nucleic acid molecule is operably integrated into the *Listeria* genome in an open reading frame with an endogenous PEST-containing gene.

[0265] Another aspect described herein provides a method of producing a recombinant *Listeria* strain expressing an antigen, the method comprising genetically fusing a first nucleic acid encoding an antigen into the *Listeria* genome in an open reading frame with an endogenous PEST-containing gene; and expressing said antigen under conditions conducive to antigenic expression in said recombinant *Listeria* strain.

[0266] Another aspect described herein provides any of the methods described hereinabove using a recombinant *Listeria* strain comprising a nucleic acid molecule encoding a heterologous antigenic polypeptide or fragment thereof, wherein said nucleic acid molecule is operably integrated into the *Listeria* genome in an open reading frame with an endogenous PEST-containing gene.

[0267] Another aspect described herein provides a kit for conveniently practicing the methods as provided herein comprising one or more *Listeria* strains as provided herein, an applicator, and instructional material that describes how to use the kit components in practicing the methods as provided herein.

[0268] The following examples are presented in order to more fully illustrate the aspects described herein

EXAMPLES

[0269] We developed a recombinant *Lm* that secretes PSA fused to tLLO (Lm-LLO-PSA), which elicits a potent PSA-specific immune response associated with regression of tumors in a mouse model for prostate cancer, wherein the expression of tLLO-PSA is derived from a plasmid based on pGG55 (Table 1), which confers antibiotic resistance to the

vector. We recently developed a new strain for the PSA vaccine based on the pADV142 plasmid, which has no antibiotic resistance markers, and referred as *LmddA-142* (Table 1). This new strain is 10 times more attenuated than *Lm*-LLO-PSA. In addition, *LmddA-142* was slightly more immunogenic and significantly more efficacious in regressing PSA expressing tumors than the *Lm*-LLO-PSA.

Table 1. Plasmids and strains

Plasmids	Features
pGG55	pAM401/pGB354 shuttle plasmid with gram(-) and gram(+) *cm* resistance, LLO-E7 expression cassette and a copy of *Lm prfA* gene
pTV3	Derived from pGG55 by deleting *cm* genes and inserting the *Lm dal* gene
pADV119	Derived from pTV3 by deleting the *prfA* gene
pADV134	Derived from pADV119 by replacing the *Lm dal* gene by the *Bacillus dal* gene
pADV142	Derived from pADV134 by replacing HPV16 *e7* with *klk3*
pADV168	Derived from pADV134 by replacing HPV16 *e7* with *hmw-maa*$_{2160\text{-}2258}$
Strains	Genotype
10403S	Wild-type *Listeria monocytogenes*:: *str*
XFL-7	10403S *prfA*$^{(-)}$
Lmdd	10403S *dal*$^{(-)}$ *dat*$^{(-)}$
LmddA	10403S *dal*$^{(-)}$ *dat*$^{(-)}$ *actA*$^{(-)}$
LmddA-134	10403S *dal*$^{(-)}$ *dat*$^{(-)}$ *actA*$^{(-)}$ *pADV134*
LmddA-142	10403S *dal*$^{(-)}$ *dat*$^{(-)}$ *actA*$^{(-)}$ *pADV142*
Lmdd-143	10403S *dal*$^{(-)}$ *dat*$^{(-)}$ *with klk3 fused to the hly gene in the chromosome*
LmddA-143	10403S *dal*$^{(-)}$ *dat*$^{(-)}$ *actA*$^{(-)}$ *with klk3 fused to the hly gene in the chromosome*
LmddA-168	10403S *dal*$^{(-)}$ *dat*$^{(-)}$ *actA*$^{(-)}$ *pADV168*
Lmdd-143/134	*Lmdd-143 pADV134*
LmddA-143/134	*LmddA-143 pADV134*
Lmdd-143/168	*Lmdd-143 pADV168*
LmddA-143/168	*LmddA-143 pADV168*

[0270] The sequence of the plasmid pAdv142 (6523 bp) was as follows:

cggagtgtatactggcttactatgttggcactgatgagggtgtcagtgaagtgcttcatgtggcaggagaaaaaaggctgcacc
ggtgcgtcagcagaatatgtgatacaggatatattccgcttcctcgctcactgactcgctacgctcggtcgttcgactgcggcgagcggaaatg
gcttacgaacggggcggagatttcctggaagatgccaggaagatacttaacagggaagtgagagggccgcggcaaagccgtttttccatag
gctccgccccccctgacaagcatcacgaaatctgacgctcaaatcagtggtggcgaaacccgacaggactataaagataccaggcgtttccc
cctggcggctccctcgtgcgctctcctgttcctgcctttcggtttaccggtgtcattccgctgttatggccgcgtttgtctcattccacgcctgaca
ctcagttccgggtaggcagttcgctccaagctggactgtatgcacgaaccccccgttcagtccgaccgctgcgccttatccggtaactatcgtc
ttgagtccaacccggaaagacatgcaaaagcaccactggcagcagccactggtaattgatttagaggagttagtcttgaagtcatgcgccggt
taaggctaaactgaaaggacaagttttggtgactgcgctcctccaagccagttacctcggttcaaagagttggtagctcagagaaccttcgaaa
aaccgccctgcaaggcggtttttcgttttcagagcaagagattacgcgcagaccaaaacgatctcaagaagatcatcttattaatcagataaaa
tatttctagccctcctttgattagtatattcctatcttaaagttacttttatgtggaggcattaacatttgttaatgacgtcaaaaggatagcaagacta
gaataaagctataaagcaagcatataatattgcgtttcatctttagaagcgaatttcgccaatattataattatcaaaagagaggggtggcaaacg
gtatttggcattattaggttaaaaaatgtagaaggagagtgaaacccatgaaaaaaataatgctagtttttattacacttatattagttagtctaccaa
ttgcgcaacaaactgaagcaaaggatgcatctgcattcaataaagaaaattcaatttcatccatggcaccaccagcatctccgcctgcaagtcc
taagacgccaatcgaaaagaaacacgcggatgaaatcgataagtatatacaaggattggattacaataaaaacaatgtattagtataccacgg
agatgcagtgacaaatgtgccgccaagaaaaggttacaaagatggaaatgaatatattgttgtggagaaaaagaagaaatccatcaatcaaa
ataatgcagacattcaagttgtgaatgcaatttcgagcctaacctatccaggtgctctcgtaaaagcgaattcggaattagtagaaaatcaacca

gatgttctccctgtaaaacgtgattcattaacactcagcattgatttgccaggtatgactaatcaagacaataaaatagttgtaaaaaatgccacta
aatcaaacgttaacaacgcagtaaatacattagtggaaagatggaatgaaaaatatgctcaagcttatccaaatgtaagtgcaaaaattgattat
gatgacgaaatggcttacagtgaatcacaattaattgcgaaatttggtacagcatttaaagctgtaaataatagcttgaatgtaaacttcggcgca
atcagtgaagggaaaatgcaagaagaagtcattagttttaaacaaatttactataacgtgaatgttaatgaacctacaagaccttccagatttttcg
gcaaagctgttactaaagagcagttgcaagcgcttggagtgaatgcagaaaatcctcctgcatatatctcaagtgtggcgtatggccgtcaagt
ttatttgaaattatcaactaattcccatagtactaaagtaaaagctgcttttgatgctgccgtaagcggaaaatctgtctcaggtgatgtagaactaa
caaatatcatcaaaaattcttccttcaaagccgtaatttacggaggttccgcaaaagatgaagttcaaatcatcgacggcaacctcggagactta
cgcgatattttgaaaaaaggcgctacttttaatcgagaaacaccaggagttcccattgcttatacaacaaacttcctaaaagacaatgaattagct
gttattaaaaacaactcagaatatattgaaacaacttcaaaagcttatacagatggaaaaattaacatcgatcactctggaggatacgttgctcaa
ttcaacatttcttgggatgaagtaaattatgatctcgagattgtgggaggctgggagtgcgagaagcattcccaaccctggcaggtgcttgtgg
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ttgctgggtcggcacagcctgtttcatcctgaagacacaggccaggtatttcaggtcagccacagcttcccacacccgctctacgatatgagc
ctcctgaagaatcgattcctcaggccaggtgatgactccagccacgacctcatgctgctccgcctgtcagagcctgccgagctcacggatgct
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ttgaccccaaagaaacttcagtgtgtggacctccatgttatttccaatgacgtgtgtgcgcaagttcaccctcagaaggtgaccaagttcatgct
gtgtgctggacgctggacaggggggcaaaagcacctgctcgggtgattctgggggcccacttgtctgttatggtgtgcttcaaggtatcacgtc
atggggcagtgaaccatgtgccctgcccgaaaggccttccctgtacaccaaggtggtgcattaccggaagtggatcaaggacaccatcgtg
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gacgtggcgttgttctgacttccgaagaagcgattcacgaaaatcaagatacatttacgcattggacaccaaacgtttatcgttatggtacgtatg
cagacgaaaaccgttcatacactaaaggacattctgaaaacaatttaagacaaatcaataccttctttattgattttgatattcacacggaaaaaga
aactatttcagcaagcgatattttaacaacagctattgatttaggttttatgcctacgttaattatcaaatctgataaaggttatcaagcatattttgtttt
agaaacgccagtctatgtgacttcaaaatcagaatttaaatctgtcaaagcagccaaaataatctcgcaaaatatccgagaatatttggaaagt
ctttgccagttgatctaacgtgcaatcattttgggattgctcgtataccaagaacggacaatgtagaatttttgatcccaattaccgttattctttcaa
agaatggcaagattggtctttcaaacaaacagataataagggctttactcgttcaagtctaacggttttaagcggtacagaaggcaaaaaacaa
gtagatgaaccctggtttaatctcttattgcacgaaacgaaattttcaggagaaaagggtttagtagggcgcaatagcgttatgtttaccctctctttt
agcctactttagttcaggctattcaatcgaaacgtgcgaatataatatgtttgagtttaataatcgattagatcaacccttagaagaaaaagaagta
atcaaaattgttagaagtgcctattcagaaaactatcaaggggctaatagggaatacattaccattctttgcaaagcttgggtatcaagtgatttaa
ccagtaaagatttatttgtccgtcaagggtggtttaaattcaagaaaaaaagaagcgaacgtcaacgtgttcatttgtcagaatggaaagaagat
ttaatggcttatattagcgaaaaaagcgatgtatacaagccttatttagcgacgaccaaaaaagagattagagaagtgctaggcattcctgaac
ggacattagataaattgctgaaggtactgaaggcgaatcaggaaattttctttaagattaaaccaggaagaaatggtggcattcaacttgctagt
gttaaatcattgttgctatcgatcattaaattaaaaaaagaagaacgagaaagctatataaaggcgctgacagcttcgtttaatttagaacgtacat
ttattcaagaaactctaaacaaattggcagaacgccccaaaacggacccacaactcgatttgtttagctacgatacaggctgaaaataaaaccc
gcactatgccattacatttatatctatgatacgtgtttgtttttctttgctggctagcttaattgcttatatttacctgcaataaaggatttcttacttccatt
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atggaaatatattcatccccctgccggcctattaatgtgacttttgtgcccggcggatattcctgatccagctccaccataaattggtccatgcaaa
ttcggccggcaattttcaggcgttttcccttcacaaggatgtcggtccctttcaattttcggagccagccgtccgcatagcctacaggcaccgtc
ccgatccatgtgtctttttccgctgtgtactcggctccgtagctgacgctctcgccttttctgatcagtttgacatgtgacagtgtcgaatgcagggt
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aaaaagccttttttcagccggagtccagcggcgctgttcgcgcagtggaccattagattctttaacggcagcggagcaatcagctctttaaagc
gctcaaactgcattaagaaatagcctctttcttttttcatccgctgtcgcaaaatgggtaaatacccctttgcactttaaacgagggttgcggtcaag
aattgccatcacgttctgaacttcttcctctgtttttacaccaagtctgttcatccccgtatcgaccttcagatgaaaatgaagagaacctttttttcgtg
tggcgggctgcctcctgaagccattcaacagaataacctgttaaggtcacgtcatactcagcagcgattgccacatactccgggggaaccgc
gccaagcaccaatataggcgccttcaatccctttttgcgcagtgaaatcgcttcatccaaaatggccacggccaagcatgaagcacctgcgtc
aagagcagcctttgctgtttctgcatcaccatgcccgtaggcgtttgctttcacaactgccatcaagtggacatgttcaccgatatgtttttttcatatt
gctgacattttcctttatcgcggacaagtcaatttccgcccacgtatctctgtaaaaaggttttgtgctcatggaaaactcctctcttttttcagaaaa
tcccagtacgtaattaagtatttgagaattaattttatattgattaatactaagtttacccagttttcacctaaaaaacaaatgatgagataatagctcc aaaggctaaa-
gaggactataccaactatttgttaattaa (SEQ ID NO: 46). This plasmid was sequenced at Genewiz facility from the E. coli strain on 2-20-08.

EXAMPLE 1: Construction of attenuated *Listeria* strain-LmddΔ*actA* and insertion of the human *klk3* gene in frame to the *hly* gene in the *Lmdd* and *Lmdda* strains.

[0271] The strain Lm *dal dat* (Lmdd) was attenuated by the irreversible deletion of the virulence factor, ActA. An in-frame deletion of *actA* in the *Lmdaldat* (Lmdd) background was constructed to avoid any polar effects on the expression of downstream genes. The Lm *dal dat* Δ*actA* contains the first 19 amino acids at the N-terminal and 28 amino acid residues of the C-terminal with a deletion of 591 amino acids of ActA.

[0272] The actA deletion mutant was produced by amplifying the chromosomal region corresponding to the upstream (657 bp-oligo's Adv 271/272) and downstream (625 bp- oligo's Adv 273/274) portions of *actA* and joining by PCR. The sequence of the primers used for this amplification is given in the Table 2. The upstream and downstream DNA regions of *actA* were cloned in the pNEB193 at the EcoRI/PstI restriction site and from this plasmid, the EcoRI/PstI was further cloned in the temperature sensitive plasmid pKSV7, resulting in ΔactA/pKSV7 (pAdv120).

Table 2: Sequence of primers that was used for the amplification of DNA sequences upstream and downstream of *actA*

Primer	Sequence	SEQ ID NO:
Adv271-actAF1	cg GAATTCGGATCCgcgccaaatcattggttgattg	47
Adv272-actAR1	gcgaGTCGACgtcggggttaatcgtaatgcaattggc	48
Adv273-actAF2	gcgaGTCGACccatacgacgttaattcttgcaatg	49
Adv274-actAR2	gataCTGCAGGGATCCttcccttctcggtaatcagtcac	50

[0273] The deletion of the gene from its chromosomal location was verified using primers that bind externally to the *actA* deletion region, which are shown in Figure 1 as primer 3 (Adv 305-tgggatggccaagaaattc, SEQ ID NO: 51) and primer 4 (Adv304-ctaccatgtcttccgttgcttg; SEQ ID NO: 52) . The PCR analysis was performed on the chromosomal DNA isolated from Lmdd and LmddΔ*actA*. The sizes of the DNA fragments after amplification with two different sets of primer pairs 1/2 and 3/4 in Lmdd chromosomal DNA was expected to be 3.0 Kb and 3.4 Kb. On the other hand, the expected sizes of PCR using the primer pairs 1/2 and 3/4 for the LmddΔ*actA* was 1.2 Kb and 1.6 Kb. Thus, PCR analysis in Figure 1 confirms that the 1.8 kb region of *actA* was deleted in the LmddΔ*actA* strain. DNA sequencing was also performed on PCR products to confirm the deletion of *actA* containing region in the strain, LmddΔ*actA*.

EXAMPLE 2: Construction of the antibiotic-independent episomal expression system for antigen delivery by *Lm* vectors.

[0274] The antibiotic-independent episomal expression system for antigen delivery by *Lm* vectors (pAdv142) is the next generation of the antibiotic-free plasmid pTV3 (Verch et al., Infect Immun, 2004. 72(11):6418-25). The gene for virulence gene transcription activator, *prfA* was deleted from pTV3 since *Listeria* strain Lmdd contains a copy of *prfA* gene in the chromosome. Additionally, the cassette for p60-*Listeria dal* at the NheI/PacI restriction site was replaced by p60-*Bacillus subtilis dal* resulting in plasmid pAdv134 (Figure 2A). The similarity of the *Listeria* and *Bacillus dal* genes is -30%, virtually eliminating the chance of recombination between the plasmid and the remaining fragment of the *dal* gene in the *Lmdd* chromosome. The plasmid pAdv134 contained the antigen expression cassette tLLO-E7. The *LmddA* strain was transformed with the pADV134 plasmid and expression of the LLO-E7 protein from selected clones confirmed by Western blot (Figure 2B). The *Lmdd* system derived from the 10403S wild-type strain lacks antibiotic resistance markers, except for the *Lmdd* streptomycin resistance.

[0275] Further, pAdv134 was restricted with XhoI/XmaI to clone human PSA, *klk3* resulting in the plasmid, pAdv142. The new plasmid, pAdv142 (Figure 2C, Table 1) contains *Bacillus dal* (B-Dal) under the control of *Listeria* p60 promoter. The shuttle plasmid, pAdv142 complemented the growth of both *E. coli ala drx* MB2159 as well as *Listeria monocytogenes* strain Lmdd in the absence of exogenous D-alanine. The antigen expression cassette in the plasmid pAdv142 consists of *hly* promoter and LLO-PSA fusion protein (Figure 2C).

[0276] The plasmid pAdv142 was transformed to the *Listeria* background strains, LmddactA strain resulting in Lm-ddA-LLO-PSA. The expression and secretion of LLO-PSA fusion protein by the strain, Lm-ddA-LLO-PSA was confirmed by Western Blot using anti-LLO and anti-PSA antibody (Figure 2D). There was stable expression and secretion of LLO-PSA fusion protein by the strain, Lm-ddA-LLO-PSA after two *in vivo* passages.

EXAMPLE 3: *In vitro* and *in vivo* stability of the strain LmddA-LLO-PSA

[0277] The *in vitro* stability of the plasmid was examined by culturing the LmddA-LLO-PSA *Listeria* strain in the presence or absence of selective pressure for eight days. The selective pressure for the strain LmddA-LLO-PSA is D-alanine. Therefore, the strain LmddA-LLO-PSA was passaged in Brain-Heart Infusion (BHI) and BHI+ 100 μg/ml D-alanine. CFUs were determined for each day after plating on selective (BHI) and non-selective (BHI+D-alanine) medium. It was expected that a loss of plasmid will result in higher CFU after plating on non-selective medium (BHI+D-alanine). As depicted in Figure 3A, there was no difference between the number of CFU in selective and non-selective medium. This suggests that the plasmid pAdv142 was stable for at least 50 generations, when the experiment was terminated.

[0278] Plasmid maintenance *in vivo* was determined by intravenous injection of 5 x 10^7 CFU LmddA-LLO-PSA, in C57BL/6 mice. Viable bacteria were isolated from spleens homogenized in PBS at 24 h and 48 h. CFUs for each sample were determined at each time point on BHI plates and BHI + 100 μg/ml D-alanine. After plating the splenocytes on selective and non-selective medium, the colonies were recovered after 24 h. Since this strain is highly attenuated, the bacterial load is cleared *in vivo* in 24 h. No significant differences of CFUs were detected on selective and non-selective plates, indicating the stable presence of the recombinant plasmid in all isolated bacteria (Figure 3B).

EXAMPLE 4: *In vivo* passaging, virulence and clearance of the strain LmddA-142 (LmddA-LLO-PSA)

[0279] *LmddA*-142 is a recombinant *Listeria* strain that secretes the episomally expressed tLLO-PSA fusion protein. To determine a safe dose, mice were immunized with LmddA-LLO-PSA at various doses and toxic effects were determined. LmddA-LLO-PSA caused minimum toxic effects (data not shown). The results suggested that a dose of 10^8 CFU of LmddA-LLO-PSA was well tolerated by mice. Virulence studies indicate that the strain LmddA-LLO-PSA was highly attenuated.

[0280] The *in vivo* clearance of LmddA-LLO-PSA after administration of the safe dose, 10^8 CFU intraperitoneally in C57BL/6 mice, was determined. There were no detectable colonies in the liver and spleen of mice immunized with LmddA-LLO-PSA after day 2. Since this strain is highly attenuated, it was completely cleared in vivo at 48 h (Figure 4A).

[0281] To determine if the attenuation of LmddA-LLO-PSA attenuated the ability of the strain LmddA-LLO-PSA to infect macrophages and grow intracellularly, we performed a cell infection assay. Mouse macrophage-like cell line such as J774A.1 were infected in vitro with *Listeria* constructs and intracellular growth was quantified. The positive control strain, wild type *Listeria* strain 10403S grows intracellularly, and the negative control XFL7, a *prfA* mutant, cannot escape the phagolysosome and thus does not grow in J774 cells. The intracytoplasmic growth of LmddA-LLO-PSA was slower than 10403S due to the loss of the ability of this strain to spread from cell to cell (Figure 4B). The results indicate that LmddA-LLO-PSA has the ability to infect macrophages and grow intracytoplasmically.

EXAMPLE 5: Immunogenicity of the strain-LmddA-LLO-PSA in C57BL/6 mice

[0282] The PSA-specific immune responses elicited by the construct LmddA-LLO-PSA in C57BL/6 mice were determined using PSA tetramer staining. Mice were immunized twice with LmddA-LLO-PSA at one week intervals and the splenocytes were stained for PSA tetramer on day 6 after the boost. Staining of splenocytes with the PSA-specific tetramer showed that LmddA-LLO-PSA elicited 23% of PSA tetramer$^+$CD8$^+$CD62L^{low} cells (Figure 5A).

[0283] The functional ability of the PSA-specific T cells to secrete IFN-γ after stimulation with PSA peptide for 5 h was examined using intracellular cytokine staining. There was a 200-fold increase in the percentage of CD8$^+$CD62L^{low}IFN-γ secreting cells stimulated with PSA peptide in the LmddA-LLO-PSA group compared to the naïve mice (Figure 5B), indicating that the LmddA-LLO-PSA strain is very immunogenic and primes high levels of functionally active PSA CD8$^+$ T cell responses against PSA in the spleen.

[0284] To determine the functional activity of cytotoxic T cells generated against PSA after immunizing mice with LmddA-LLO-PSA, we tested the ability of PSA-specific CTLs to lyse cells EL4 cells pulsed with H-2D^b peptide in an *in vitro* assay. A FACS-based caspase assay (Figure 5C) and Europium release (Figure 5D) were used to measure cell lysis. Splenocytes of mice immunized with LmddA-LLO-PSA contained CTLs with high cytolytic activity for the cells that display PSA peptide as a target antigen.

[0285] Elispot was performed to determine the functional ability of effector T cells to secrete IFN-γ after 24 h stimulation with antigen. Using ELISpot, we observed there was a 20-fold increase in the number of spots for IFN-γ in splenocytes from mice immunized with LmddA-LLO-PSA stimulated with specific peptide when compared to the splenocytes of the naïve mice (Figure 5E).

EXAMPLE 6: Immunization with the *LmddA*-142 strains induces regression of a tumor expressing PSA and infiltration of the tumor by PSA-specific CTLs.

[0286] The therapeutic efficacy of the construct *LmddA*-142 (LmddA-LLO-PSA) was determined using a prostrate adenocarcinoma cell line engineered to express PSA (Tramp-CI-PSA (TPSA); Shahabi et al., 2008). Mice were subcutaneously implanted with 2 x 10^6 TPSA cells. When tumors reached the palpable size of 4-6 mm, on day 6 after tumor inoculation, mice were immunized three times at one week intervals with 10^8 CFU LmddA-142, 10^7 CFU Lm-LLO-PSA (positive control) or left untreated. The naive mice developed tumors gradually (Figure 6A). The mice immunized with LmddA-142 were all tumor-free until day 35 and gradually 3 out of 8 mice developed tumors, which grew at a much slower rate as compared to the naive mice (Figure 6B). Five out of eight mice remained tumor free through day 70. As expected, Lm-LLO-PSA-vaccinated mice had fewer tumors than naive controls and tumors developed more slowly than in controls (Figure 6C). Thus, the construct LmddA-LLO-PSA could regress 60 % of the tumors established by TPSA cell line and slow the growth of tumors in other mice. Cured mice that remained tumor free were rechallenged with TPSA tumors on day 68.

[0287] Immunization of mice with the *LmddA*-142 can control the growth and induce regression of 7-day established Tramp-CI tumors that were engineered to express PSA in more than 60% of the experimental animals (Figure 6B), compared to none in the untreated group (Figure 6A). The *LmddA*-142 was constructed using a highly attenuated vector (*LmddA*) and the plasmid pADV142 (Table 1).

[0288] Further, the ability of PSA-specific CD8 lymphocytes generated by the LmddA-LLO-PSA construct to infiltrate tumors was investigated. Mice were subcutaneously implanted with a mixture of tumors and matrigel followed by two immunizations at seven day intervals with naiive or control (Lm-LLO-E7) *Listeria,* or with LmddA-LLO-PSA. Tumors were excised on day 21 and were analyzed for the population of $CD8^+CD62L^{low}$ $PSA^{tetramer+}$ and $CD4^+$ $CD25^+FoxP3^+$ regulatory T cells infiltrating in the tumors.

[0289] A very low number of $CD8^+CD62L^{low}$ $PSA^{tetramer+}$ tumor infiltrating lymphocytes (TILs) specific for PSA that were present in the both naive and Lm-LLO-E7 control immunized mice was observed. However, there was a 10-30-fold increase in the percentage of PSA-specific $CD8^+CD62L^{low}$ $PSA^{tetramer+}$ TILs in the mice immunized with LmddA-LLO-PSA (Figure 7A). Interestingly, the population of $CD8^+CD62L^{low}$ $PSA^{tetramer+}$ cells in spleen was 7.5 fold less than in tumor (Figure 7A).

[0290] In addition, the presence of $CD4^+/CD25^+/Foxp3^+$ T regulatory cells (regs) in the tumors of untreated mice and *Listeria* immunized mice was determined. Interestingly, immunization with *Listeria* resulted in a considerable decrease in the number of $CD4^+$ $CD25^+FoxP3^+$ T-regs in tumor but not in spleen (Figure 7B). However, the construct LmddA-LLO-PSA had a stronger impact in decreasing the frequency of $CD4^+$ $CD25^+FoxP3^+$ T-regs in tumors when compared to the naive and Lm-LLO-E7 immunized group (Figure 7B).

[0291] Thus, the *LmddA*-142 vaccine can induce PSA-specific $CD8^+$ T cells that are able to infiltrate the tumor site (Figure 7A). Interestingly, Immunization with *LmddA*-142 was associated with a decreased number of regulatory T cells in the tumor (Figure 7B), probably creating a more favorable environment for an efficient anti-tumor CTL activity.

EXAMPLE 7: *Lmdd*-143 and *LmddA*-143 secretes a functional LLO despite the PSA fusion.

[0292] The *Lmdd*-143 and *LmddA*-143 contain the full-length human *klk3* gene, which encodes the PSA protein, inserted by homologous recombination downstream and in frame with the *hly* gene in the chromosome. These constructs were made by homologous recombination using the pKSV7 plasmid (Smith and Youngman, Biochimie. 1992; 74 (7-8) p705-711), which has a temperature-sensitive replicon, carrying the *hly-klk3-mpl* recombination cassette. Because of the plasmid excision after the second recombination event, the antibiotic resistance marker used for integration selection is lost. Additionally, the *actA* gene is deleted in the *LmddA*-143 strain (Figure 8A). The insertion of *klk3* in frame with *hly* into the chromosome was verified by PCR (Figure 8B) and sequencing (data not shown) in both constructs.

[0293] One important aspect of these chromosomal constructs is that the production of LLO-PSA would not completely abolish the function of LLO, which is required for escape of Listeria from the phagosome, cytosol invasion and efficient immunity generated by *L. monocytogenes*. Western-blot analysis of secreted proteins from *Lmdd*-143 and *LmddA*-143 culture supernatants revealed an ~81 kDa band corresponding to the LLO-PSA fusion protein and an -60 kDa band, which is the expected size of LLO (Figure 9A), indicating that LLO is either cleaved from the LLO-PSA fusion or still produced as a single protein by *L. monocytogenes,* despite the fusion gene in the chromosome. The LLO secreted by *Lmdd*-143 and *LmddA*-143 retained 50% of the hemolytic activity, as compared to the wild-type *L. monocytogenes* 10403S (Figure 9B). In agreement with these results, both *Lmdd*-143 and *LmddA*-143 were able to replicate intracellularly in the macrophage-like J774 cell line (Figure 9C).

EXAMPLE 8: Both *Lmdd*-143 and *LmddA*-143 elicit cell-mediated immune responses against the PSA antigen.

[0294] After showing that both *Lmdd*-143 and *LmddA*-143 are able to secrete PSA fused to LLO, we investigated if these strains could elicit PSA-specific immune responses *in vivo*. C57B1/6 mice were either left untreated or immunized twice with the *Lmdd*-143, *LmddA*-143 or *LmddA*-142. PSA-specific $CD8^+$ T cell responses were measured by stimulating splenocytes with the PSA_{65-74} peptide and intracellular staining for IFN-γ. As shown in Figure 10, the immune response induced by the chromosomal and the plasmid-based vectors is similar.

EXAMPLE 9: A recombinant *Lm* strain secreting a LLO-HMW-MAA fusion protein results in a broad antitumor response.

[0295] Three *Lm*-based vaccines expressing distinct HMW-MAA fragments based on the position of previously mapped and predicted HLA-A2 epitopes were designed (Figure 11A). The L_m-tLLO-HMW-$MMA_{2160-2258}$ (also referred as *Lm*-LLO-HMW-MAA-C) is based on the avirulent *Lm* XFL-7 strain and a pGG55-based plasmid. This strain secretes a ~62 kDa band corresponding to the tLLO-HMW-$MAA_{2160-2258}$ fusion protein (Figure 11B). The secretion of tLLO-HMW-$MAA_{2160-2258}$ is relatively weak likely due to the high hydrophobicity of this fragment, which corresponds to the HMW-MAA transmembrane domain. Using B16F10 melanoma cells transfected with the full-length HMW-MAA gene, we observed that up to 62.5% of the mice immunized with the *Lm*-LLO-HMW-MAA-C could impede the growth of established tumors (Figure 11C). This result shows that HMW-MAA can be used as a target antigen in vaccination strategies. Interestingly, we also observed that immunization of mice with *Lm*-LLO-HMW-MAA-C significantly impaired the growth of tumors not engineered to express HMW-MAA, such as B16F10, RENCA and NT-2 (Figure 11D), which were derived from distinct mouse strains. In the NT-2 tumor model, which is a mammary carcinoma cell line expressing the rat HER-2/neu protein and is derived from the FVB/N transgenic mice, immunization with *Lm*-LLO-HMW-MAA-C 7 days after tumor inoculation not only impaired tumor growth but also induced regression of the tumor in 1 out of 5 mice (Figure 11D).

EXAMPLE 10: Immunization of mice with *Lm*-LLO-HMW-MAA-C induces infiltration of the tumor stroma by $CD8^+$ T cells and a significant reduction in the pericyte coverage in the tumor vasculature.

[0296] Although NT-2 cells do not express the HMW-MAA homolog NG2, immunization of FVB/N mice with *Lm*-LLO-HMW-MAA-C significantly impaired the growth of NT-2 tumors and eventually led to tumor regression (Figure 11D). This tumor model was used to evaluate $CD8^+$ T cells and pericytes in the tumor site by immunofluorescence. Staining of NT-2 tumor sections for CD8 showed infiltration of $CD8^+$ T cells into the tumors and around blood vessels in mice immunized with the *Lm*-LLO-HMW-MAA-C vaccine, but not in mice immunized with the control vaccine (Figure 12A). Pericytes in NT-2 tumors were also analyzed by double staining with αSMA and NG2 (murine homolog of HMW-MAA) antibodies. Data analysis from three independent NT-2 tumors showed a significant decrease in the number of pericytes in mice immunized with *Lm*-LLO-HMW-MAA-C, as compared to control ($P \leq 0.05$) (Figure 12B). Similar results were obtained when the analysis was restricted to cells stained for αSMA, which is not targeted by the vaccine (data not shown). Thus, *Lm*-LLO-HMW-MAA-C vaccination impacts blood vessel formation in the tumor site by targeting pericytes.

EXAMPLE 11: Development of a recombinant *L. monocytogenes* vector with enhanced anti-tumor activity by concomitant expression and secretion of LLO-PSA and tLLO-HMW-$MAA_{2160-2258}$ fusion proteins, eliciting immune responses to both heterologous antigens.

Materials and Methods:

[0297] Construction of the pADV168 plasmid. The HMW-MAA-C fragment is excised from a pCR2.1-HMW-$MAA_{2160-2258}$ plasmid by double digestion with XhoI and XmaI restriction endonucleases. This fragment is cloned in the pADV134 plasmid already digested with XhoI and XmaI to excise the E7 gene. The pADV168 plasmid is electroporated into electrocompetent the $dal^{(-)}$ $dat^{(-)}$ *E. coli* strain MB2159 and positive clones screened for RFLP and sequence analysis.

[0298] Construction of *Lmdd*-143/168, *LmddA*-143/168 and the control strains *LmddA*-168, *Lmdd*-143/134 and *LmddA*-143/134. *Lmdd, Lmdd*-143 and *LmddA*-143 is transformed with either pADV168 or pADV134 plasmid. Transformants are selected on Brain-Heart Infusion-agar plates supplemented with streptomycin (250 μg/ml) and without D-alanine (BHIs medium). Individual clones are screened for LLO-PSA, tLLO-HMW-$MAA_{2160-2258}$ and tLLO-E7 secretion in bacterial culture supernatants by Western-blot using an anti-LLO, anti-PSA or anti-E7 antibody. A selected clone from each strain will be evaluated for *in vitro* and *in vivo* virulence. Each strain is passaged twice *in vivo* to select the most stable recombinant clones. Briefly, a selected clone from each construct is grown and injected i.p to a group of 4 mice at $1x10^8$ CFU/mouse. Spleens are harvested on days 1 and 3, homogenized and plated on BHIs-agar plates. After the first passage, one colony from each strain is selected and passaged *in vivo* for a second time. To prevent further

attenuation of the vector, to a level impairing its viability, constructs in two vectors with distinct attenuation levels (*Lmdd*-143/168, *LmddA*-143/168) are generated.

[0299] ***In vitro* virulence determination by intracellular replication in J774 cells.** Uptake of *Lm* by macrophages, followed by cytosolic invasion and intracellular proliferation are required for successful antigen delivery and presentation by *Lm*-based vaccines. An *in vitro* invasion assay, using a macrophage-like J774 cell line is used to test these properties in new recombinant *Lm* strains. Briefly, J774 cells are infected for 1 hour in medium without antibiotics at MOI of 1:1 with either the control wild-type *Lm* strain 10403S or the new *Lm* strains to be tested. Extracellular bacteria are killed by 1 hour incubation in medium 10 μg/ml of gentamicin. Samples are harvested at regular intervals and cells lysed with water. Ten-fold serial dilutions of the lysates are plated in duplicates on BHIs plates and colony-forming units (CFU) counted in each sample.

[0300] ***In vivo* virulence studies.** Groups of four C57BL/6 mice (7 weeks old) are injected i.p. with two different doses (1 x 10^8 and 1 x 10^9 CFUs/dose) of *Lmdd*-143/168, *LmddA*-143/168, *LmddA*-168, *Lmdd*-143/134 or *LmddA*-143/134 strains. Mice are followed-up for 2 weeks for survival and LD_{50} estimation. An LD_{50} of >1 x 10^8 constitutes an acceptable value based on previous experience with other *Lm*-based vaccines.

RESULTS

[0301] Once the pADV168 plasmid is successfully constructed, it is sequenced for the presence of the correct HMW-MAA sequence. This plasmid in these new strains express and secrete the LLO fusion proteins specific for each construct. These strains are highly attenuated, with an LD50 of at least 1x10^8 CFU and likely higher than 1x10^9 CFU for the actA-deficient (*LmddA*) strains, which lack the actA gene and consequently the ability of cell-to-cell spread. The construct is tested and the one that has a better balance between attenuation and therapeutic efficacy is selected.

EXAMPLE 12: Detection of immune responses and anti-tumor effects elicited upon immunization with *Lmdd*-143/168 and *LmddA*-143/168.

[0302] Immune responses to PSA and HMW-MAA are studied in mice upon immunization with *Lmdd*-143/168 and *LmddA*-143/168 strains using standard methods, such as detection of IFN-γ production and specific CTL activity against these antigens. The therapeutic efficacy of dual-expression vectors are tested in the TPSA23 tumor model.

[0303] **Intracellular cytokine staining for IFN-γ.** C57BL/6 mice (3 mice per treatment group) are immunized twice at 1-week intervals with the *Lmdd*-143/168 and *LmddA*-143/168 strains. As controls for this experiment, mice are immunized with *Lmdd*-143, *LmddA*-143, *LmddA*-142, *LmddA*-168, *Lmdd*-143/134, *LmddA*-143/134 or left untreated (naive group). Spleens are harvested after 7 days and a single cell suspension of splenocytes are prepared. These splenocytes are plated at 2x10^6 cells/well in a round bottom 96-well plate, in freshly prepared complete RPMI medium with IL-2 (50U/ml) and stimulated with either the PSA H-2Db peptide, HCIRNKSVIL, (SEQ ID NO: 53), or the HPV16 E7 H-2Db control peptide RAHYNIVTF (SEQ ID NO: 54) at a final concentration of 1μM. Since HMW-MAA-epitopes have not been mapped in the C57B1/6 mouse, HMW-MAA-specific immune responses are detected by incubating 2x10^6 splenocytes with 2x10^5 EL4-HMW-MAA cells. The cells are incubated for 5 hours in the presence of monensin to retain the intracellular IFN-γ in the cells. After incubation, cells are stained with anti-mouse CD8-FITC, CD3-PerCP, CD62L-APC antibodies. They are then permeabilized and stained for IFNγ-PE and analyzed in a four-color FACS Calibur (BD Biosciences).

[0304] **Cytotoxicity assay.** To investigate the effector activity of the PSA and HMW-MAA specific T cells generated upon vaccinations, isolated splenocytes are incubated for 5 days in complete RPMI medium containing 20 U/ml of mouse IL-2 (Sigma), in the presence of stimulator cells (mitomycin C treated MC57G cells infected with either PSA or HMW-MAA vaccinia). For the cytotoxicity assay, EL4 target cells are labeled for 15 minutes with DDAO-SE (0.6 μM) (Molecular Probes) and washed twice with complete medium. The labeled target cells are pulsed for 1 hour with either the PSA H-2Db peptide, or the HPV16 E7 H-2Db control peptide, at a final concentration of 5μM. For HMW-MAA-specific cytotoxic responses, the EL4-HMW-MAA cells are used as targets. The cytotoxicity assay is performed for 2 hours by incubating the target cells (T) with effector cells (E) at different E:T ratios for 2-3 hours. Cells are fixed with formalin, permeabilized and stained for cleaved caspase-3 to detect induction of apoptosis in the target cells.

[0305] **Anti-tumor efficacy.** The anti-tumor efficacy of the *Lmdd*-143/168 and *LmddA*-143/168 strains are compared to that of *LmddA*-142 and *LmddA*-168, using the T-PSA23 tumor model (TrampC-1/PSA). Groups of 8 male C57BL/6 mice (6-8 weeks old) are inoculated s.c. with 2 x 10^6 T-PSA23 cells and 7 days later immunized i.p. with 0.1 x LD50 dose of *Lmdd*-143/168, *LmddA*-143/168, *LmddA*-142 and *LmddA*-168. As controls, mice are either left untreated or immunized with an *Lm* control strain (*LmddA*-134). Each group receives two additional doses of the vaccines with 7 day intervals. Tumors are monitored for 60 days or until they reach a size of 2 cm, at which point mice are sacrificed.

RESULTS

[0306] Immunization of mice with *LmddA*-168 results in the induction of specific responses against HMW-MAA. Similarly, *Lmdd*-143/168 and *LmddA*-143/168 elicits an immune response against PSA and HMW-MAA that is comparable to the immune responses generated by *L. monocytogenes* vectors expressing each antigen individually. Immunization of T-PSA-23-bearing mice with the *Lmdd*-143/168 and *LmddA*-143/168 results in a better anti-tumor therapeutic efficacy than the immunization with either *LmddA*-142 or *LmddA*-168.

EXAMPLE 13: Immunization with either *Lmdd*-143/168 or *LmddA*-143/168 results in pericyte destruction, up-regulation of adhesion molecules in endothelial cells and enhanced infiltration of TILs specific for PSA.

[0307] Characterization of tumor infiltrating lymphocytes and endothelial cell-adhesion molecules induced upon immunization with *Lmdd*-143/168 or *LmddA*-143/168. The tumors from mice immunized with either *Lmdd*-143/168 or *LmddA*-143/168 are analyzed by immunofluorescence to study expression of adhesion molecules by endothelial cells, blood vessel density and pericyte coverage in the tumor vasculature, as well as infiltration of the tumor by immune cells, including CD8 and CD4 T cells. TILs specific for the PSA antigen are characterized by tetramer analysis and functional tests.

[0308] Analysis of tumor infiltrating lymphocytes (TILs). TPSA23 cells embedded in matrigel are inoculated s.c in mice (n=3 per group), which are immunized on days 7 and 14 with either *Lmdd*-143/168 or *LmddA*-143/168, depending on which one is the more effective according to results obtained in anti-tumor studies. For comparison, mice are immunized with *LmddA*-142, *LmddA*-168, a control *Lm* vaccine or left untreated. On day 21, the tumors are surgically excised, washed in ice-cold PBS and minced with a scalpel. The tumors are treated with dispase to solubilize the Matrigel and release single cells for analysis. PSA-specific $CD8^+$ T cells are stained with a PSA65-74 H-2Db tetramer-PE and anti-mouse CD8-FITC, CD3-PerCP-Cy5.5 and CD62L-APC antibodies. To analyze regulatory T cell in the tumor, TILs are stained with CD4-FITC, CD3-PerCP-Cy5.5 and CD25-APC and subsequently permeabilized for FoxP3 staining (anti-FoxP3-PE, Milteny Biotec). Cells are analyzed by a FACS Calibur cytometer and CellQuestPro software (BD Biosciences).

[0309] Immunofluorescence. On day 21 post tumor inoculation, the TPSA23 tumors embedded in matrigel are surgically excised and a fragment immediately cryopreserved in OCT freezing medium. The tumor fragments are cryosectioned for 8-10μm thick sections. For immunofluorescence, samples are thawed and fixed using 4% formalin. After blocking, sections are stained with antibodies in blocking solution in a humidified chamber at 37°C for 1 hour. DAPI (Invitrogen) staining are performed according to manufacturer instructions. For intracellular stains (αSMA), incubation is performed in PBS / 0.1% Tween / 1% BSA solution. Slides are cover-slipped using a mounting solution (Biomeda) with anti-fading agents, set for 24 hours and kept at 4°C until imaging using Spot Image Software (2006) and BX51 series Olympus fluorescent microscope. CD8, CD4, FoxP3, αSMA, NG2, CD31, ICAM-1, VCAM-1 and VAP-1 are evaluated by immunofluorescence.

[0310] Statistical analysis: Non-parametric Mann-Whitney and Kruskal-Wallis tests are applied to compare tumor sizes among different treatment groups. Tumor sizes are compared at the latest time-point with the highest number of mice in each group (8 mice). A p-value of less than 0.05 is considered statistically significant in these analyses.

RESULTS

[0311] Immunization of TPSA23-bearing mice with the *Lmdd*-143/168 and *LmddA*-143/168 results in higher numbers of effector TILs specific to PSA and also decreases pericyte coverage of the tumor vasculature. Further, cell-adhesion markers are significantly up-regulated in immunized mice.

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<211> 416
<212> PRT
<213> Listeria monocytogenes

<400> 11

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

Pro Ile Ala Gln Gln Thr Glu Ala Lys Asp Ala Ser Ala Phe Asn Lys
            20                  25                  30

Glu Asn Ser Ile Ser Ser Val Ala Pro Pro Ala Ser Pro Pro Ala Ser
        35                  40                  45

Pro Lys Thr Pro Ile Glu Lys Lys His Ala Asp Glu Ile Asp Lys Tyr
    50                  55                  60

Ile Gln Gly Leu Asp Tyr Asn Lys Asn Asn Val Leu Val Tyr His Gly
65                  70                  75                  80

Asp Ala Val Thr Asn Val Pro Pro Arg Lys Gly Tyr Lys Asp Gly Asn
                85                  90                  95

Glu Tyr Ile Val Val Glu Lys Lys Lys Lys Ser Ile Asn Gln Asn Asn
            100                 105                 110

Ala Asp Ile Gln Val Val Asn Ala Ile Ser Ser Leu Thr Tyr Pro Gly
        115                 120                 125

Ala Leu Val Lys Ala Asn Ser Glu Leu Val Glu Asn Gln Pro Asp Val
    130                 135                 140
```

```
Leu Pro Val Lys Arg Asp Ser Leu Thr Leu Ser Ile Asp Leu Pro Gly
145                 150                 155                 160

Met Thr Asn Gln Asp Asn Lys Ile Val Val Lys Asn Ala Thr Lys Ser
                165                 170                 175

Asn Val Asn Asn Ala Val Asn Thr Leu Val Glu Arg Trp Asn Glu Lys
            180                 185                 190

Tyr Ala Gln Ala Tyr Ser Asn Val Ser Ala Lys Ile Asp Tyr Asp Asp
        195                 200                 205

Glu Met Ala Tyr Ser Glu Ser Gln Leu Ile Ala Lys Phe Gly Thr Ala
    210                 215                 220

Phe Lys Ala Val Asn Asn Ser Leu Asn Val Asn Phe Gly Ala Ile Ser
225                 230                 235                 240

Glu Gly Lys Met Gln Glu Glu Val Ile Ser Phe Lys Gln Ile Tyr Tyr
                245                 250                 255

Asn Val Asn Val Asn Glu Pro Thr Arg Pro Ser Arg Phe Phe Gly Lys
            260                 265                 270

Ala Val Thr Lys Glu Gln Leu Gln Ala Leu Gly Val Asn Ala Glu Asn
        275                 280                 285

Pro Pro Ala Tyr Ile Ser Ser Val Ala Tyr Gly Arg Gln Val Tyr Leu
    290                 295                 300

Lys Leu Ser Thr Asn Ser His Ser Thr Lys Val Lys Ala Ala Phe Asp
305                 310                 315                 320

Ala Ala Val Ser Gly Lys Ser Val Ser Gly Asp Val Glu Leu Thr Asn
                325                 330                 335

Ile Ile Lys Asn Ser Ser Phe Lys Ala Val Ile Tyr Gly Gly Ser Ala
            340                 345                 350

Lys Asp Glu Val Gln Ile Ile Asp Gly Asn Leu Gly Asp Leu Arg Asp
        355                 360                 365

Ile Leu Lys Lys Gly Ala Thr Phe Asn Arg Glu Thr Pro Gly Val Pro
    370                 375                 380

Ile Ala Tyr Thr Thr Asn Phe Leu Lys Asp Asn Glu Leu Ala Val Ile
385                 390                 395                 400
```

Lys Asn Asn Ser Glu Tyr Ile Glu Thr Thr Ser Lys Ala Tyr Thr Asp
405 410 415

<210> 12
<211> 529
<212> PRT
<213> Listeria monocytogenes

<400> 12

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

Pro Ile Ala Gln Gln Thr Glu Ala Lys Asp Ala Ser Ala Phe Asn Lys
20 25 30

Glu Asn Ser Ile Ser Ser Met Ala Pro Pro Ala Ser Pro Pro Ala Ser
35 40 45

Pro Lys Thr Pro Ile Glu Lys Lys His Ala Asp Glu Ile Asp Lys Tyr
50 55 60

Ile Gln Gly Leu Asp Tyr Asn Lys Asn Asn Val Leu Val Tyr His Gly
65 70 75 80

Asp Ala Val Thr Asn Val Pro Pro Arg Lys Gly Tyr Lys Asp Gly Asn
85 90 95

Glu Tyr Ile Val Val Glu Lys Lys Lys Lys Ser Ile Asn Gln Asn Asn
100 105 110

Ala Asp Ile Gln Val Val Asn Ala Ile Ser Ser Leu Thr Tyr Pro Gly
115 120 125

Ala Leu Val Lys Ala Asn Ser Glu Leu Val Glu Asn Gln Pro Asp Val
130 135 140

Leu Pro Val Lys Arg Asp Ser Leu Thr Leu Ser Ile Asp Leu Pro Gly
145 150 155 160

Met Thr Asn Gln Asp Asn Lys Ile Val Val Lys Asn Ala Thr Lys Ser
165 170 175

Asn Val Asn Asn Ala Val Asn Thr Leu Val Glu Arg Trp Asn Glu Lys
180 185 190

Tyr Ala Gln Ala Tyr Pro Asn Val Ser Ala Lys Ile Asp Tyr Asp Asp
195 200 205

```
Glu Met Ala Tyr Ser Glu Ser Gln Leu Ile Ala Lys Phe Gly Thr Ala
    210                 215                 220

Phe Lys Ala Val Asn Asn Ser Leu Asn Val Asn Phe Gly Ala Ile Ser
225                 230                 235                 240

Glu Gly Lys Met Gln Glu Glu Val Ile Ser Phe Lys Gln Ile Tyr Tyr
                245                 250                 255

Asn Val Asn Val Asn Glu Pro Thr Arg Pro Ser Arg Phe Phe Gly Lys
            260                 265                 270

Ala Val Thr Lys Glu Gln Leu Gln Ala Leu Gly Val Asn Ala Glu Asn
        275                 280                 285

Pro Pro Ala Tyr Ile Ser Ser Val Ala Tyr Gly Arg Gln Val Tyr Leu
    290                 295                 300

Lys Leu Ser Thr Asn Ser His Ser Thr Lys Val Lys Ala Ala Phe Asp
305                 310                 315                 320

Ala Ala Val Ser Gly Lys Ser Val Ser Gly Asp Val Glu Leu Thr Asn
                325                 330                 335

Ile Ile Lys Asn Ser Ser Phe Lys Ala Val Ile Tyr Gly Gly Ser Ala
            340                 345                 350

Lys Asp Glu Val Gln Ile Ile Asp Gly Asn Leu Gly Asp Leu Arg Asp
        355                 360                 365

Ile Leu Lys Lys Gly Ala Thr Phe Asn Arg Glu Thr Pro Gly Val Pro
    370                 375                 380

Ile Ala Tyr Thr Thr Asn Phe Leu Lys Asp Asn Glu Leu Ala Val Ile
385                 390                 395                 400

Lys Asn Asn Ser Glu Tyr Ile Glu Thr Thr Ser Lys Ala Tyr Thr Asp
                405                 410                 415

Gly Lys Ile Asn Ile Asp His Ser Gly Gly Tyr Val Ala Gln Phe Asn
            420                 425                 430

Ile Ser Trp Asp Glu Val Asn Tyr Asp Pro Glu Gly Asn Glu Ile Val
        435                 440                 445

Gln His Lys Asn Trp Ser Glu Asn Asn Lys Ser Lys Leu Ala His Phe
    450                 455                 460
```

```
Thr Ser Ser Ile Tyr Leu Pro Gly Asn Ala Arg Asn Ile Asn Val Tyr
465                 470                 475                 480

Ala Lys Glu Cys Thr Gly Leu Ala Trp Glu Trp Trp Arg Thr Val Ile
                485                 490                 495

Asp Asp Arg Asn Leu Pro Leu Val Lys Asn Arg Asn Ile Ser Ile Trp
            500                 505                 510

Gly Thr Thr Leu Tyr Pro Lys Tyr Ser Asn Lys Val Asp Asn Pro Ile
        515                 520                 525

Glu
```

<210> 13
<211> 416
<212> PRT
<213> Listeria monocytogenes

<400> 13

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

Pro Ile Ala Gln Gln Thr Glu Ala Lys Asp Ala Ser Ala Phe Asn Lys
            20                  25                  30

Glu Asn Ser Ile Ser Ser Val Ala Pro Pro Ala Ser Pro Pro Ala Ser
        35                  40                  45

Pro Lys Thr Pro Ile Glu Lys Lys His Ala Asp Glu Ile Asp Lys Tyr
    50                  55                  60

Ile Gln Gly Leu Asp Tyr Asn Lys Asn Asn Val Leu Val Tyr His Gly
65                  70                  75                  80

Asp Ala Val Thr Asn Val Pro Pro Arg Lys Gly Tyr Lys Asp Gly Asn
                85                  90                  95

Glu Tyr Ile Val Val Glu Lys Lys Lys Lys Ser Ile Asn Gln Asn Asn
            100                 105                 110

Ala Asp Ile Gln Val Val Asn Ala Ile Ser Ser Leu Thr Tyr Pro Gly
        115                 120                 125

Ala Leu Val Lys Ala Asn Ser Glu Leu Val Glu Asn Gln Pro Asp Val
    130                 135                 140
```

```
Leu Pro Val Lys Arg Asp Ser Leu Thr Leu Ser Ile Asp Leu Pro Gly
145                 150                 155                 160

Met Thr Asn Gln Asp Asn Lys Ile Val Val Lys Asn Ala Thr Lys Ser
                165                 170                 175

Asn Val Asn Asn Ala Val Asn Thr Leu Val Glu Arg Trp Asn Glu Lys
            180                 185                 190

Tyr Ala Gln Ala Tyr Ser Asn Val Ser Ala Lys Ile Asp Tyr Asp Asp
        195                 200                 205

Glu Met Ala Tyr Ser Glu Ser Gln Leu Ile Ala Lys Phe Gly Thr Ala
    210                 215                 220

Phe Lys Ala Val Asn Asn Ser Leu Asn Val Asn Phe Gly Ala Ile Ser
225                 230                 235                 240

Glu Gly Lys Met Gln Glu Glu Val Ile Ser Phe Lys Gln Ile Tyr Tyr
                245                 250                 255

Asn Val Asn Val Asn Glu Pro Thr Arg Pro Ser Arg Phe Phe Gly Lys
            260                 265                 270

Ala Val Thr Lys Glu Gln Leu Gln Ala Leu Gly Val Asn Ala Glu Asn
        275                 280                 285

Pro Pro Ala Tyr Ile Ser Ser Val Ala Tyr Gly Arg Gln Val Tyr Leu
    290                 295                 300

Lys Leu Ser Thr Asn Ser His Ser Thr Lys Val Lys Ala Ala Phe Asp
305                 310                 315                 320

Ala Ala Val Ser Gly Lys Ser Val Ser Gly Asp Val Glu Leu Thr Asn
                325                 330                 335

Ile Ile Lys Asn Ser Ser Phe Lys Ala Val Ile Tyr Gly Gly Ser Ala
            340                 345                 350

Lys Asp Glu Val Gln Ile Ile Asp Gly Asn Leu Gly Asp Leu Arg Asp
        355                 360                 365

Ile Leu Lys Lys Gly Ala Thr Phe Asn Arg Glu Thr Pro Gly Val Pro
    370                 375                 380

Ile Ala Tyr Thr Thr Asn Phe Leu Lys Asp Asn Glu Leu Ala Val Ile
```

385 390 395 400

Lys Asn Asn Ser Glu Tyr Ile Glu Thr Thr Ser Lys Ala Tyr Thr Asp
405 410 415

<210> 14
<211> 390
<212> PRT
<213> Listeria monocytogenes

<400> 14

```
Met Arg Ala Met Met Val Val Phe Ile Thr Ala Asn Cys Ile Thr Ile
1               5                  10                  15

Asn Pro Asp Ile Ile Phe Ala Ala Thr Asp Ser Glu Asp Ser Ser Leu
            20                  25                  30

Asn Thr Asp Glu Trp Glu Glu Glu Lys Thr Glu Glu Gln Pro Ser Glu
        35                  40                  45

Val Asn Thr Gly Pro Arg Tyr Glu Thr Ala Arg Glu Val Ser Ser Arg
    50                  55                  60

Asp Ile Lys Glu Leu Glu Lys Ser Asn Lys Val Arg Asn Thr Asn Lys
65                  70                  75                  80

Ala Asp Leu Ile Ala Met Leu Lys Glu Lys Ala Glu Lys Gly Pro Asn
                85                  90                  95

Ile Asn Asn Asn Asn Ser Glu Gln Thr Glu Asn Ala Ala Ile Asn Glu
            100                 105                 110

Glu Ala Ser Gly Ala Asp Arg Pro Ala Ile Gln Val Glu Arg Arg His
        115                 120                 125

Pro Gly Leu Pro Ser Asp Ser Ala Ala Glu Ile Lys Lys Arg Arg Lys
    130                 135                 140

Ala Ile Ala Ser Ser Asp Ser Glu Leu Glu Ser Leu Thr Tyr Pro Asp
145                 150                 155                 160

Lys Pro Thr Lys Val Asn Lys Lys Lys Val Ala Lys Glu Ser Val Ala
                165                 170                 175

Asp Ala Ser Glu Ser Asp Leu Asp Ser Ser Met Gln Ser Ala Asp Glu
            180                 185                 190

Ser Ser Pro Gln Pro Leu Lys Ala Asn Gln Gln Pro Phe Phe Pro Lys
```

```
                195                 200                 205

Val Phe Lys Lys Ile Lys Asp Ala Gly Lys Trp Val Arg Asp Lys Ile
    210                 215                 220

Asp Glu Asn Pro Glu Val Lys Lys Ala Ile Val Asp Lys Ser Ala Gly
225                 230                 235                 240

Leu Ile Asp Gln Leu Leu Thr Lys Lys Lys Ser Glu Glu Val Asn Ala
                245                 250                 255

Ser Asp Phe Pro Pro Pro Pro Thr Asp Glu Glu Leu Arg Leu Ala Leu
            260                 265                 270

Pro Glu Thr Pro Met Leu Leu Gly Phe Asn Ala Pro Ala Thr Ser Glu
        275                 280                 285

Pro Ser Ser Phe Glu Phe Pro Pro Pro Pro Thr Asp Glu Glu Leu Arg
    290                 295                 300

Leu Ala Leu Pro Glu Thr Pro Met Leu Leu Gly Phe Asn Ala Pro Ala
305                 310                 315                 320

Thr Ser Glu Pro Ser Ser Phe Glu Phe Pro Pro Pro Pro Thr Glu Asp
                325                 330                 335

Glu Leu Glu Ile Ile Arg Glu Thr Ala Ser Ser Leu Asp Ser Ser Phe
            340                 345                 350

Thr Arg Gly Asp Leu Ala Ser Leu Arg Asn Ala Ile Asn Arg His Ser
        355                 360                 365

Gln Asn Phe Ser Asp Phe Pro Pro Ile Pro Thr Glu Glu Glu Leu Asn
    370                 375                 380

Gly Arg Gly Gly Arg Pro
385                 390
```

<210> 15
<211> 1200
<212> DNA
<213> Listeria monocytogenes

<400> 15

atgcgtgcga tgatggtggt tttcattact gccaattgca ttacgattaa ccccgacata 60

atatttgcag cgacagatag cgaagattct agtctaaaca cagatgaatg ggaagaagaa 120

aaaacagaag agcaaccaag cgaggtaaat acgggaccaa gatacgaaac tgcacgtgaa 180

gtaagttcac gtgatattaa agaactagaa aaatcgaata aagtgagaaa tacgaacaaa 240

gcagacctaa tagcaatgtt gaaagaaaaa gcagaaaaag gtccaaatat caataataac 300

aacagtgaac aaactgagaa tgcggctata aatgaagagg cttcaggagc cgaccgacca 360

gctatacaag tggagcgtcg tcatccagga ttgccatcgg atagcgcagc ggaaattaaa 420

aaaagaagga aagccatagc atcatcggat agtgagcttg aaagccttac ttatccggat 480

aaaccaacaa aagtaaataa gaaaaaagtg gcgaaagagt cagttgcgga tgcttctgaa 540

agtgacttag attctagcat gcagtcagca gatgagtctt caccacaacc tttaaaagca 600

aaccaacaac catttttccc taaagtattt aaaaaaataa aagatgcggg gaaatgggta 660

cgtgataaaa tcgacgaaaa tcctgaagta aagaaagcga ttgttgataa aagtgcaggg 720

ttaattgacc aattattaac caaaaagaaa agtgaagagg taaatgcttc ggacttcccg 780

ccaccaccta cggatgaaga gttaagactt gctttgccag agacaccaat gcttcttggt 840

tttaatgctc ctgctacatc agaaccgagc tcattcgaat ttccaccacc acctacggat 900

gaagagttaa gacttgcttt gccagagacg ccaatgcttc ttggttttaa tgctcctgct 960

acatcggaac cgagctcgtt cgaatttcca ccgcctccaa cagaagatga actagaaatc 1020

atccgggaaa cagcatcctc gctagattct agttttacaa gaggggattt agctagtttg 1080

agaaatgcta ttaatcgcca tagtcaaaat ttctctgatt tcccaccaat cccaacagaa 1140

gaagagttga acgggagagg cggtagacca gaagagttga acgggagagg cggtagacca 1200

<210> 16
<211> 390
<212> PRT
<213> Listeria monocytogenes

<400> 16

```
Met Arg Ala Met Met Val Val Phe Ile Thr Ala Asn Cys Ile Thr Ile
1               5                  10                  15

Asn Pro Asp Ile Ile Phe Ala Ala Thr Asp Ser Glu Asp Ser Ser Leu
            20                  25                  30

Asn Thr Asp Glu Trp Glu Glu Glu Lys Thr Glu Glu Gln Pro Ser Glu
        35                  40                  45

Val Asn Thr Gly Pro Arg Tyr Glu Thr Ala Arg Glu Val Ser Ser Arg
    50                  55                  60

Asp Ile Glu Glu Leu Glu Lys Ser Asn Lys Val Lys Asn Thr Asn Lys
65                  70                  75                  80

Ala Asp Leu Ile Ala Met Leu Lys Ala Lys Ala Glu Lys Gly Pro Asn
```

85 90 95

Asn Asn Asn Asn Asn Gly Glu Gln Thr Gly Asn Val Ala Ile Asn Glu
100 105 110

Glu Ala Ser Gly Val Asp Arg Pro Thr Leu Gln Val Glu Arg Arg His
115 120 125

Pro Gly Leu Ser Ser Asp Ser Ala Ala Glu Ile Lys Lys Arg Arg Lys
130 135 140

Ala Ile Ala Ser Ser Asp Ser Glu Leu Glu Ser Leu Thr Tyr Pro Asp
145 150 155 160

Lys Pro Thr Lys Ala Asn Lys Arg Lys Val Ala Lys Glu Ser Val Val
165 170 175

Asp Ala Ser Glu Ser Asp Leu Asp Ser Ser Met Gln Ser Ala Asp Glu
180 185 190

Ser Thr Pro Gln Pro Leu Lys Ala Asn Gln Lys Pro Phe Phe Pro Lys
195 200 205

Val Phe Lys Lys Ile Lys Asp Ala Gly Lys Trp Val Arg Asp Lys Ile
210 215 220

Asp Glu Asn Pro Glu Val Lys Lys Ala Ile Val Asp Lys Ser Ala Gly
225 230 235 240

Leu Ile Asp Gln Leu Leu Thr Lys Lys Lys Ser Glu Glu Val Asn Ala
245 250 255

Ser Asp Phe Pro Pro Pro Pro Thr Asp Glu Glu Leu Arg Leu Ala Leu
260 265 270

Pro Glu Thr Pro Met Leu Leu Gly Phe Asn Ala Pro Thr Pro Ser Glu
275 280 285

Pro Ser Ser Phe Glu Phe Pro Pro Pro Pro Thr Asp Glu Glu Leu Arg
290 295 300

Leu Ala Leu Pro Glu Thr Pro Met Leu Leu Gly Phe Asn Ala Pro Ala
305 310 315 320

Thr Ser Glu Pro Ser Ser Phe Glu Phe Pro Pro Pro Pro Thr Glu Asp
325 330 335

```
Glu Leu Glu Ile Met Arg Glu Thr Ala Pro Ser Leu Asp Ser Ser Phe
            340                 345                 350

Thr Ser Gly Asp Leu Ala Ser Leu Arg Ser Ala Ile Asn Arg His Ser
        355                 360                 365

Glu Asn Phe Ser Asp Phe Pro Leu Ile Pro Thr Glu Glu Glu Leu Asn
    370                 375                 380

Gly Arg Gly Gly Arg Pro
385                 390
```

<210> 17
<211> 1200
<212> DNA
<213> Listeria monocytogenes

<400> 17

atgcgtgcga tgatggtagt tttcattact gccaactgca ttacgattaa ccccgacata 60

atatttgcag cgacagatag cgaagattcc agtctaaaca cagatgaatg ggaagaagaa 120

aaaacagaag agcagccaag cgaggtaaat acgggaccaa gatacgaaac tgcacgtgaa 180

gtaagttcac gtgatattga ggaactagaa aaatcgaata aagtgaaaaa tacgaacaaa 240

gcagacctaa tagcaatgtt gaaagcaaaa gcagagaaag gtccgaataa caataataac 300

aacggtgagc aaacaggaaa tgtggctata aatgaagagg cttcaggagt cgaccgacca 360

actctgcaag tggagcgtcg tcatccaggt ctgtcatcgg atagcgcagc ggaaattaaa 420

aaaagaagaa aagccatagc gtcgtcggat agtgagcttg aaagccttac ttatccagat 480

aaaccaacaa aagcaaataa gagaaaagtg gcgaaagagt cagttgtgga tgcttctgaa 540

agtgacttag attctagcat gcagtcagca gacgagtcta caccacaacc tttaaaagca 600

aatcaaaaac cattttccc taaagtattt aaaaaaataa aagatgcggg gaaatgggta 660

cgtgataaaa tcgacgaaaa tcctgaagta aagaaagcga ttgttgataa aagtgcaggg 720

ttaattgacc aattattaac caaaaagaaa agtgaagagg taaatgcttc ggacttcccg 780

ccaccaccta cggatgaaga gttaagactt gctttgccag agacaccgat gcttctcggt 840

tttaatgctc ctactccatc ggaaccgagc tcattcgaat ttccgccgcc acctacggat 900

gaagagttaa gacttgcttt gccagagacg ccaatgcttc ttggttttaa tgctcctgct 960

acatcggaac cgagctcatt cgaatttcca ccgcctccaa cagaagatga actagaaatt 1020

atgcgggaaa cagcaccttc gctagattct agttttacaa gcggggattt agctagtttg 1080

agaagtgcta ttaatcgcca tagcgaaaat ttctctgatt tcccactaat cccaacagaa 1140

gaagagttga acgggagagg cggtagacca gaagagttga acgggagagg cggtagacca 1200

<210> 18
<211> 1256
<212> DNA
<213> Listeria monocytogenes

<400> 18

gcgccaaatc attggttgat tggtgaggat gtctgtgtgc gtgggtcgcg agatgggcga 60

ataagaagca ttaaagatcc tgacaaatat aatcaagcgg ctcatatgaa agattacgaa 120

tcgcttccac tcacagagga aggcgactgg ggcggagttc attataatag tggtatcccg 180

aataaagcag cctataatac tatcactaaa cttggaaaag aaaaaacaga acagctttat 240

tttcgcgcct taaagtacta tttaacgaaa aaatcccagt ttaccgatgc gaaaaaagcg 300

cttcaacaag cagcgaaaga tttatatggt gaagatgctt ctaaaaaagt tgctgaagct 360

tgggaagcag ttggggttaa ctgattaaca aatgttagag aaaaattaat tctccaagtg 420

atattcttaa aataattcat gaatattttt tcttatatta gctaattaag aagataacta 480

actgctaatc caatttttaa cggaacaaat tagtgaaaat gaaggccgaa ttttccttgt 540

tctaaaaagg ttgtattagc gtatcacgag gagggagtat aagtgggatt aaacagattt 600

atgcgtgcga tgatggtggt tttcattact gccaattgca ttacgattaa ccccgacgtc 660

gacccatacg acgttaattc ttgcaatgtt agctattggc gtgttctctt taggggcgtt 720

tatcaaaatt attcaattaa gaaaaaataa ttaaaaacac agaacgaaag aaaaagtgag 780

gtgaatgata tgaaattcaa aaaggtggtt ctaggtatgt gcttgatcgc aagtgttcta 840

gtctttccgg taacgataaa agcaaatgcc tgttgtgatg aatacttaca aacacccgca 900

gctccgcatg atattgacag caaattacca cataaactta gttggtccgc ggataacccg 960

acaaatactg acgtaaatac gcactattgg ctttttaaac aagcggaaaa aatactagct 1020

aaagatgtaa atcatatgcg agctaattta atgaatgaac ttaaaaaatt cgataaacaa 1080

atagctcaag gaatatatga tgcggatcat aaaaatccat attatgatac tagtacattt 1140

ttatctcatt tttataatcc tgatagagat aatacttatt tgccgggttt tgctaatgcg 1200

aaaataacag gagcaaagta tttcaatcaa tcggtgactg attaccgaga agggaa 1256

<210> 19
<211> 2322
<212> PRT
<213> Homo sapiens

<400> 19

Met Gln Ser Gly Arg Gly Pro Pro Leu Pro Ala Pro Gly Leu Ala Leu
1 5 10 15

Ala Leu Thr Leu Thr Met Leu Ala Arg Leu Ala Ser Ala Ala Ser Phe
20 25 30

```
Phe Gly Glu Asn His Leu Glu Val Pro Val Ala Thr Ala Leu Thr Asp
        35                  40                  45

Ile Asp Leu Gln Leu Gln Phe Ser Thr Ser Gln Pro Glu Ala Leu Leu
    50                  55                  60

Leu Leu Ala Ala Gly Pro Ala Asp His Leu Leu Leu Gln Leu Tyr Ser
65                  70                  75                  80

Gly Arg Leu Gln Val Arg Leu Val Leu Gly Gln Glu Glu Leu Arg Leu
                85                  90                  95

Gln Thr Pro Ala Glu Thr Leu Leu Ser Asp Ser Ile Pro His Thr Val
            100                 105                 110

Val Leu Thr Val Val Glu Gly Trp Ala Thr Leu Ser Val Asp Gly Phe
        115                 120                 125

Leu Asn Ala Ser Ser Ala Val Pro Gly Ala Pro Leu Glu Val Pro Tyr
    130                 135                 140

Gly Leu Phe Val Gly Gly Thr Gly Thr Leu Gly Leu Pro Tyr Leu Arg
145                 150                 155                 160

Gly Thr Ser Arg Pro Leu Arg Gly Cys Leu His Ala Ala Thr Leu Asn
                165                 170                 175

Gly Arg Ser Leu Leu Arg Pro Leu Thr Pro Asp Val His Glu Gly Cys
            180                 185                 190

Ala Glu Glu Phe Ser Ala Ser Asp Asp Val Ala Leu Gly Phe Ser Gly
        195                 200                 205

Pro His Ser Leu Ala Ala Phe Pro Ala Trp Gly Thr Gln Asp Glu Gly
    210                 215                 220

Thr Leu Glu Phe Thr Leu Thr Thr Gln Ser Arg Gln Ala Pro Leu Ala
225                 230                 235                 240

Phe Gln Ala Gly Gly Arg Arg Gly Asp Phe Ile Tyr Val Asp Ile Phe
                245                 250                 255

Glu Gly His Leu Arg Ala Val Val Glu Lys Gly Gln Gly Thr Val Leu
            260                 265                 270

Leu His Asn Ser Val Pro Val Ala Asp Gly Gln Pro His Glu Val Ser
        275                 280                 285
```

```
Val His Ile Asn Ala His Arg Leu Glu Ile Ser Val Asp Gln Tyr Pro
    290                 295                 300

Thr His Thr Ser Asn Arg Gly Val Leu Ser Tyr Leu Glu Pro Arg Gly
305                 310                 315                 320

Ser Leu Leu Leu Gly Gly Leu Asp Ala Glu Ala Ser Arg His Leu Gln
                325                 330                 335

Glu His Arg Leu Gly Leu Thr Pro Glu Ala Thr Asn Ala Ser Leu Leu
            340                 345                 350

Gly Cys Met Glu Asp Leu Ser Val Asn Gly Gln Arg Arg Gly Leu Arg
        355                 360                 365

Glu Ala Leu Leu Thr Arg Asn Met Ala Ala Gly Cys Arg Leu Glu Glu
    370                 375                 380

Glu Glu Tyr Glu Asp Asp Ala Tyr Gly His Tyr Glu Ala Phe Ser Thr
385                 390                 395                 400

Leu Ala Pro Glu Ala Trp Pro Ala Met Glu Leu Pro Glu Pro Cys Val
                405                 410                 415

Pro Glu Pro Gly Leu Pro Pro Val Phe Ala Asn Phe Thr Gln Leu Leu
            420                 425                 430

Thr Ile Ser Pro Leu Val Val Ala Glu Gly Gly Thr Ala Trp Leu Glu
        435                 440                 445

Trp Arg His Val Gln Pro Thr Leu Asp Leu Met Glu Ala Glu Leu Arg
    450                 455                 460

Lys Ser Gln Val Leu Phe Ser Val Thr Arg Gly Ala Arg His Gly Glu
465                 470                 475                 480

Leu Glu Leu Asp Ile Pro Gly Ala Gln Ala Arg Lys Met Phe Thr Leu
                485                 490                 495

Leu Asp Val Val Asn Arg Lys Ala Arg Phe Ile His Asp Gly Ser Glu
            500                 505                 510

Asp Thr Ser Asp Gln Leu Val Leu Glu Val Ser Val Thr Ala Arg Val
        515                 520                 525

Pro Met Pro Ser Cys Leu Arg Arg Gly Gln Thr Tyr Leu Leu Pro Ile
```

530 535 540

Gln Val Asn Pro Val Asn Asp Pro Pro His Ile Ile Phe Pro His Gly
545 550 555 560

Ser Leu Met Val Ile Leu Glu His Thr Gln Lys Pro Leu Gly Pro Glu
565 570 575

Val Phe Gln Ala Tyr Asp Pro Asp Ser Ala Cys Glu Gly Leu Thr Phe
580 585 590

Gln Val Leu Gly Thr Ser Ser Gly Leu Pro Val Glu Arg Arg Asp Gln
595 600 605

Pro Gly Glu Pro Ala Thr Glu Phe Ser Cys Arg Glu Leu Glu Ala Gly
610 615 620

Ser Leu Val Tyr Val His Arg Gly Gly Pro Ala Gln Asp Leu Thr Phe
625 630 635 640

Arg Val Ser Asp Gly Leu Gln Ala Ser Pro Pro Ala Thr Leu Lys Val
645 650 655

Val Ala Ile Arg Pro Ala Ile Gln Ile His Arg Ser Thr Gly Leu Arg
660 665 670

Leu Ala Gln Gly Ser Ala Met Pro Ile Leu Pro Ala Asn Leu Ser Val
675 680 685

Glu Thr Asn Ala Val Gly Gln Asp Val Ser Val Leu Phe Arg Val Thr
690 695 700

Gly Ala Leu Gln Phe Gly Glu Leu Gln Lys Gln Gly Ala Gly Gly Val
705 710 715 720

Glu Gly Ala Glu Trp Trp Ala Thr Gln Ala Phe His Gln Arg Asp Val
725 730 735

Glu Gln Gly Arg Val Arg Tyr Leu Ser Thr Asp Pro Gln His His Ala
740 745 750

Tyr Asp Thr Val Glu Asn Leu Ala Leu Glu Val Gln Val Gly Gln Glu
755 760 765

Ile Leu Ser Asn Leu Ser Phe Pro Val Thr Ile Gln Arg Ala Thr Val
770 775 780

```
Trp Met Leu Arg Leu Glu Pro Leu His Thr Gln Asn Thr Gln Gln Glu
785                 790                 795                 800

Thr Leu Thr Thr Ala His Leu Glu Ala Thr Leu Glu Glu Ala Gly Pro
                805                 810                 815

Ser Pro Pro Thr Phe His Tyr Glu Val Val Gln Ala Pro Arg Lys Gly
            820                 825                 830

Asn Leu Gln Leu Gln Gly Thr Arg Leu Ser Asp Gly Gln Gly Phe Thr
        835                 840                 845

Gln Asp Asp Ile Gln Ala Gly Arg Val Thr Tyr Gly Ala Thr Ala Arg
    850                 855                 860

Ala Ser Glu Ala Val Glu Asp Thr Phe Arg Phe Arg Val Thr Ala Pro
865                 870                 875                 880

Pro Tyr Phe Ser Pro Leu Tyr Thr Phe Pro Ile His Ile Gly Gly Asp
                885                 890                 895

Pro Asp Ala Pro Val Leu Thr Asn Val Leu Leu Val Val Pro Glu Gly
            900                 905                 910

Gly Glu Gly Val Leu Ser Ala Asp His Leu Phe Val Lys Ser Leu Asn
        915                 920                 925

Ser Ala Ser Tyr Leu Tyr Glu Val Met Glu Arg Pro Arg His Gly Arg
    930                 935                 940

Leu Ala Trp Arg Gly Thr Gln Asp Lys Thr Thr Met Val Thr Ser Phe
945                 950                 955                 960

Thr Asn Glu Asp Leu Leu Arg Gly Arg Leu Val Tyr Gln His Asp Asp
                965                 970                 975

Ser Glu Thr Thr Glu Asp Asp Ile Pro Phe Val Ala Thr Arg Gln Gly
            980                 985                 990

Glu Ser Ser Gly Asp Met Ala Trp  Glu Glu Val Arg Gly  Val Phe Arg
        995                 1000                1005

Val Ala  Ile Gln Pro Val Asn  Asp His Ala Pro Val  Gln Thr Ile
    1010                1015                1020

Ser Arg  Ile Phe His Val Ala  Arg Gly Gly Arg Arg  Leu Leu Thr
    1025                1030                1035
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Thr Asp  Asp Val Ala Phe Ser  Asp Ala Asp Ser Gly  Phe Ala Asp
    1040                1045                1050

Ala Gln  Leu Val Leu Thr Arg  Lys Asp Leu Leu Phe  Gly Ser Ile
    1055                1060                1065

Val Ala  Val Asp Glu Pro Thr  Arg Pro Ile Tyr Arg  Phe Thr Gln
    1070                1075                1080

Glu Asp  Leu Arg Lys Arg Arg  Val Leu Phe Val His  Ser Gly Ala
    1085                1090                1095

Asp Arg  Gly Trp Ile Gln Leu  Gln Val Ser Asp Gly  Gln His Gln
    1100                1105                1110

Ala Thr  Ala Leu Leu Glu Val  Gln Ala Ser Glu Pro  Tyr Leu Arg
    1115                1120                1125

Val Ala  Asn Gly Ser Ser Leu  Val Val Pro Gln Gly  Gly Gln Gly
    1130                1135                1140

Thr Ile  Asp Thr Ala Val Leu  His Leu Asp Thr Asn  Leu Asp Ile
    1145                1150                1155

Arg Ser  Gly Asp Glu Val His  Tyr His Val Thr Ala  Gly Pro Arg
    1160                1165                1170

Trp Gly  Gln Leu Val Arg Ala  Gly Gln Pro Ala Thr  Ala Phe Ser
    1175                1180                1185

Gln Gln  Asp Leu Leu Asp Gly  Ala Val Leu Tyr Ser  His Asn Gly
    1190                1195                1200

Ser Leu  Ser Pro Arg Asp Thr  Met Ala Phe Ser Val  Glu Ala Gly
    1205                1210                1215

Pro Val  His Thr Asp Ala Thr  Leu Gln Val Thr Ile  Ala Leu Glu
    1220                1225                1230

Gly Pro  Leu Ala Pro Leu Lys  Leu Val Arg His Lys  Lys Ile Tyr
    1235                1240                1245

Val Phe  Gln Gly Glu Ala Ala  Glu Ile Arg Arg Asp  Gln Leu Glu
    1250                1255                1260

Ala Ala  Gln Glu Ala Val Pro  Pro Ala Asp Ile Val  Phe Ser Val
    1265                1270                1275
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```
Lys Ser  Pro Pro Ser Ala Gly  Tyr Leu Val Met Val  Ser Arg Gly
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Ala Leu  Ala Asp Glu Pro Pro  Ser Leu Asp Pro Val  Gln Ser Phe
    1295                 1300                 1305

Ser Gln  Glu Ala Val Asp Thr  Gly Arg Val Leu Tyr  Leu His Ser
    1310                 1315                 1320

Arg Pro  Glu Ala Trp Ser Asp  Ala Phe Ser Leu Asp  Val Ala Ser
    1325                 1330                 1335

Gly Leu  Gly Ala Pro Leu Glu  Gly Val Leu Val Glu  Leu Glu Val
    1340                 1345                 1350

Leu Pro  Ala Ala Ile Pro Leu  Glu Ala Gln Asn Phe  Ser Val Pro
    1355                 1360                 1365

Glu Gly  Gly Ser Leu Thr Leu  Ala Pro Pro Leu Leu  Arg Val Ser
    1370                 1375                 1380

Gly Pro  Tyr Phe Pro Thr Leu  Leu Gly Leu Ser Leu  Gln Val Leu
    1385                 1390                 1395

Glu Pro  Pro Gln His Gly Ala  Leu Gln Lys Glu Asp  Gly Pro Gln
    1400                 1405                 1410

Ala Arg  Thr Leu Ser Ala Phe  Ser Trp Arg Met Val  Glu Glu Gln
    1415                 1420                 1425

Leu Ile  Arg Tyr Val His Asp  Gly Ser Glu Thr Leu  Thr Asp Ser
    1430                 1435                 1440

Phe Val  Leu Met Ala Asn Ala  Ser Glu Met Asp Arg  Gln Ser His
    1445                 1450                 1455

Pro Val  Ala Phe Thr Val Thr  Val Leu Pro Val Asn  Asp Gln Pro
    1460                 1465                 1470

Pro Ile  Leu Thr Thr Asn Thr  Gly Leu Gln Met Trp  Glu Gly Ala
    1475                 1480                 1485

Thr Ala  Pro Ile Pro Ala Glu  Ala Leu Arg Ser Thr  Asp Gly Asp
    1490                 1495                 1500

Ser Gly  Ser Glu Asp Leu Val  Tyr Thr Ile Glu Gln  Pro Ser Asn
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Gly Arg  Val Val Leu Arg Gly  Ala Pro Gly Thr Glu  Val Arg Ser
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Phe Thr  Gln Ala Gln Leu Asp  Gly Gly Leu Val Leu  Phe Ser His
    1535                1540                1545

Arg Gly  Thr Leu Asp Gly Gly  Phe Arg Phe Arg Leu  Ser Asp Gly
    1550                1555                1560

Glu His  Thr Ser Pro Gly His  Phe Phe Arg Val Thr  Ala Gln Lys
    1565                1570                1575

Gln Val  Leu Leu Ser Leu Lys  Gly Ser Gln Thr Leu  Thr Val Cys
    1580                1585                1590

Pro Gly  Ser Val Gln Pro Leu  Ser Ser Gln Thr Leu  Arg Ala Ser
    1595                1600                1605

Ser Ser  Ala Gly Thr Asp Pro  Gln Leu Leu Leu Tyr  Arg Val Val
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Arg Gly  Pro Gln Leu Gly Arg  Leu Phe His Ala Gln  Gln Asp Ser
    1625                1630                1635

Thr Gly  Glu Ala Leu Val Asn  Phe Thr Gln Ala Glu  Val Tyr Ala
    1640                1645                1650

Gly Asn  Ile Leu Tyr Glu His  Glu Met Pro Pro Glu  Pro Phe Trp
    1655                1660                1665

Glu Ala  His Asp Thr Leu Glu  Leu Gln Leu Ser Ser  Pro Pro Ala
    1670                1675                1680

Arg Asp  Val Ala Ala Thr Leu  Ala Val Ala Val Ser  Phe Glu Ala
    1685                1690                1695

Ala Cys  Pro Gln Arg Pro Ser  His Leu Trp Lys Asn  Lys Gly Leu
    1700                1705                1710

Trp Val  Pro Glu Gly Gln Arg  Ala Arg Ile Thr Val  Ala Ala Leu
    1715                1720                1725

Asp Ala  Ser Asn Leu Leu Ala  Ser Val Pro Ser Pro  Gln Arg Ser
    1730                1735                1740
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Glu His  Asp Val Leu Phe Gln  Val Thr Gln Phe Pro  Ser Arg Gly
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Gln Leu  Leu Val Ser Glu Glu  Pro Leu His Ala Gly  Gln Pro His
    1760                 1765                 1770

Phe Leu  Gln Ser Gln Leu Ala  Ala Gly Gln Leu Val  Tyr Ala His
    1775                 1780                 1785

Gly Gly  Gly Gly Thr Gln Gln  Asp Gly Phe His Phe  Arg Ala His
    1790                 1795                 1800

Leu Gln  Gly Pro Ala Gly Ala  Ser Val Ala Gly Pro  Gln Thr Ser
    1805                 1810                 1815

Glu Ala  Phe Ala Ile Thr Val  Arg Asp Val Asn Glu  Arg Pro Pro
    1820                 1825                 1830

Gln Pro  Gln Ala Ser Val Pro  Leu Arg Leu Thr Arg  Gly Ser Arg
    1835                 1840                 1845

Ala Pro  Ile Ser Arg Ala Gln  Leu Ser Val Val Asp  Pro Asp Ser
    1850                 1855                 1860

Ala Pro  Gly Glu Ile Glu Tyr  Glu Val Gln Arg Ala  Pro His Asn
    1865                 1870                 1875

Gly Phe  Leu Ser Leu Val Gly  Gly Gly Leu Gly Pro  Val Thr Arg
    1880                 1885                 1890

Phe Thr  Gln Ala Asp Val Asp  Ser Gly Arg Leu Ala  Phe Val Ala
    1895                 1900                 1905

Asn Gly  Ser Ser Val Ala Gly  Ile Phe Gln Leu Ser  Met Ser Asp
    1910                 1915                 1920

Gly Ala  Ser Pro Pro Leu Pro  Met Ser Leu Ala Val  Asp Ile Leu
    1925                 1930                 1935

Pro Ser  Ala Ile Glu Val Gln  Leu Arg Ala Pro Leu  Glu Val Pro
    1940                 1945                 1950

Gln Ala  Leu Gly Arg Ser Ser  Leu Ser Gln Gln Gln  Leu Arg Val
    1955                 1960                 1965

Val Ser  Asp Arg Glu Glu Pro  Glu Ala Ala Tyr Arg  Leu Ile Gln
    1970                 1975                 1980
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Gly Pro  Gln Tyr Gly His Leu  Leu Val Gly Gly Arg  Pro Thr Ser
    1985                 1990                 1995

Ala Phe  Ser Gln Phe Gln Ile  Asp Gln Gly Glu Val  Val Phe Ala
    2000                 2005                 2010

Phe Thr  Asn Phe Ser Ser Ser  His Asp His Phe Arg  Val Leu Ala
    2015                 2020                 2025

Leu Ala  Arg Gly Val Asn Ala  Ser Ala Val Val Asn  Val Thr Val
    2030                 2035                 2040

Arg Ala  Leu Leu His Val Trp  Ala Gly Gly Pro Trp  Pro Gln Gly
    2045                 2050                 2055

Ala Thr  Leu Arg Leu Asp Pro  Thr Val Leu Asp Ala  Gly Glu Leu
    2060                 2065                 2070

Ala Asn  Arg Thr Gly Ser Val  Pro Arg Phe Arg Leu  Leu Glu Gly
    2075                 2080                 2085

Pro Arg  His Gly Arg Val Val  Arg Val Pro Arg Ala  Arg Thr Glu
    2090                 2095                 2100

Pro Gly  Gly Ser Gln Leu Val  Glu Gln Phe Thr Gln  Gln Asp Leu
    2105                 2110                 2115

Glu Asp  Gly Arg Leu Gly Leu  Glu Val Gly Arg Pro  Glu Gly Arg
    2120                 2125                 2130

Ala Pro  Gly Pro Ala Gly Asp  Ser Leu Thr Leu Glu  Leu Trp Ala
    2135                 2140                 2145

Gln Gly  Val Pro Pro Ala Val  Ala Ser Leu Asp Phe  Ala Thr Glu
    2150                 2155                 2160

Pro Tyr  Asn Ala Ala Arg Pro  Tyr Ser Val Ala Leu  Leu Ser Val
    2165                 2170                 2175

Pro Glu  Ala Ala Arg Thr Glu  Ala Gly Lys Pro Glu  Ser Ser Thr
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Pro Thr  Gly Glu Pro Gly Pro  Met Ala Ser Ser Pro  Glu Pro Ala
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Val Ala  Lys Gly Gly Phe Leu  Ser Phe Leu Glu Ala  Asn Met Phe
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Ser Val  Ile Ile Pro Met Cys  Leu Val Leu Leu Leu  Leu Ala Leu
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Ile Leu  Pro Leu Leu Phe Tyr  Leu Arg Lys Arg Asn  Lys Thr Gly
    2240                2245                2250

Lys His  Asp Val Gln Val Leu  Thr Ala Lys Pro Arg  Asn Gly Leu
    2255                2260                2265

Ala Gly  Asp Thr Glu Thr Phe  Arg Lys Val Glu Pro  Gly Gln Ala
    2270                2275                2280

Ile Pro  Leu Thr Ala Val Pro  Gly Gln Gly Pro Pro  Pro Gly Gly
    2285                2290                2295

Gln Pro  Asp Pro Glu Leu Leu  Gln Phe Cys Arg Thr  Pro Asn Pro
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Ala Leu  Lys Asn Gly Gln Tyr  Trp Val
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Val Cys Gly Gly Val Leu Val His Pro Gln Trp Val Leu Thr Ala Ala
    50                  55                  60

His Cys Ile Arg Asn Lys Ser Val Ile Leu Leu Gly Arg His Ser Leu
65                  70                  75                  80

Phe His Pro Glu Asp Thr Gly Gln Val Phe Gln Val Ser His Ser Phe
                85                  90                  95

Pro His Pro Leu Tyr Asp Met Ser Leu Leu Lys Asn Arg Phe Leu Arg
            100                 105                 110

Pro Gly Asp Asp Ser Ser His Asp Leu Met Leu Leu Arg Leu Ser Glu
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Pro Ala Glu Leu Thr Asp Ala Val Lys Val Met Asp Leu Pro Thr Gln
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Glu Pro Ala Leu Gly Thr Thr Cys Tyr Ala Ser Gly Trp Gly Ser Ile
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Cys Ser Gly Asp Ser Gly Gly Pro Leu Val Cys Asn Gly Val Leu Gln
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Gly Ile Thr Ser Trp Gly Ser Glu Pro Cys Ala Leu Pro Glu Arg Pro
225                 230                 235                 240

Ser Leu Tyr Thr Lys Val Val His Tyr Arg Lys Trp Ile Lys Asp Thr
                245                 250                 255

Ile Val Ala Asn Pro
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<210> 22
<211> 237
<212> PRT
<213> Homo sapiens

<400> 22

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1               5                  10                  15

Leu Val Ala Ser Arg Gly Arg Ala Val Cys Gly Gly Val Leu Val His
            20                  25                  30

Pro Gln Trp Val Leu Thr Ala Ala His Cys Ile Arg Asn Lys Ser Val
        35                  40                  45

Ile Leu Leu Gly Arg His Ser Leu Phe His Pro Glu Asp Thr Gly Gln
    50                  55                  60

Val Phe Gln Val Ser His Ser Phe Pro His Pro Leu Tyr Asp Met Ser
65                  70                  75                  80

Leu Leu Lys Asn Arg Phe Leu Arg Pro Gly Asp Asp Ser Ser His Asp
                85                  90                  95

Leu Met Leu Leu Arg Leu Ser Glu Pro Ala Glu Leu Thr Asp Ala Val

            100                 105                 110

Lys Val Met Asp Leu Pro Thr Gln Glu Pro Ala Leu Gly Thr Thr Cys
        115                 120                 125

Tyr Ala Ser Gly Trp Gly Ser Ile Glu Pro Glu Glu Phe Leu Thr Pro
    130                 135                 140

Lys Lys Leu Gln Cys Val Asp Leu His Val Ile Ser Asn Asp Val Cys
145                 150                 155                 160

Ala Gln Val His Pro Gln Lys Val Thr Lys Phe Met Leu Cys Ala Gly
                165                 170                 175

Arg Trp Thr Gly Gly Lys Ser Thr Cys Ser Gly Asp Ser Gly Gly Pro
            180                 185                 190

Leu Val Cys Tyr Gly Val Leu Gln Gly Ile Thr Ser Trp Gly Ser Glu
        195                 200                 205

Pro Cys Ala Leu Pro Glu Arg Pro Ser Leu Tyr Thr Lys Val Val His
    210                 215                 220

Tyr Arg Lys Trp Ile Lys Asp Thr Ile Val Ala Asn Pro
225                 230                 235
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<210> 23

<211> 237
<212> PRT
<213> Homo sapiens

<400> 23

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Ile Val Gly Gly Trp Glu Cys Glu Lys His Ser Gln Pro Trp Gln Val
1               5                  10                  15

Leu Val Ala Ser Arg Gly Arg Ala Val Cys Gly Gly Val Leu Val His
            20                  25                  30

Pro Gln Trp Val Leu Thr Ala Ala His Cys Ile Arg Asn Lys Ser Val
        35                  40                  45

Ile Leu Leu Gly Arg His Ser Leu Phe His Pro Glu Asp Thr Gly Gln
    50                  55                  60

Val Phe Gln Val Ser His Ser Phe Pro His Pro Leu Tyr Asp Met Ser
65                  70                  75                  80

Leu Leu Lys Asn Arg Phe Leu Arg Pro Gly Asp Asp Ser Ser His Asp
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85 90 95

Leu Met Leu Leu Arg Leu Ser Glu Pro Ala Glu Leu Thr Asp Ala Val
100 105 110

Lys Val Met Asp Leu Pro Thr Gln Glu Pro Ala Leu Gly Thr Thr Cys
115 120 125

Tyr Ala Ser Gly Trp Gly Ser Ile Glu Pro Glu Glu Phe Leu Thr Pro
130 135 140

Lys Lys Leu Gln Cys Val Asp Leu His Val Ile Ser Asn Asp Val Cys
145 150 155 160

Ala Gln Val His Pro Gln Lys Val Thr Lys Phe Met Leu Cys Ala Gly
165 170 175

Arg Trp Thr Gly Gly Lys Ser Thr Cys Ser Gly Asp Ser Gly Gly Pro
180 185 190

Leu Val Cys Asn Gly Val Leu Gln Gly Ile Thr Ser Trp Gly Ser Glu
195 200 205

Pro Cys Ala Leu Pro Glu Arg Pro Ser Leu Tyr Thr Lys Val Val His
210 215 220

Tyr Arg Lys Trp Ile Lys Asp Thr Ile Val Ala Asn Pro
225 230 235

<210> 24
<211> 5873
<212> DNA
<213> Homo sapiens

<400> 24

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<210> 25
<211> 238
<212> PRT
<213> Homo sapiens

<400> 25

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            20                  25                  30

Lys His Ser Gln Pro Trp Gln Val Leu Val Ala Ser Arg Gly Arg Ala
        35                  40                  45

Val Cys Gly Gly Val Leu Val His Pro Gln Trp Val Leu Thr Ala Ala
    50                  55                  60

His Cys Ile Arg Asn Lys Ser Val Ile Leu Leu Gly Arg His Ser Leu
65                  70                  75                  80

Phe His Pro Glu Asp Thr Gly Gln Val Phe Gln Val Ser His Ser Phe
                85                  90                  95

Pro His Pro Leu Tyr Asp Met Ser Leu Leu Lys Asn Arg Phe Leu Arg
            100                 105                 110

Pro Gly Asp Asp Ser Ser His Asp Leu Met Leu Leu Arg Leu Ser Glu
        115                 120                 125

Pro Ala Glu Leu Thr Asp Ala Val Lys Val Met Asp Leu Pro Thr Gln
    130                 135                 140

Glu Pro Ala Leu Gly Thr Thr Cys Tyr Ala Ser Gly Trp Gly Ser Ile
145                 150                 155                 160

Glu Pro Glu Glu Phe Leu Thr Pro Lys Lys Leu Gln Cys Val Asp Leu
                165                 170                 175

His Val Ile Ser Asn Asp Val Cys Ala Gln Val His Pro Gln Lys Val
            180                 185                 190

Thr Lys Phe Met Leu Cys Ala Gly Arg Trp Thr Gly Gly Lys Ser Thr
        195                 200                 205

Cys Ser Trp Val Ile Leu Ile Thr Glu Leu Thr Met Pro Ala Leu Pro
    210                 215                 220

Met Val Leu His Gly Ser Leu Val Pro Trp Arg Gly Gly Val
225                 230                 235
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<210> 26
<211> 1906
<212> DNA
<213> Homo sapiens

<400> 26

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<210> 27
<211> 69
<212> PRT
<213> Homo sapiens

<400> 27

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Met Trp Val Pro Val Val Phe Leu Thr Leu Ser Val Thr Trp Ile Gly
1               5                  10                  15

Ala Ala Pro Leu Ile Leu Ser Arg Ile Val Gly Gly Trp Glu Cys Glu
            20                  25                  30

Lys His Ser Gln Pro Trp Gln Val Leu Val Ala Ser Arg Gly Arg Ala
        35                  40                  45

Val Cys Gly Gly Val Leu Val His Pro Gln Trp Val Leu Thr Ala Ala
    50                  55                  60

His Cys Ile Arg Lys
65
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<210> 28
<211> 554
<212> DNA
<213> Homo sapiens

<400> 28

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<210> 29
<211> 220
<212> PRT
<213> Homo sapiens

<400> 29

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Met Trp Val Pro Val Val Phe Leu Thr Leu Ser Val Thr Trp Ile Gly
1               5                  10                  15

Ala Ala Pro Leu Ile Leu Ser Arg Ile Val Gly Gly Trp Glu Cys Glu
            20                  25                  30

Lys His Ser Gln Pro Trp Gln Val Leu Val Ala Ser Arg Gly Arg Ala
        35                  40                  45

Val Cys Gly Gly Val Leu Val His Pro Gln Trp Val Leu Thr Ala Ala
    50                  55                  60

His Cys Ile Arg Asn Lys Ser Val Ile Leu Leu Gly Arg His Ser Leu
65                  70                  75                  80

Phe His Pro Glu Asp Thr Gly Gln Val Phe Gln Val Ser His Ser Phe
                85                  90                  95

Pro His Pro Leu Tyr Asp Met Ser Leu Leu Lys Asn Arg Phe Leu Arg
            100                 105                 110

Pro Gly Asp Asp Ser Ser Ile Glu Pro Glu Glu Phe Leu Thr Pro Lys
        115                 120                 125

Lys Leu Gln Cys Val Asp Leu His Val Ile Ser Asn Asp Val Cys Ala
    130                 135                 140

Gln Val His Pro Gln Lys Val Thr Lys Phe Met Leu Cys Ala Gly Arg
145                 150                 155                 160

Trp Thr Gly Gly Lys Ser Thr Cys Ser Gly Asp Ser Gly Gly Pro Leu
                165                 170                 175

Val Cys Asn Gly Val Leu Gln Gly Ile Thr Ser Trp Gly Ser Glu Pro
            180                 185                 190

Cys Ala Leu Pro Glu Arg Pro Ser Leu Tyr Thr Lys Val Val His Tyr
        195                 200                 205

Arg Lys Trp Ile Lys Asp Thr Ile Val Ala Asn Pro
    210                 215                 220
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<210> 30
<211> 1341
<212> DNA
<213> Homo sapiens

<400> 30

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<210> 31
<211> 218
<212> PRT
<213> Homo sapiens

<400> 31

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Met Trp Val Pro Val Val Phe Leu Thr Leu Ser Val Thr Trp Ile Gly
1               5                  10                  15

Ala Ala Pro Leu Ile Leu Ser Arg Ile Val Gly Gly Trp Glu Cys Glu
            20                  25                  30

Lys His Ser Gln Pro Trp Gln Val Leu Val Ala Ser Arg Gly Arg Ala
        35                  40                  45

Val Cys Gly Gly Val Leu Val His Pro Gln Trp Val Leu Thr Ala Ala
    50                  55                  60

His Cys Ile Arg Lys Pro Gly Asp Asp Ser Ser His Asp Leu Met Leu
65                  70                  75                  80

Leu Arg Leu Ser Glu Pro Ala Glu Leu Thr Asp Ala Val Lys Val Met
                85                  90                  95

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

Gly Trp Gly Ser Ile Glu Pro Glu Glu Phe Leu Thr Pro Lys Lys Leu
        115                 120                 125

Gln Cys Val Asp Leu His Val Ile Ser Asn Asp Val Cys Ala Gln Val
    130                 135                 140

His Pro Gln Lys Val Thr Lys Phe Met Leu Cys Ala Gly Arg Trp Thr
145                 150                 155                 160

Gly Gly Lys Ser Thr Cys Ser Gly Asp Ser Gly Gly Pro Leu Val Cys
                165                 170                 175

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

Leu Pro Glu Arg Pro Ser Leu Tyr Thr Lys Val Val His Tyr Arg Lys
        195                 200                 205

Trp Ile Lys Asp Thr Ile Val Ala Asn Pro
    210                 215
```

<210> 32
<211> 1325
<212> DNA
<213> Homo sapiens

<400> 32

agccccaagc ttaccacctg cacccggaga gctgtgtcac catgtgggtc ccggttgtct 60

tcctcaccct gtccgtgacg tggattggtg ctgcacccct catcctgtct cggattgtgg 120

gaggctggga gtgcgagaag cattcccaac cctggcaggt gcttgtggcc tctcgtggca 180

gggcagtctg cggcggtgtt ctggtgcacc cccagtgggt cctcacagct gcccactgca 240

tcaggaagcc aggtgatgac tccagccacg acctcatgct gctccgcctg tcagagcctg 300

ccgagctcac ggatgctgtg aaggtcatgg acctgcccac ccaggagcca gcactgggga 360

ccacctgcta cgcctcaggc tggggcagca ttgaaccaga ggagttcttg accccaaaga 420

aacttcagtg tgtggacctc catgttattt ccaatgacgt gtgtgcgcaa gttcaccctc 480

agaaggtgac caagttcatg ctgtgtgctg gacgctggac aggggggcaaa agcacctgct 540

cgggtgattc tgggggccca cttgtctgta atggtgtgct tcaaggtatc acgtcatggg 600

gcagtgaacc atgtgccctg cccgaaaggc cttccctgta caccaaggtg gtgcattacc 660

caaggacacc atcgtggcca acccctgagc acccctatca acccccctatt gtagtaaact 720

tggaaccttg gaaatgacca ggccaagact caagcctccc cagttctact gacctttgtc 780

cttaggtgtg aggtccaggg ttgctaggaa aagaaatcag cagacacagg tgtagaccag 840

agtgtttctt aaatggtgta attttgtcct ctctgtgtcc tggggaatac tggccatgcc 900

tggagacata tcactcaatt tctctgagga cacagatagg atggggtgtc tgtgttattt 960

gtggggtaca gagatgaaag aggggtggga tccacactga gagagtggag agtgacatgt 1020

gctggacact gtccatgaag cactgagcag aagctggagg cacaacgcac cagacactca 1080

cagcaaggat ggagctgaaa acataaccca ctctgtcctg gaggcactgg gaagcctaga 1140

gaaggctgtg agccaaggag ggagggtctt cctttggcat gggatgggga tgaagtaagg 1200

agagggactg gacccccctgg aagctgattc actatggggg gaggtgtatt gaagtcctcc 1260

agacaaccct cagatttgat gatttcctag tagaactcac agaaataaag agctgttata 1320

ctgtg 1325

<210> 33
<211> 261
<212> PRT
<213> Homo sapiens

<400> 33

```
Met Trp Val Pro Val Val Phe Leu Thr Leu Ser Val Thr Trp Ile Gly
1               5                  10                  15

Ala Ala Pro Leu Ile Leu Ser Arg Ile Val Gly Gly Trp Glu Cys Glu
            20                  25                  30

Lys His Ser Gln Pro Trp Gln Val Leu Val Ala Ser Arg Gly Arg Ala
        35                  40                  45

Val Cys Gly Gly Val Leu Val His Pro Gln Trp Val Leu Thr Ala Ala
    50                  55                  60

His Cys Ile Arg Asn Lys Ser Val Ile Leu Leu Gly Arg His Ser Leu
65                  70                  75                  80
```

```
Phe His Pro Glu Asp Thr Gly Gln Val Phe Gln Val Ser His Ser Phe
                85                  90                  95

Pro His Pro Leu Tyr Asp Met Ser Leu Leu Lys Asn Arg Phe Leu Arg
            100                 105                 110

Pro Gly Asp Asp Ser Ser His Asp Leu Met Leu Leu Arg Leu Ser Glu
        115                 120                 125

Pro Ala Glu Leu Thr Asp Ala Val Lys Val Met Asp Leu Pro Thr Gln
    130                 135                 140

Glu Pro Ala Leu Gly Thr Thr Cys Tyr Ala Ser Gly Trp Gly Ser Ile
145                 150                 155                 160

Glu Pro Glu Glu Phe Leu Thr Pro Lys Lys Leu Gln Cys Val Asp Leu
                165                 170                 175

His Val Ile Ser Asn Asp Val Cys Ala Gln Val His Pro Gln Lys Val
            180                 185                 190

Thr Lys Phe Met Leu Cys Ala Gly Arg Trp Thr Gly Gly Lys Ser Thr
        195                 200                 205

Cys Ser Gly Asp Ser Gly Gly Pro Leu Val Cys Asn Gly Val Leu Gln
    210                 215                 220

Gly Ile Thr Ser Trp Gly Ser Glu Pro Cys Ala Leu Pro Glu Arg Pro
225                 230                 235                 240

Ser Leu Tyr Thr Lys Val Val His Tyr Arg Lys Trp Ile Lys Asp Thr
                245                 250                 255

Ile Val Ala Asn Pro
            260
```

<210> 34
<211> 1464
<212> DNA
<213> Homo sapiens

<400> 34

agccccaagc ttaccacctg cacccggaga gctgtgtcac catgtgggtc ccggttgtct 60

tcctcaccct gtccgtgacg tggattggtg ctgcacccct catcctgtct cggattgtgg 120

gaggctggga gtgcgagaag cattcccaac cctggcaggt gcttgtggcc tctcgtggca 180

gggcagtctg cggcggtgtt ctggtgcacc cccagtgggt cctcacagct gcccactgca 240

tcaggaacaa aagcgtgatc ttgctgggtc ggcacagcct gtttcatcct gaagacacag 300

gccaggtatt tcaggtcagc cacagcttcc cacacccgct ctacgatatg agcctcctga 360

agaatcgatt cctcaggcca ggtgatgact ccagccacga cctcatgctg ctccgcctgt 420

cagagcctgc cgagctcacg gatgctgtga aggtcatgga cctgcccacc caggagccag 480

cactggggac cacctgctac gcctcaggct ggggcagcat tgaaccagag gagttcttga 540

ccccaaagaa acttcagtgt gtggacctcc atgttatttc caatgacgtg tgtgcgcaag 600

ttcaccctca gaaggtgacc aagttcatgc tgtgtgctgg acgctggaca gggggcaaaa 660

gcacctgctc gggtgattct gggggcccac ttgtctgtaa tggtgtgctt caaggtatca 720

cgtcatgggg cagtgaacca tgtgccctgc ccgaaaggcc ttccctgtac accaaggtgg 780

tgcattaccg gaagtggatc aaggacacca tcgtggccaa cccctgagca cccctatcaa 840

ccccctattg tagtaaactt ggaaccttgg aaatgaccag gccaagactc aagcctcccc 900

agttctactg acctttgtcc ttaggtgtga ggtccagggt tgctaggaaa agaaatcagc 960

agacacaggt gtagaccaga gtgtttctta aatggtgtaa ttttgtcctc tctgtgtcct 1020

ggggaatact ggccatgcct ggagacatat cactcaattt ctctgaggac acagatagga 1080

tggggtgtct gtgttatttg tggggtacag agatgaaaga ggggtgggat ccacactgag 1140

agagtggaga gtgacatgtg ctggacactg tccatgaagc actgagcaga agctggaggc 1200

acaacgcacc agacactcac agcaaggatg gagctgaaaa cataacccac tctgtcctgg 1260

aggcactggg aagcctagag aaggctgtga gccaaggagg gagggtcttc ctttggcatg 1320

ggatggggat gaagtaagga gagggactgg acccccctgga agctgattca ctatgggggg 1380

aggtgtattg aagtcctcca gacaaccctc agatttgatg atttcctagt agaactcaca 1440

gaaataaaga gctgttatac tgtg 1464

<210> 35
<211> 261
<212> PRT
<213> Homo sapiens

<400> 35

```
Met Trp Val Pro Val Val Phe Leu Thr Leu Ser Val Thr Trp Ile Gly
1               5                  10                  15

Ala Ala Pro Leu Ile Leu Ser Arg Ile Val Gly Gly Trp Glu Cys Glu
            20                  25                  30

Lys His Ser Gln Pro Trp Gln Val Leu Val Ala Ser Arg Gly Arg Ala
        35                  40                  45

Val Cys Gly Gly Val Leu Val His Pro Gln Trp Val Leu Thr Ala Ala
    50                  55                  60
```

```
His Cys Ile Arg Asn Lys Ser Val Ile Leu Leu Gly Arg His Ser Leu
65                  70                  75                  80

Phe His Pro Glu Asp Thr Gly Gln Val Phe Gln Val Ser His Ser Phe
                85                  90                  95

Pro His Pro Leu Tyr Asp Met Ser Leu Leu Lys Asn Arg Phe Leu Arg
            100                 105                 110

Pro Gly Asp Asp Ser Ser His Asp Leu Met Leu Leu Arg Leu Ser Glu
        115                 120                 125

Pro Ala Glu Leu Thr Asp Ala Val Lys Val Met Asp Leu Pro Thr Gln
    130                 135                 140

Glu Pro Ala Leu Gly Thr Thr Cys Tyr Ala Ser Gly Trp Gly Ser Ile
145                 150                 155                 160

Glu Pro Glu Glu Phe Leu Thr Pro Lys Lys Leu Gln Cys Val Asp Leu
                165                 170                 175

His Val Ile Ser Asn Asp Val Cys Ala Gln Val His Pro Gln Lys Val
            180                 185                 190

Thr Lys Phe Met Leu Cys Ala Gly Arg Trp Thr Gly Gly Lys Ser Thr
        195                 200                 205

Cys Ser Gly Asp Ser Gly Gly Pro Leu Val Cys Asn Gly Val Leu Gln
    210                 215                 220

Gly Ile Thr Ser Trp Gly Ser Glu Pro Cys Ala Leu Pro Glu Arg Pro
225                 230                 235                 240

Ser Leu Tyr Thr Lys Val Val His Tyr Arg Lys Trp Ile Lys Asp Thr
                245                 250                 255

Ile Val Ala Asn Pro
            260
```

<210> 36
<211> 1495
<212> DNA
<213> Homo sapiens

<400> 36

gggggagccc caagcttacc acctgcaccc ggagagctgt gtcaccatgt gggtcccggt 60

tgtcttcctc accctgtccg tgacgtggat tggtgctgca cccctcatcc tgtctcggat 120

tgtgggaggc tgggagtgcg agaagcattc ccaaccctgg caggtgcttg tggcctctcg 180

tggcagggca gtctgcggcg gtgttctggt gcacccccag tgggtcctca cagctgccca 240

ctgcatcagg aacaaaagcg tgatcttgct gggtcggcac agcctgtttc atcctgaaga 300

cacaggccag gtatttcagg tcagccacag cttcccacac ccgctctacg atatgagcct 360

cctgaagaat cgattcctca ggccaggtga tgactccagc cacgacctca tgctgctccg 420

cctgtcagag cctgccgagc tcacggatgc tgtgaaggtc atggacctgc ccacccagga 480

gccagcactg gggaccacct gctacgcctc aggctggggc agcattgaac cagaggagtt 540

cttgacccca aagaaacttc agtgtgtgga cctccatgtt atttccaatg acgtgtgtgc 600

gcaagttcac cctcagaagg tgaccaagtt catgctgtgt gctggacgct ggacaggggg 660

caaaagcacc tgctcgggtg attctggggg cccacttgtc tgtaatggtg tgcttcaagg 720

tatcacgtca tggggcagtg aaccatgtgc cctgcccgaa aggccttccc tgtacaccaa 780

ggtggtgcat taccggaagt ggatcaagga caccatcgtg gccaacccct gagcacccct 840

atcaactccc tattgtagta aacttggaac cttggaaatg accaggccaa gactcaggcc 900

tccccagttc tactgacctt tgtccttagg tgtgaggtcc agggttgcta ggaaaagaaa 960

tcagcagaca caggtgtaga ccagagtgtt tcttaaatgg tgtaattttg tcctctctgt 1020

gtcctgggga atactggcca tgcctggaga catatcactc aatttctctg aggacacaga 1080

taggatgggg tgtctgtgtt atttgtgggg tacagagatg aaagaggggt gggatccaca 1140

ctgagagagt ggagagtgac atgtgctgga cactgtccat gaagcactga gcagaagctg 1200

gaggcacaac gcaccagaca ctcacagcaa ggatggagct gaaaacataa cccactctgt 1260

cctggaggca ctgggaagcc tagagaaggc tgtgagccaa ggagggaggg tcttcctttg 1320

gcatgggatg gggatgaagt agggagaggg actggacccc ctggaagctg attcactatg 1380

gggggaggtg tattgaagtc ctccagacaa ccctcagatt tgatgatttc ctagtagaac 1440

tcacagaaat aaagagctgt tatactgcga aaaaaaaaaa aaaaaaaaaa aaaaa 1495

<210> 37
<211> 218
<212> PRT
<213> Homo sapiens

<400> 37

```
Met Trp Val Pro Val Val Phe Leu Thr Leu Ser Val Thr Trp Ile Gly
1               5                  10                  15

Ala Ala Pro Leu Ile Leu Ser Arg Ile Val Gly Gly Trp Glu Cys Glu
            20                  25                  30

Lys His Ser Gln Pro Trp Gln Val Leu Val Ala Ser Arg Gly Arg Ala
        35                  40                  45

Val Cys Gly Gly Val Leu Val His Pro Gln Trp Val Leu Thr Ala Ala
    50                  55                  60

His Cys Ile Arg Asn Lys Ser Val Ile Leu Leu Gly Arg His Ser Leu
65                  70                  75                  80

Phe His Pro Glu Asp Thr Gly Gln Val Phe Gln Val Ser His Ser Phe
                85                  90                  95

Pro His Pro Leu Tyr Asp Met Ser Leu Leu Lys Asn Arg Phe Leu Arg
            100                 105                 110

Pro Gly Asp Asp Ser Ser Ile Glu Pro Glu Glu Phe Leu Thr Pro Lys
        115                 120                 125

Lys Leu Gln Cys Val Asp Leu His Val Ile Ser Asn Asp Val Cys Ala
    130                 135                 140

Gln Val His Pro Gln Lys Val Thr Lys Phe Met Leu Cys Ala Gly Arg
145                 150                 155                 160

Trp Thr Gly Gly Lys Ser Thr Cys Ser Gly Asp Ser Gly Gly Pro Leu
                165                 170                 175

Val Cys Asn Gly Val Leu Gln Gly Ile Thr Ser Trp Gly Ser Glu Pro
            180                 185                 190

Cys Ala Leu Pro Glu Arg Pro Ser Leu Tyr Thr Lys Val Val His Tyr
        195                 200                 205

Arg Lys Trp Ile Lys Asp Thr Ile Val Ala
    210                 215
```

<210> 38
<211> 227
<212> PRT
<213> Homo sapiens

<400> 38

```
Met Trp Val Pro Val Val Phe Leu Thr Leu Ser Val Thr Trp Ile Gly
1               5                  10                  15

Ala Ala Pro Leu Ile Leu Ser Arg Ile Val Gly Gly Trp Glu Cys Glu
            20                  25                  30

Lys His Ser Gln Pro Trp Gln Val Leu Val Ala Ser Arg Gly Arg Ala
        35                  40                  45

Val Cys Gly Gly Val Leu Val His Pro Gln Trp Val Leu Thr Ala Ala
    50                  55                  60

His Cys Ile Arg Asn Lys Ser Val Ile Leu Leu Gly Arg His Ser Leu
65                  70                  75                  80

Phe His Pro Glu Asp Thr Gly Gln Val Phe Gln Val Ser His Ser Phe
                85                  90                  95

Pro His Pro Leu Tyr Asp Met Ser Leu Leu Lys Asn Arg Phe Leu Arg
            100                 105                 110

Pro Gly Asp Asp Ser Ser His Asp Leu Met Leu Leu Arg Leu Ser Glu
        115                 120                 125

Pro Ala Glu Leu Thr Asp Ala Val Lys Val Met Asp Leu Pro Thr Gln
    130                 135                 140

Glu Pro Ala Leu Gly Thr Thr Cys Tyr Ala Ser Gly Trp Gly Ser Ile
145                 150                 155                 160

Glu Pro Glu Glu Phe Leu Thr Pro Lys Lys Leu Gln Cys Val Asp Leu
                165                 170                 175

His Val Ile Ser Asn Asp Val Cys Ala Gln Val His Pro Gln Lys Val
            180                 185                 190

Thr Lys Phe Met Leu Cys Ala Gly Arg Trp Thr Gly Gly Lys Ser Thr
        195                 200                 205

Cys Ser Val Ser His Pro Tyr Ser Gln Asp Leu Glu Gly Lys Gly Glu
    210                 215                 220

Trp Gly Pro
225
```

<210> 39
<211> 104
<212> PRT

<213> Homo sapiens

<400> 39

```
Met Trp Val Pro Val Val Phe Leu Thr Leu Ser Val Thr Trp Ile Gly
1               5                  10                  15

Glu Arg Gly His Gly Trp Gly Asp Ala Gly Glu Gly Ala Ser Pro Asp
            20                  25                  30

Cys Gln Ala Glu Ala Leu Ser Pro Pro Thr Gln His Pro Ser Pro Asp
        35                  40                  45

Arg Glu Leu Gly Ser Phe Leu Ser Leu Pro Ala Pro Leu Gln Ala His
    50                  55                  60

Thr Pro Ser Pro Ser Ile Leu Gln Gln Ser Ser Leu Pro His Gln Val
65                  70                  75                  80

Pro Ala Pro Ser His Leu Pro Gln Asn Phe Leu Pro Ile Ala Gln Pro
                85                  90                  95

Ala Pro Cys Ser Gln Leu Leu Tyr
            100
```

<210> 40
<211> 261
<212> PRT
<213> Homo sapiens

<400> 40

```
Met Trp Val Pro Val Val Phe Leu Thr Leu Ser Val Thr Trp Ile Gly
1               5                  10                  15

Ala Ala Pro Leu Ile Leu Ser Arg Ile Val Gly Gly Trp Glu Cys Glu
            20                  25                  30

Lys His Ser Gln Pro Trp Gln Val Leu Val Ala Ser Arg Gly Arg Ala
        35                  40                  45

Val Cys Gly Gly Val Leu Val His Pro Gln Trp Val Leu Thr Ala Ala
    50                  55                  60

His Cys Ile Arg Asn Lys Ser Val Ile Leu Leu Gly Arg His Ser Leu
65                  70                  75                  80

Phe His Pro Glu Asp Thr Gly Gln Val Phe Gln Val Ser His Ser Phe
                85                  90                  95

Pro His Pro Leu Tyr Asp Met Ser Leu Leu Lys Asn Arg Phe Leu Arg
            100                 105                 110

Pro Gly Asp Asp Ser Ser His Asp Leu Met Leu Leu Arg Leu Ser Glu
        115                 120                 125
```

```
Pro Ala Glu Leu Thr Asp Ala Val Lys Val Met Asp Leu Pro Thr Gln
    130                 135                 140

Glu Pro Ala Leu Gly Thr Thr Cys Tyr Ala Ser Gly Trp Gly Ser Ile
145                 150                 155                 160

Glu Pro Glu Glu Phe Leu Thr Pro Lys Lys Leu Gln Cys Val Asp Leu
                165                 170                 175

His Val Ile Ser Asn Asp Val Cys Ala Gln Val His Pro Gln Lys Val
            180                 185                 190

Thr Lys Phe Met Leu Cys Ala Gly Arg Trp Thr Gly Gly Lys Ser Thr
        195                 200                 205

Cys Ser Gly Asp Ser Gly Gly Pro Leu Val Cys Asn Gly Val Leu Gln
    210                 215                 220

Gly Ile Thr Ser Trp Gly Ser Glu Pro Cys Ala Leu Pro Glu Arg Pro
225                 230                 235                 240

Ser Leu Tyr Thr Lys Val Val His Tyr Arg Lys Trp Ile Lys Asp Thr
                245                 250                 255

Ile Val Ala Asn Pro
            260
```

<210> 41
<211> 1778
<212> DNA
<213> Homo sapiens

<400> 41

aagtttccct tctcccagtc caagacccca atcaccaca aaggacccaa tccccagact 60

caagatatgg tctgggcgct gtcttgtgtc tcctaccctg atccctgggt tcaactctgc 120

tcccagagca tgaagcctct ccaccagcac cagccaccaa cctgcaaacc tagggaagat 180

tgacagaatt cccagccttt cccagctccc cctgcccatg tcccaggact cccagccttg 240

gttctctgcc cccgtgtctt ttcaaaccca catcctaaat ccatctccta tccgagtccc 300

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ccctcatcct gtctcggatt gtgggaggct gggagtgcga gaagcattcc caaccctggc 420

aggtgcttgt agcctctcgt ggcagggcag tctgcggcgg tgttctggtg caccccccagt 480

gggtcctcac agctacccac tgcatcagga acaaaagcgt gatcttgctg ggtcggcaca 540

gcctgtttca tcctgaagac acaggccagg tatttcaggt cagccacagc ttcccacacc 600

cgctctacga tatgagcctc ctgaagaatc gattcctcag gccaggtgat gactccagcc 660

acgacctcat gctgctccgc ctgtcagagc ctgccgagct cacggatgct atgaaggtca 720

tggacctgcc cacccaggag ccagcactgg ggaccacctg ctacgcctca ggctggggca 780

gcattgaacc agaggagttc ttgaccccaa agaaacttca gtgtgtggac ctccatgtta 840

tttccaatga cgtgtgtgcg caagttcacc ctcagaaggt gaccaagttc atgctgtgtg 900

ctggacgctg gacaggggggc aaaagcacct gctcgggtga ttctgggggc ccacttgtct 960

gtaatggtgt gcttcaaggt atcacgtcat ggggcagtga accatgtgcc ctgcccgaaa 1020

ggccttccct gtacaccaag gtggtgcatt accggaagtg gatcaaggac accatcgtgg 1080

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aaaacataac ccactctgtc ctggaggcac tgggaagcct agagaaggct gtgaaccaag 1560

gagggagggt cttcctttgg catgggatgg ggatgaagta aggagaggga ctgacccct 1620

ggaagctgat tcactatggg gggaggtgta ttgaagtcct ccagacaacc ctcagatttg 1680

atgatttcct agtagaactc acagaaataa agagctgtta tactgtgaaa tgatttccta 1740

gtagaactca cagaaataaa gagctgttat actgtgaa 1778

<210> 42

<211> 1134
<212> DNA
<213> Listeria monocytogenes

<400> 42

atggtgacag gctggcatcg tccaacatgg attgaaatag accgcgcagc aattcgcgaa 60

aatataaaaa atgaacaaaa taaactcccg gaaagtgtcg acttatgggc agtagtcaaa 120

gctaatgcat atggtcacgg aattatcgaa gttgctagga cggcgaaaga agctggagca 180

aaaggtttct gcgtagccat tttagatgag gcactggctc ttagagaagc tggatttcaa 240

gatgacttta ttcttgtgct tggtgcaacc agaaaagaag atgctaatct ggcagccaaa 300

aaccacattt cacttactgt ttttagagaa gattggctag agaatctaac gctagaagca 360

acacttcgaa ttcatttaaa agtagatagc ggtatggggc gtctcggtat tcgtacgact 420

gaagaagcac ggcgaattga agcaaccagt actaatgatc accaattaca actggaaggt 480

atttacacgc attttgcaac agccgaccag ctagaaacta gttattttga acaacaatta 540

gctaagttcc aaacgatttt aacgagttta aaaaaacgac caacttatgt tcatacagcc 600

aattcagctg cttcattgtt acagccacaa atcgggtttg atgcgattcg ctttggtatt 660

tcgatgtatg gattaactcc ctccacagaa atcaaaacta gcttgccgtt tgagcttaaa 720

cctgcacttg cactctatac cgagatggtt catgtgaaag aacttgcacc aggcgatagc 780

gttagctacg gagcaactta tacagcaaca gagcgagaat gggttgcgac attaccaatt 840

ggctatgcgg atggattgat tcgtcattac agtggtttcc atgttttagt agacggtgaa 900

ccagctccaa tcattggtcg agtttgtatg gatcaaacca tcataaaact accacgtgaa 960

tttcaaactg gttcaaaagt aacgataatt ggcaaagatc atggtaacac ggtaacagca 1020

gatgatgccg ctcaatattt agatacaatt aattatgagg taacttgttt gttaaatgag 1080

cgcataccta gaaaatacat ccattagcgc atacctagaa aatacatcca ttag 1134

<210> 43
<211> 368
<212> PRT
<213> Listeria monocytogenes

<400> 43

```
Met Val Thr Gly Trp His Arg Pro Thr Trp Ile Glu Ile Asp Arg Ala
1               5                  10                  15

Ala Ile Arg Glu Asn Ile Lys Asn Glu Gln Asn Lys Leu Pro Glu Ser
            20                  25                  30

Val Asp Leu Trp Ala Val Val Lys Ala Asn Ala Tyr Gly His Gly Ile
        35                  40                  45

Ile Glu Val Ala Arg Thr Ala Lys Glu Ala Gly Ala Lys Gly Phe Cys
    50                  55                  60

Val Ala Ile Leu Asp Glu Ala Leu Ala Leu Arg Glu Ala Gly Phe Gln
65                  70                  75                  80

Asp Asp Phe Ile Leu Val Leu Gly Ala Thr Arg Lys Glu Asp Ala Asn
                85                  90                  95

Leu Ala Ala Lys Asn His Ile Ser Leu Thr Val Phe Arg Glu Asp Trp
            100                 105                 110

Leu Glu Asn Leu Thr Leu Glu Ala Thr Leu Arg Ile His Leu Lys Val
        115                 120                 125

Asp Ser Gly Met Gly Arg Leu Gly Ile Arg Thr Thr Glu Glu Ala Arg
    130                 135                 140
```

```
Arg Ile Glu Ala Thr Ser Thr Asn Asp His Gln Leu Gln Leu Glu Gly
145                 150                 155                 160

Ile Tyr Thr His Phe Ala Thr Ala Asp Gln Leu Glu Thr Ser Tyr Phe
                165                 170                 175

Glu Gln Gln Leu Ala Lys Phe Gln Thr Ile Leu Thr Ser Leu Lys Lys
            180                 185                 190

Arg Pro Thr Tyr Val His Thr Ala Asn Ser Ala Ala Ser Leu Leu Gln
        195                 200                 205

Pro Gln Ile Gly Phe Asp Ala Ile Arg Phe Gly Ile Ser Met Tyr Gly
    210                 215                 220

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Claims

1. A recombinant *Listeria* strain comprising a nucleic acid molecule encoding an antigenic listeriolysin O (LLO) - prostate specific antigen (PSA) fusion polypeptide, wherein said recombinant *Listeria* is an attenuated, auxotrophic dal/dat mutant, that comprises an episomal expression vector comprising a metabolic enzyme that complements the auxotrophy of said auxotrophic mutant and wherein said recombinant *Listeria* further comprises a deletion of the endogenous ActA gene.

2. The recombinant *Listeria* of claim 1, wherein said nucleic acid molecule is operably integrated into the *Listeria* genome in an open reading frame with an endogenous LLO gene.

3. The recombinant *Listeria* of claim 1, wherein said nucleic acid molecule is in a plasmid in said recombinant *Listeria* strain.

4. The recombinant *Listeria* strain of claim 3, wherein said fused PSA-LLO polypeptide comprises an N-terminal LLO fragment.

5. The recombinant *Listeria* of claim 3 or 4, wherein said plasmid is stably maintained in said recombinant *Listeria* vaccine strain in the absence of antibiotic selection.

6. The recombinant *Listeria* of any of claims 3-5, wherein said plasmid does not confer antibiotic resistance upon said recombinant *Listeria.*

7. The recombinant *Listeria* strain of any one of claims 1-6, wherein said metabolic enzyme is an alanine racemase enzyme or a D-amino acid transferase enzyme.

8. The recombinant *Listeria* strain of any one of claims 1-7, wherein said *Listeria* strain is a *Listeria monocytogenes* strain.

9. An immunogenic composition comprising the recombinant *Listeria* strain of any one of claims 1-8, and an adjuvant.

10. An immunogenic composition according to claim 9, wherein said adjuvant comprises QS21 or a CpG-containing oligonucleotide.

11. A recombinant *Listeria* strain according to any one of claims 1-10, for use as a medicament.

12. A recombinant *Listeria* strain according to any one of claims 1-10, for use in inducing an immune response to PSA in a subject.

13. A recombinant *Listeria* strain according to any one of claims 1 to 10, for use in a method of preventing or delaying onset of a cancer in a subject.

14. A recombinant *Listeria* strain according to any one of claims 1-10, for use in treating, suppressing, or inhibiting a cancer or tumor in a subject.

15. The recombinant *Listeria* strain according to claim 13 or 14 for use according to claim 13 or 14, wherein said cancer or tumour is a prostate cancer or tumor.

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

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Patentkrav

1. Rekombinant *Listeria*-stamme, der omfatter et nukleinsyremolekyle, der koder for et antigent listeriolysin O- (LLO) prostataspecifikt antigen- (PSA) fusionspolypeptid, hvor den rekombinante *Listeria* er en svækket, auxotrof dal/dat-mutant, der omfatter en episomal ekspressionsvektor, der omfatter et metabolisk enzym, som komplementerer den auxotrofe mutants auxotrofi, og hvor den rekombinante *Listeria* endvidere omfatter en deletion af det endogene ActA-gen.

2. Rekombinant *Listeria* ifølge krav 1, hvor nukleinsyremolekylet er operativt integreret i *Listeria*-genomet i en åben læseramme med et endogent LLO-gen.

3. Rekombinant *Listeria* ifølge krav 1, hvor nukleinsyremolekylet er i et plasmid i den rekombinante *Listeria*-stamme.

4. Rekombinant *Listeria*-stamme ifølge krav 3, hvor det kondenserede PSA-LLO- polypeptid omfatter et N-terminalt LLO-fragment.

5. Rekombinant *Listeria* ifølge krav 3 eller 4, hvor plasmidet er stabilt bevaret i den rekombinante *Listeria*-vaccinestamme ved fravær af valg af antibiotikum.

6. Rekombinant *Listeria* ifølge et hvilket som helst af krav 3-5, hvor plasmidet ikke forårsager antibiotiresistens på grund af den rekombinante *Listeria.*

7. Rekombinant *Listeria*-stamme ifølge et hvilket som helst af krav 1-6, hvor det metaboliske enzym er et alaninracemaseenzym eller et D-aminosyretransferaseenzym.

8. Rekombinant *Listeria*-stamme ifølge et hvilket som helst af krav 1-7, hvor *Listeria*-stammen er en *Listeria monocytogenes*-stamme.

9. Immunogen sammensætning, der omfatter den rekombinante *Listeria*-stamme ifølge et hvilket som helst af krav 1-8 og et adjuvans.

10. Immunogen sammensætning ifølge krav 9, hvor adjuvansen omfatter QS21 eller et CpG-holdigt oligonukleotid.

11. Rekombinant *Listeria*-stamme ifølge et hvilket som helst af krav 1-10 til anvendelse som lægemiddel.

12. Rekombinant *Listeria*-stamme ifølge et hvilket som helst af krav 1-10 til anvendelse i induktion af et immunrespons mod PSA hos et individ.

13. Rekombinant *Listeria*-stamme ifølge et hvilket som helst af krav 1 til 10 til anvendelse i en fremgangsmåde til forebyggelse eller forsinket indtræden af en cancer hos et individ.

14. Rekombinant *Listeria*-stamme ifølge et hvilket som helst af krav 1-10 til anvendelse i behandling, undertrykkelse eller hæmning af en cancer eller tumor hos et individ.

15. Rekombinant *Listeria*-stamme ifølge krav 13 eller 14 til anvendelse ifølge krav 13 eller 14, hvor canceren eller tumoren er en prostatacancer eller -tumor.

DRAWINGS

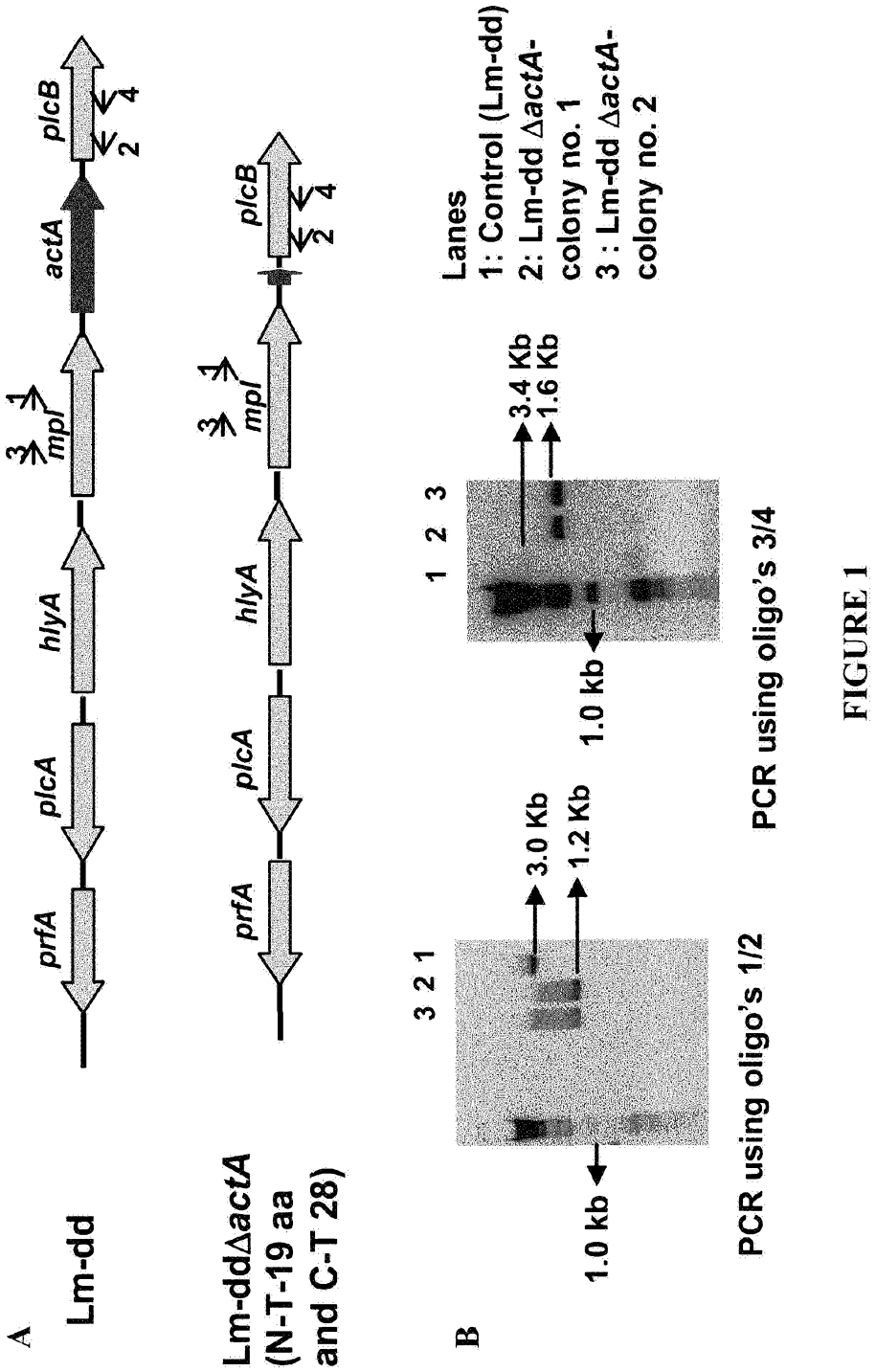


FIGURE 1

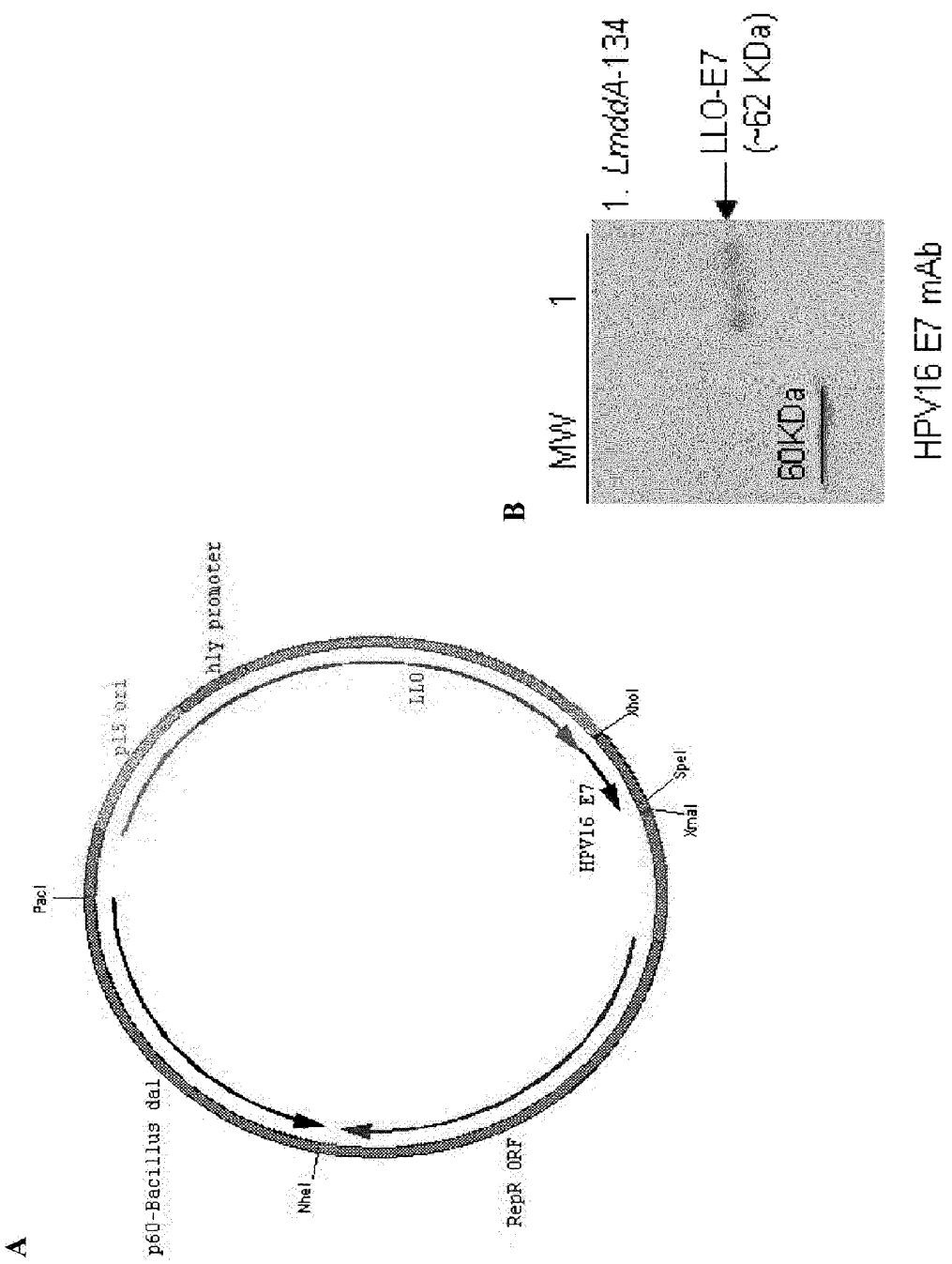

A
p60-Bacillus dal
p15 ori
hly promoter
LLO
HPV16 E7
RepR ORF
PacI
NheI
XhoI
SpeI
XmaI
B
MW
1
1. LmddA-134
LLO-E7
(~62 KDa)
60kDa
HPV16 E7 mAb

FIGURE 2

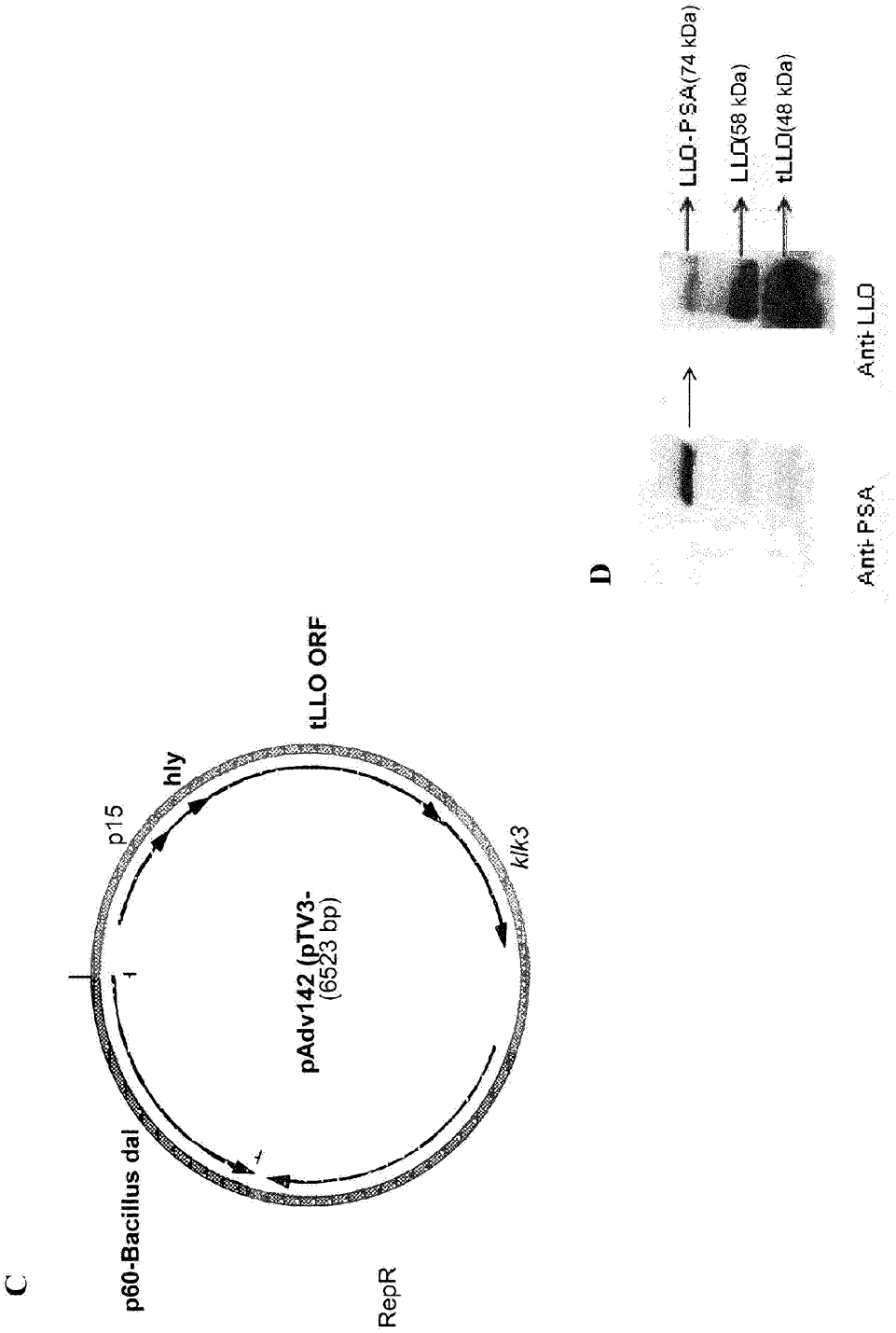


FIGURE 2 (cont.)

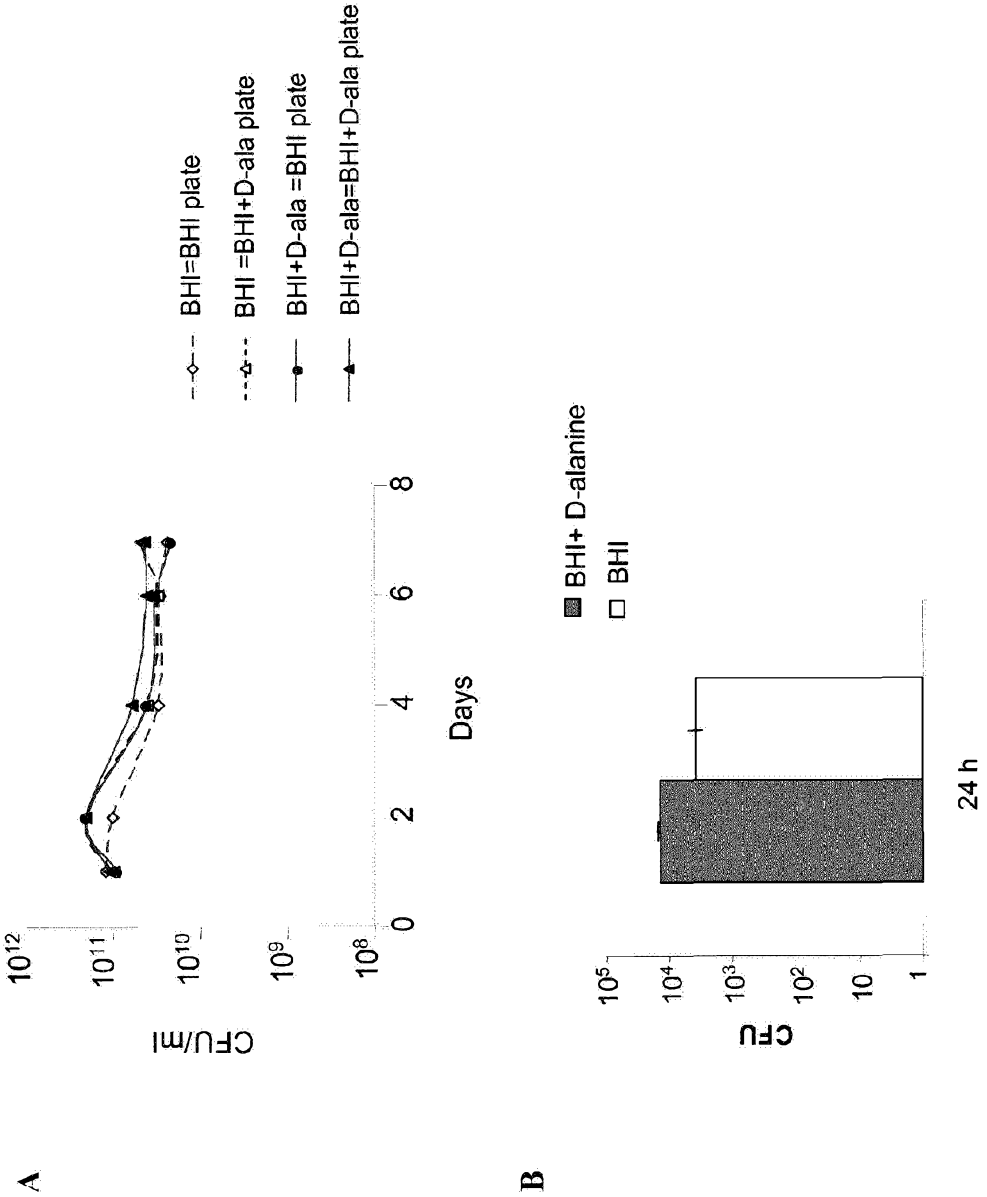

A
CFU/ml
10^{12}
10^{11}
10^{10}
10^{9}
10^{8}
0
2
4
6
8
Days
BHI=BHI plate
BHI =BHI+D-ala plate
BHI+D-ala =BHI plate
BHI+D-ala=BHI+D-ala plate
B
BHI+ D-alanine
BHI
CFU
10^{5}
10^{4}
10^{3}
10^{2}
10
1
24 h

FIGURE 3

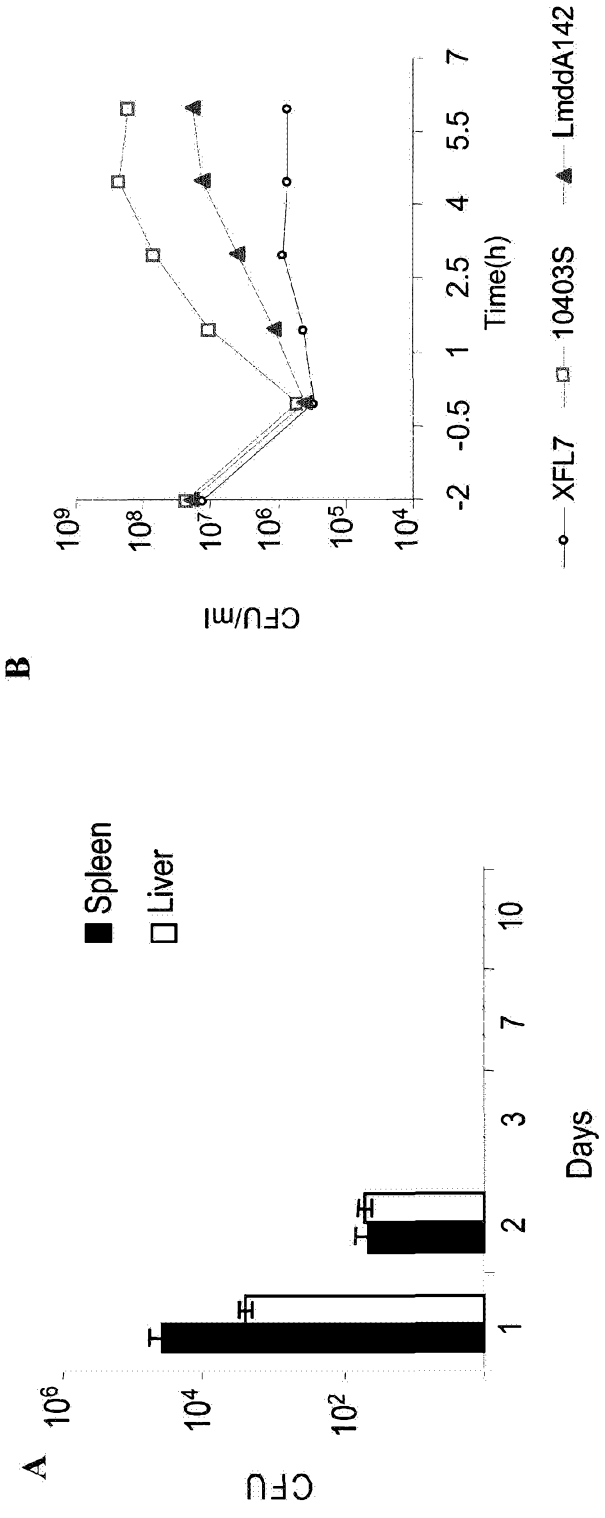

A
CFU
10^6
10^4
10^2
Spleen
Liver
1
2
3
7
10
Days
B
CFU/ml
10^9
10^8
10^7
10^6
10^5
10^4
-2
-0.5
1
2.5
4
5.5
7
Time(h)
XFL7
10403S
LmddA142

FIGURE 4

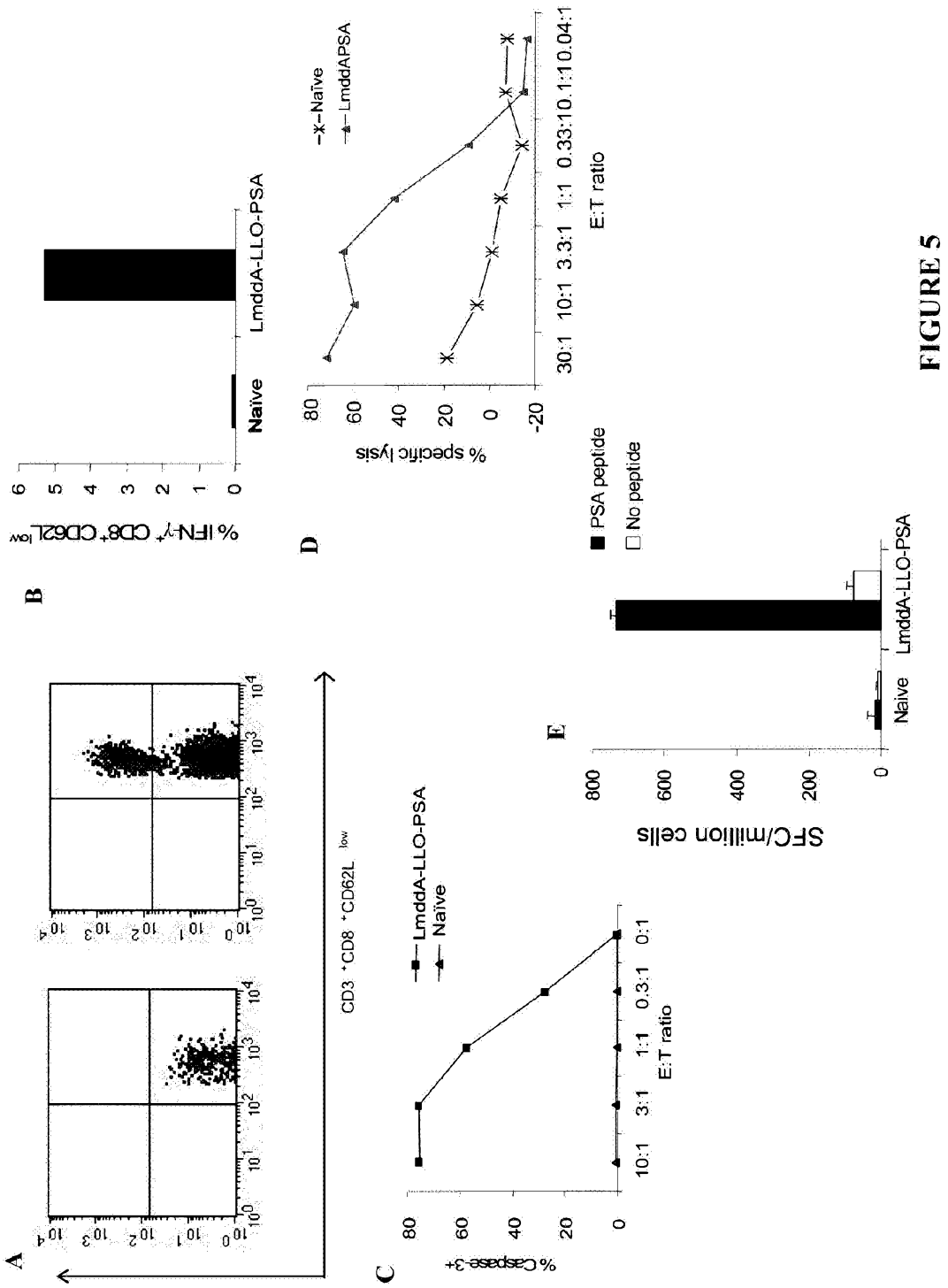

A
CD3 +CD8 +CD62L low
B
% IFN-γ+ CD8+ CD62Llow
Naive
LmddA-LLO-PSA
C
% Caspase-3+
LmddA-LLO-PSA
Naive
E:T ratio
10:1 3:1 1:1 0.3:1 0:1
D
% specific lysis
Naïve
LmddAPSA
E:T ratio
30:1 10:1 3:1 1:1 0.33:1 0.1:1 0.04:1
E
SFC/million cells
PSA peptide
No peptide
Naive
LmddA-LLO-PSA

FIGURE 5

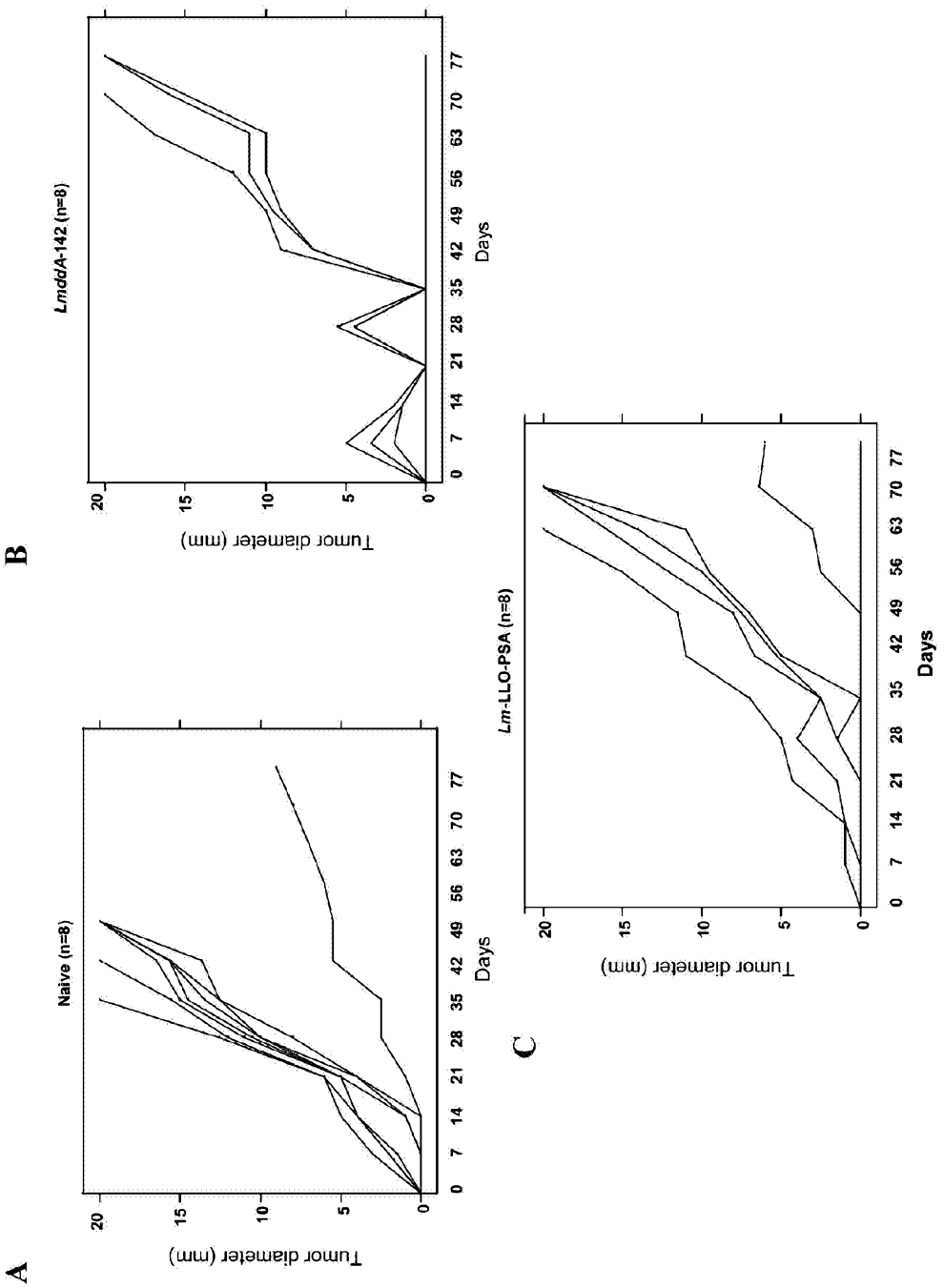

A
Naive (n=8)
Tumor diameter (mm)
0
5
10
15
20
0 7 14 21 28 35 42 49 56 63 70 77
Days
B
LmddA-142 (n=8)
Tumor diameter (mm)
0
5
10
15
20
0 7 14 21 28 35 42 49 56 63 70 77
Days
C
Lm-LLO-PSA (n=8)
Tumor diameter (mm)
0
5
10
15
20
0 7 14 21 28 35 42 49 56 63 70 77
Days

FIGURE 6

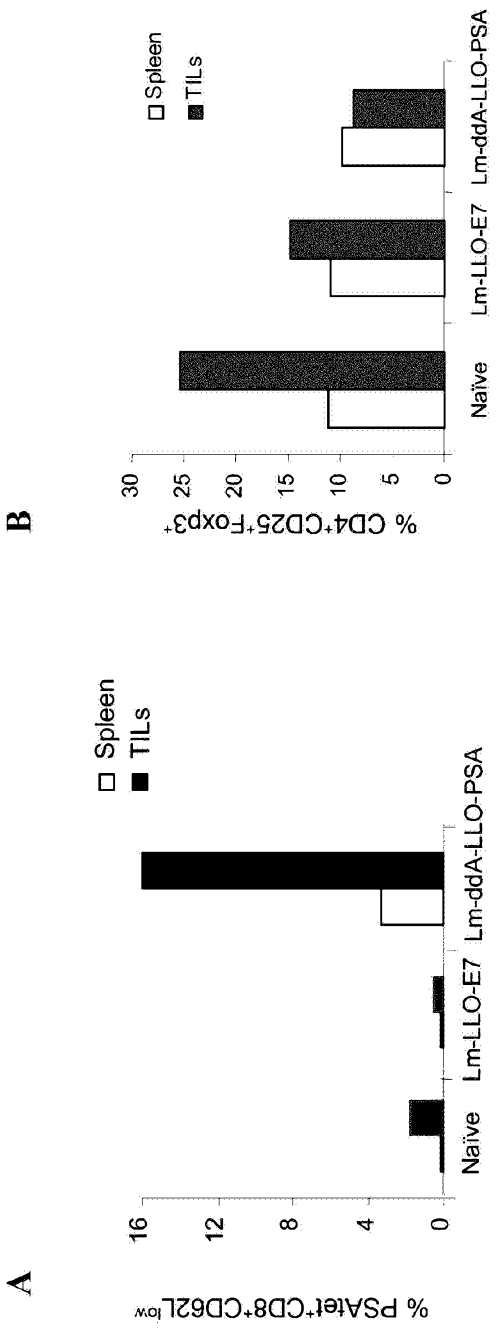
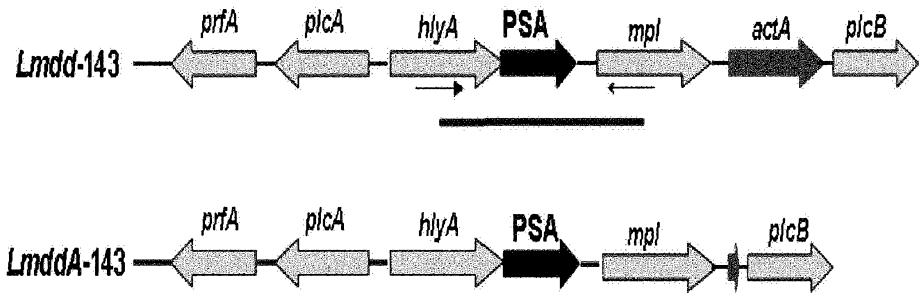

A
% PSAtet+CD8+CD62Llow
16
12
8
4
0
Naïve
Lm-LLO-E7
Lm-ddA-LLO-PSA
Spleen
TILs
B
% CD4+CD25+Foxp3+
30
25
20
15
10
5
0
Naïve
Lm-LLO-E7
Lm-ddA-LLO-PSA
Spleen
TILs

FIGURE 7

A



B

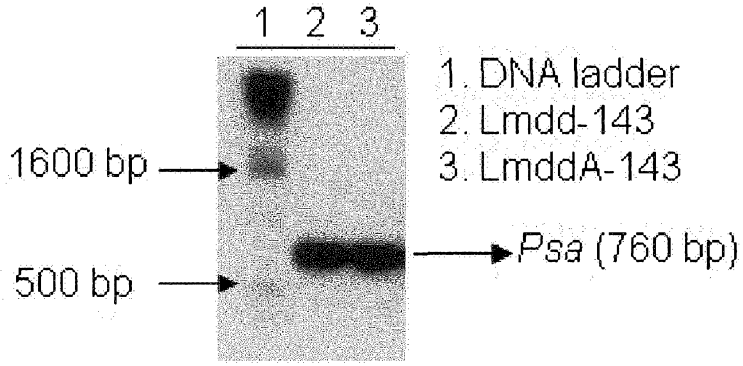


FIGURE 8

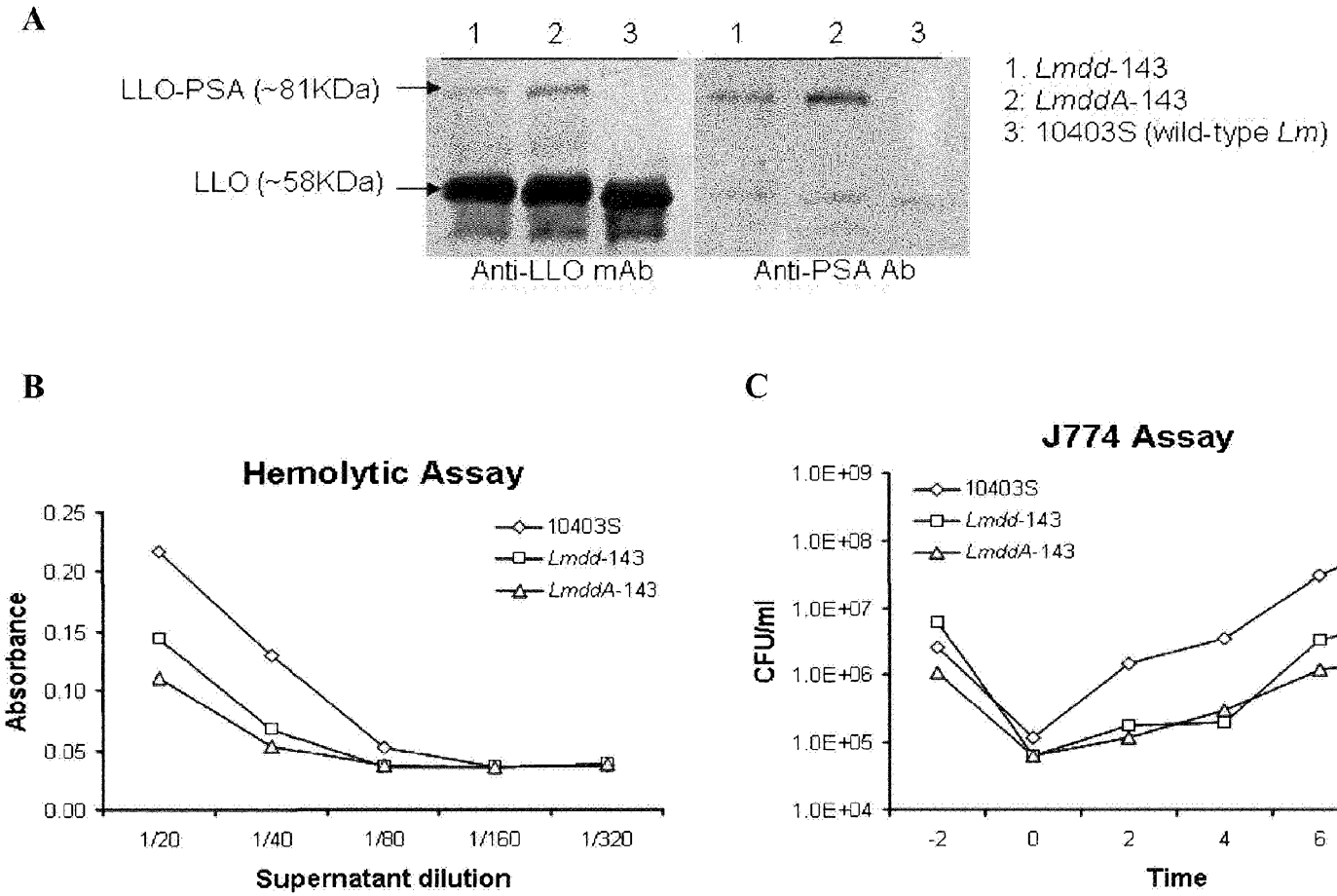


FIGURE 9

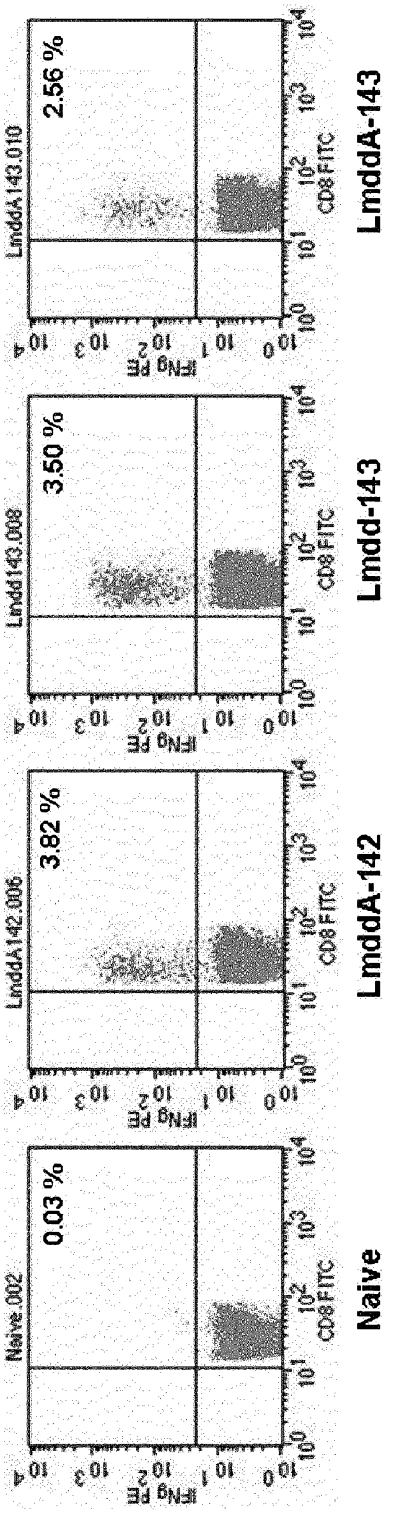

Naive.002
0.03 %
LmddA142.006
3.82 %
Lmdd143.008
3.50 %
LmddA143.010
2.56 %
IFNg PE
CD8 FITC
Naive
LmddA-142
Lmdd-143
LmddA-143

FIGURE 10

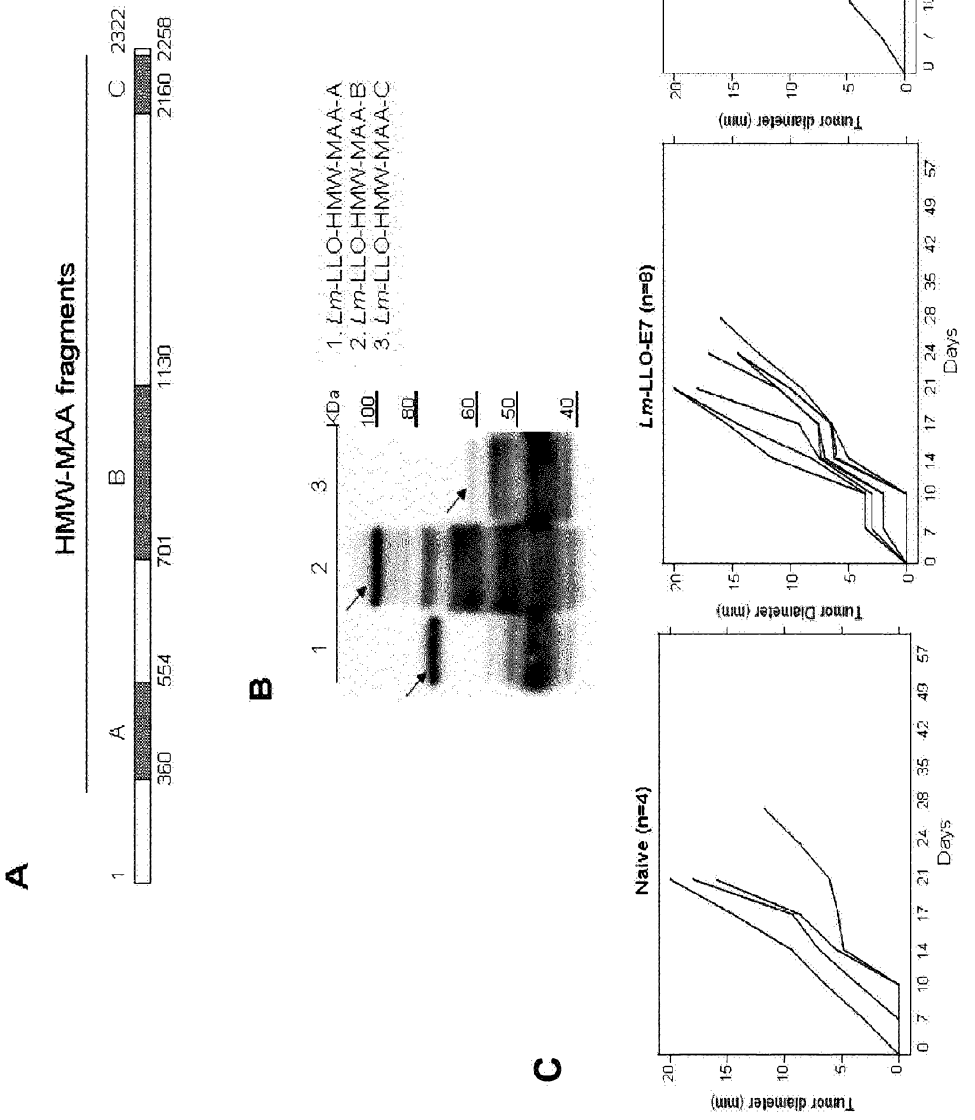
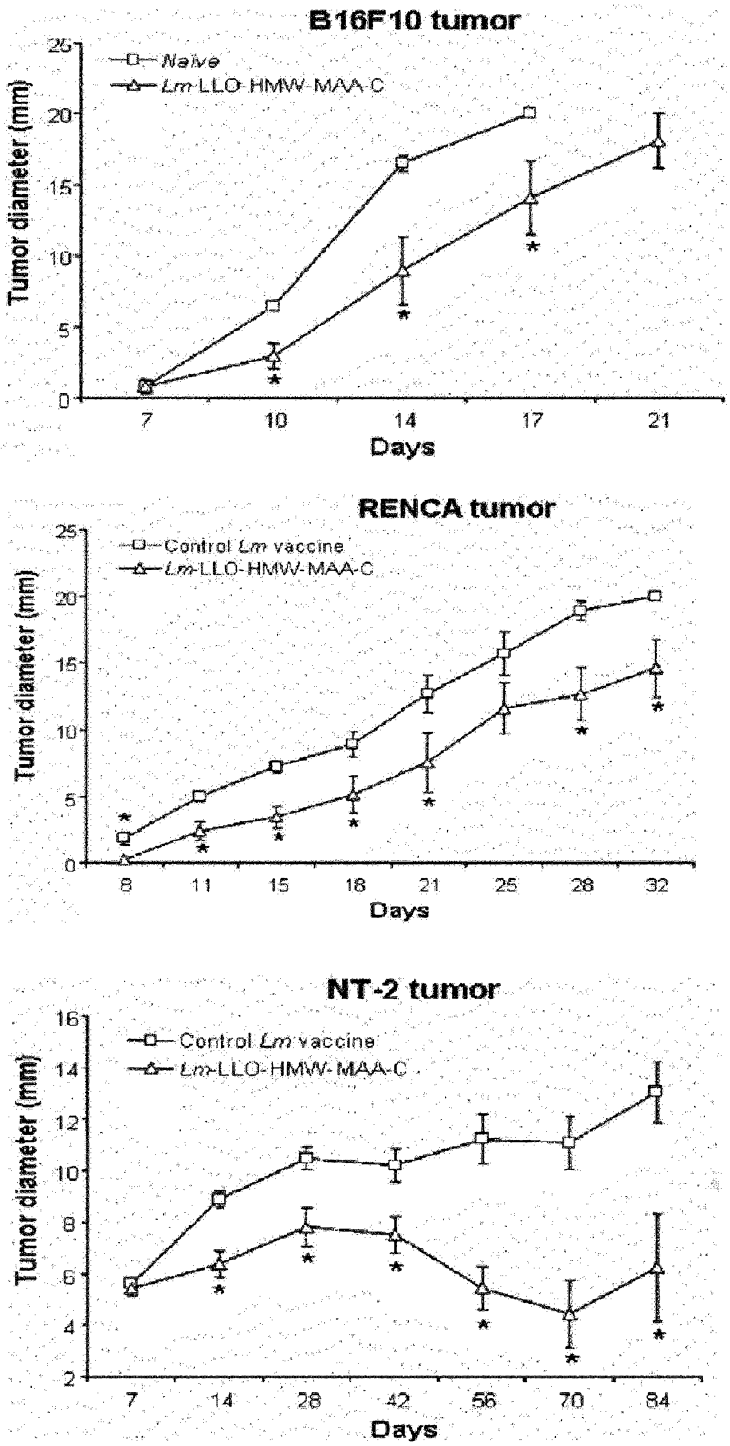

A
HMW-MAA fragments
1
A
B
C
2322
360
554
701
1130
2160
2258
B
KDa
1. Lm-LLO-HMW-MAA-A
2. Lm-LLO-HMW-MAA-B
3. Lm-LLO-HMW-MAA-C
100
80
80
50
40
C
Naive (n=4)
Lm-LLO-E7 (n=8)
Lm-LLO-HMW-MAA-C (n=8)
Tumor diameter (mm)
Days

FIGURE 11

Fig 11D


B16F10 tumor
Naïve
Lm-LLO-HMW-MAA-C
Tumor diameter (mm)
Days
RENCA tumor
Control Lm vaccine
Lm-LLO-HMW-MAA-C
Tumor diameter (mm)
Days
NT-2 tumor
Control Lm vaccine
Lm-LLO-HMW-MAA-C
Tumor diameter (mm)
Days

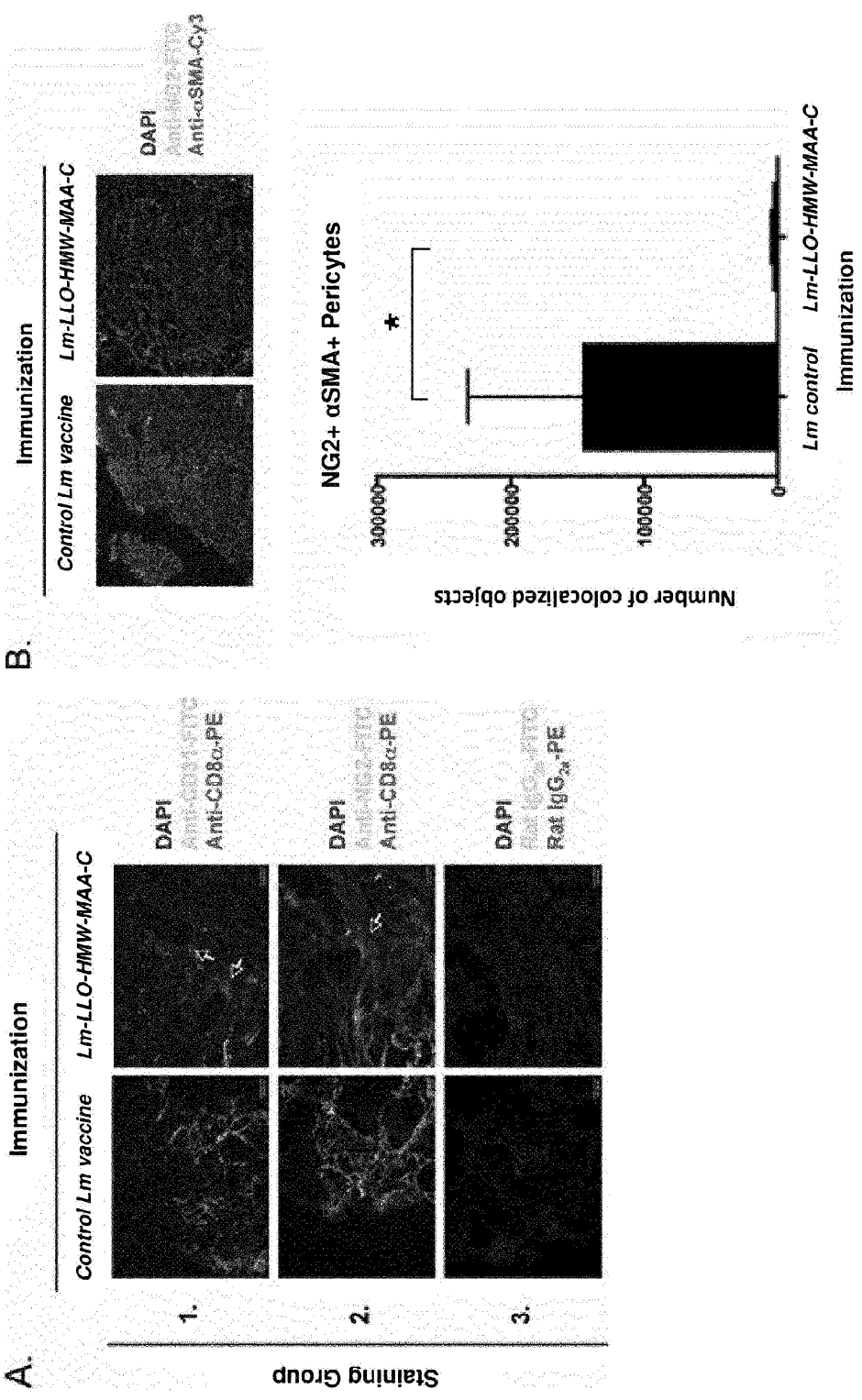

A.
Immunization
Control Lm vaccine
Lm-LLO-HMW-MAA-C
Staining Group
1.
2.
3.
DAPI
Anti-CD8α-PE
DAPI
Anti-CD8α-PE
DAPI
Rat IgG2a-PE
B.
Immunization
Control Lm vaccine
Lm-LLO-HMW-MAA-C
DAPI
Anti-αSMA-Cy3
NG2+ αSMA+ Pericytes
Number of colocalized objects
300000
200000
100000
0
*
Lm control
Lm-LLO-HMW-MAA-C
Immunization

FIGURE 12